

Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN
BIODIVERSITÀ ED EVOLUZIONE

Ciclo XXVI

Settore Concorsuale di afferenza: 05/B1 - ZOOLOGIA E ANTROPOLOGIA

Settore Scientifico disciplinare: BIO/05 - ZOOLOGIA

TITOLO TESI

**DEMERSAL COMMUNITIES IN THE MEDITERRANEAN SEA:
A CASE STUDY OF TRIGLIDAE (OSTEICHTHYES,
SCORPAENIFORMES) ON THE CONSERVATION AND
SUSTAINABLE USE OF MARINE RESOURCES**

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Esame finale anno 2015

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Chapter I. General Introduction

This project focuses on the catch and biology of the family Triglidae (Teleostei, Scorpaeniformes) from north-middle Adriatic (north-east Mediterranean) as a tool to increase the knowledge and to develop the management of a demersal resource.

Gurnards (Scorpaeniformes, Triglidae) are demersal fishes which inhabit the continental and insular shelves of tropical and temperate seas (along the Mediterranean Sea, the Black Sea and the eastern Atlantic Ocean from Norway to the west African coast) to depths of 500 m, found on sandy, muddy or rubble substrates (Fischer et al. 1987). There are seven different species (*Chelidonichthys* (sin. *Aspitrigla*) *cuculus*, *Chelidonichthys lucerna*, *Eutrigla gurnardus*, *Lepidotrigla cavillone*, *Lepidotrigla dieuzeidei*, *Trigla lyra* and *Trigloporus lastoviza*) in the north-middle Adriatic Sea (13°-15°E, 42°-45°N)) from the Gulf of Trieste to the line joining the Gargano promontory (Italy) to the borderline between Croatia and Montenegro. These species in other Mediterranean areas show differences related to biometric features and ecological ones such as diet, spawning period and depth distribution (Tsimenides et al. 1992, Colloca et al. 1994, 2003). Red gurnard (*C. cuculus* Linnaeus, 1758), tub gurnard (*C. lucerna* L., 1758) grey gurnard (*E. gurnardus* L., 1758) and rock gurnard (*T. lastoviza* Bonnaterre, 1788) have been included in the list of reference species of the European MEDITS project which are studied for their biological parameters (Relini et al. 2008). *C. lucerna* is the most landed and commercially important gurnard species for Italian fishery and in particular for Adriatic Sea (Relini et al. 1999) and along with *C. cuculus* and *E. gurnardus* have been mentioned by the International Council for the Exploration of the Sea as new MOU (Memorandum Of Understanding) species and has recommended that monitoring programs should be conducted to derive information on biological parameters for stock assessment purposes (ICES 2006). Triglidae family, with the decline in traditionally exploited fish stock, have been considered new landed species by trawlers (Boudaya et al. 2008 – South Mediterranean), and emergent key predator of commercially important shelf stocks such as hake (as the case of *Eutrigla gurnardus* in the North Sea reported by Floeter et al. (2005) and Weinert et al. (2010)). However, there is a lack of knowledge on the life cycle of this species and its ecological characteristics in the eastern Italian

coast (north Mediterranean). Available data for Adriatic Sea are very limited, dating back several decades (Valiani 1934, Frogliani 1976). The change of many genera within Triglidae family occurred in the last years also has been discussed by Italian Society of Marine Biology (Relini and Lanteri 2010). Therefore, as a commercially important species, increasingly exploited and poorly known, the study of gurnards biology, ecology and taxonomy has become of particular importance for fisheries management purposes and in order to improve knowledge of demersal communities. All these information are made operational for fisheries research and could overcome the inadequacy of “traditional” single-species models, that is amplified by the lack of long-term routine fisheries data and the poor level of scientific information, in contrast to the considerable increase in fishing activity over time (Stergiou 2000). Furthermore will be useful for an ecosystem-based management of Adriatic Sea (north-eastern Mediterranean).

The Northern and Central Adriatic (GSA 17) is one of the largest and best defined areas of occurrence of demersal and small pelagic shared stocks in the Mediterranean. In this area bottom trawl fishing is a well-developed activity since the beginning of 20th century along the whole basin. Demersal resources are exploited by Italian, Croatian and Slovenian fishing fleets which often operate on the same stocks and fishing grounds. Because of that cooperation among all the countries involved in fisheries, the knowledge of spatial distribution of species, as well as the knowledge of status and the evolution of populations, assume crucial importance in developing and supporting management plans for sustainable fishery and rationale exploitation and protection of demersal stocks in the Adriatic (Piccinetti et al. 2012). The management of resources adopted until now on world wide scale, and based on a population approach, has failed as showed by different signals, i.e.: the decline of global fishery catch (Caddy and Garibaldi 2000; FAO 2010), the depauperation and over-exploitation of worldwide stocks (Gislason et al. 2000; FAO 2010), the decline in the mean trophic level of organism landed (Pauly et al. 1998); the overall consequence is the ecological and economic collapse of numerous important fishery in many parts of the world (Pauly et al. 2002). In the Mediterranean Sea the most important commercial species are fully exploited or

overexploited (Leonart 2005; FAO 2010); although Mediterranean fisheries have shown a surprisingly high resilience to human exploitation compared to some areas of the Atlantic (Leonart 2005), important negative impacts have also been measured at level of habitats and communities (Tudela 2004). The state of heavy exploitation of Adriatic fishery resources is also evident and for some stocks is critical (Mannini et al. 2004). The effects of this failure also affect biodiversity, structure and functioning of ecosystems (Gislason et al. 2000), and this impact of fishing on the environment has been abundantly described. The ecosystem effect of fishing can be classified as direct or indirect effects. More specifically, capture fisheries reduce abundance, spawning potential and modify size structure of the target resources, as well as of their associated and dependent species; as a matter of fact by-catch and discarding results in mortality of non-target species while reductions in target species may allow competitors to increase in abundance. Moreover the removal of top predators, which may release an usually large abundance of preys at lower levels, deeply modify the flows of biomass and energy across the ecosystem, with cascading and feedback effects on the trophic structure and species composition; conversely, the decline of primary productivity consumers low in the food chain removes important forage species needed higher in the food web, with cascading effects for the ecosystem. Fishery can also alter habitats, most notably by destroying and disturbing the topography of bottoms and the associated habitats and benthic communities, and may lead to increased abundance of scavengers and other opportunistic species in disturbed areas (Bianchi et al. 2000). On the other hand, it is true that it is often difficult to separate out the effects of fishing from the anthropogenic influences (i.e. pollution and habitat modification) and from the effects of natural environmental variability (Gislason et al. 2000).

GOALS OF THE THESIS

The present PhD work was conducted at BiGeA, Department of Biological, Geological and Environmental Sciences and Laboratory of Marine Biology and Fisheries of Fano (University of Bologna). Collaboration with Chemistry Department (University of Bologna) and IMEDEA (CSIC-

UIB) Institute (Spain) were held. The project was born in 2007 supported by MiPAAF, studying the feeding biology of principal demersal species in Adriatic Sea, included tub gurnard *Chelidonichthys lucerna*. The opportunity to collect gurnard samples from MEDITS (European) and GRUND (national) trawl surveys in collaboration with Laboratory of Marine Biology and Fisheries of Fano (Andrea scientific vessel) and the care for the family Triglidae still little studied, prompted to create a literature for the Adriatic population for better understanding the status of this resource, supported by Canziani funds. This project is included in the research line of BiGeA Department (University of Bologna): “Biodiversity and autoecology of bony fishes”. Several aspects were taken into account as summarized as followed.

Main objectives:

- To study the biology and ecology of the family in the light of specie-specific patterns;
- Given the information on their biology and utilisation, to consider their potential for future sustainable utilisation and management in the trawl fishery in north-central Adriatic Sea.

These objectives were achieved by:

- a) investigating aspects of the population dynamics of the whole family: depth and spatial distribution, preliminary studies on nursery areas, length-weight relationships, size at first maturity (see CHAPTER II);
- b) studying the feeding biology through the examination of stomach contents to determine diet, trophic interactions, and potential ecological impact of directed commercial utilisation on other species (CHAPTER III);
- c) using otoliths as potential marker of species life cycle: sclerochronological studies (DGI), morphometries and shape analysis, ultrastructural analysis (SEM and TEM), chemical analysis on bio-mineralization process (TGA, XRD, proteomic analyses) (see CHAPTER IV);
- d) preliminary data on mDNA phylogeny (see CHAPTER V);

e) briefly summarizing all the results for a general conclusion (see CHAPTER VI).

Chapter II. Investigating aspects of the population dynamics of the Triglidae family: depth and spatial distribution, preliminary studies on nursery areas, length-weight relationships, size at first maturity

Scientific papers are reported below the summary of chapter II as follows:

VALLISNERI M., TOMMASINI S., STAGIONI M., MANFREDI C., ISAJLOVIC I., **MONTANINI S.** (2014). Distribution and some biological parameters of the red gurnard, *Chelidonichthys cuculus* (Actinopterygii, Scorpaeniformes, Triglidae) in the north-central Adriatic Sea. *Acta Ichthyol. Pisc.*, 44 (3): 173-180.

VALLISNERI M., STAGIONI M., **MONTANINI S.**, TOMMASINI S. (2011). Body size, sexual maturity and diet in *Chelidonichthys lucerna* (Osteichthyes: Triglidae) from the Adriatic Sea, north eastern Mediterranean. *Acta Adriat.*, 51 (1): 141-148.

MANFREDI C., **MONTANINI S.**, STAGIONI M., TOMMASINI S., VALLISNERI M. (2013). Note su distribuzione e “aree di nursery” di due specie di triglidi (Scorpaeniformes) nel nord-centro Adriatico. *Atti Soc. Nat. Mat. Modena*, 144: p. 156. 74° Congresso UZI, Modena 30 settembre-3 ottobre 2013.

MANFREDI C., VALLISNERI M., STAGIONI M., ISAJLOVIC I., **MONTANINI S.** (2014). Depth distribution of gurnard target species (Scorpaeniformes: Triglidae) from GSA17. *Biol. Mar. Mediterr.*, 21 (1): 281-282.

VALLISNERI M., **MONTANINI S.**, STAGIONI M. (2012). Size at maturity of triglid fishes in the Adriatic Sea, northeastern Mediterranean. *J. Appl. Ichthyol.*, 28: 123-125.

Scientific papers before 2011-2014 years:

VALLISNERI M., **MONTANINI S.**, STAGIONI M. (2010). Length–weight relationships for the family Triglidae in the Adriatic Sea, northeastern Mediterranean. *J. Appl. Ichthyol.*, 26:460-462.

VALLISNERI M., **MONTANINI S.**, STAGIONI M., TOMMASINI S. (2009). Length–weight relationships for seven species of the family Triglidae from the middle and north Adriatic Sea. *Biol. Mar. Mediterr.*, 16 (1): 370-371.

MONTANINI S., STAGIONI M., VALLISNERI M. (2008). Notes on the biology of *Chelidonichthys lucernus* (Teleostei: Triglidae) in the Northern-Middle Adriatic Sea. *Biol. Mar. Mediterr.*, 15 (1): 340-341.

SUMMARY OF CHAPTER II.

Bathymetric and spatial distribution showed intra- and inter-specific pattern linked to biological and ecological behaviour of target gurnard species. *C. cuculus* (red gurnard) showed preference for deeper water according to Tsimenides et al. (1992) and Marriot et al. (2010). It has been widely distributed over the whole Central Adriatic. There was only restricted areas in water deeper than 200 meters and along shallow coastal water where it was not caught. *C. cuculus* recruits and spawners also showing preference for areas at different depth, as also observed in other seas (Serena et al. 1990). Recruits were found at shallower depth (mostly between 76-150 m) while adults were observed at greater depth (mostly deeper than 150 m). The juveniles of red gurnard were mainly distributed in the open Central Adriatic Sea and annual high-density patches of recruits were found in the whole distribution area of the species. The area showing the greatest persistence ($PI > 0.75$) was detected on soft bottom to the North of Pomo/Jabuka Pit at depth range between 100 and 200 m. In this zone the main incidence of recruits was highlighted by values of the R/Tot variable always ≥ 0.8 . *C. lucerna* (tub gurnard) has been mainly distributed along shallow coastal water along the Western side of the basin. Length–frequency distribution of all samples ranged from 63 to 415 mm (mean 208.8 ± 58.9 mm) and articulates on two principal cohorts: the first one includes small samples more frequent in the summer and next to the coast, the second individuals of average and big size, more frequent in winter and to great depth. *E. gurnardus* (grey gurnard) has been widely distributed in the whole basin with the greatest abundance steadily found on clay and relict sand bottoms at 100 meters of depth. For *E. gurnardus* (catches depth range: 14-266 m) no differences about trend of abundance along depth were found between recruits and spawners, their depth abundances varying concordantly. *T. lastoviza* (streaked gurnard) has been mainly distributed in the median and eastern part of the North Adriatic, and in the channel area from Split to Dubrovnik, generally down to 100 meters. For *T. lastoviza* no differences in bathymetric distribution were found between recruits and spawners, their depth abundance being strictly correlated. Annual patches of

great density of the streaked gurnard recruits were mainly found in the whole distribution area of the species throughout the study period, although juveniles were mostly concentrated along the north-eastern coast; the area showing the greatest persistence ($PI > 0.75$) was pinpointed off the Pag Island. In this area ratio R/Tot was > 0.5 . For no target gurnard species (*L. cavillone*, *L. dieuzeidei* and *T. lyra*) were taken into account bathymetric and spatial data from Piccinetti et al. (2012).

Length-weight relationships were estimated for seven species of gurnards (Triglidae) based on data collected during the last twenty-seven years (1982-2010) in the Adriatic Sea for the first time. The L-W relationship was determined by the equation: $\log W = \log(a) + b \log(L)$, where W is the total weight of the fish (g), L is the total length (cm), a is the intercept on the Y-axis of the regression curve and b is the regression coefficient. In order to verify if b was significantly different from the isometric value ($b=3$), the Student's t-test ($H_0: b=3$) with a confidence level of $\pm 95\%$ ($\alpha=0.05$) and so for sexes or life-stages was employed (Sokal and Rohlf 1987). Geographic cline is tested by $\log(a)$ over b plot (Froese 2006). Results showed significantly different relationships between juveniles and adults (female and male combined) for *C. cuculus*, *E. gurnardus*, *C. lucerna* and *L. cavillone*. Isometric growth of juveniles were indicated for four species, allometric growth of adults for all species. There was no evidence of geographic cline for *C. lucerna* about slope values even if b values increased from north to south (latitude).

Size at first maturity was calculated for three target gurnard species (*C. cuculus*, *C. lucerna* and *E. gurnardus*) collected in the north-middle Adriatic sea from 2005 to 2009. The percentage of mature individuals in 10 mm class intervals was calculated for both sexes. Cumulative frequency curve and maturity ogive plots were used in order to estimate the size at which 50% of individuals were sexually mature. The sex and maturity stages were determined by the macroscopic examination of the gonads (eight maturity stages were distinguished for the females and males) according to the manual elaborated in the frame of an international project to harmonise the samplings by bottom

trawling of the demersal resources in the Mediterranean Sea (MEDIT project); spawning begins in 2c and 3 gonad maturity stages (maturing and mature/spawner) (Relini et al. 2008). According to literature, considered gurnard species showed a critical size species related, coinciding with the start of sexual maturity, the tendency to migrate to greater depths, a change of diet from crustaceans to fish and an increase of variety of food items eaten. Such behaviour probably depends on the predator's changing energetic requirements in relation to fish size, dietary protein levels influencing the size of fish at first maturity. Data from various stocks throughout Northwest Europe and the Mediterranean according to latitude were also investigated. Results presented shows that males attain their sexual maturity at smaller sizes than females as other stocks in different areas and showed a tendency towards a geographical cline, particularly for *C. lucerna*. These differences are usually attributed to different ecological and hydrographical conditions, particularly temperature that stimulates sexual maturation. For Mediterranean fishes, differences seem to be more related to seasonal variation in feeding intensity according to Colloca et al. (2003).

**DISTRIBUTION AND SOME BIOLOGICAL PARAMETERS OF THE RED GURNARD,
CHELIDONICHTHYS CUCULUS (ACTINOPTERYGII, SCORPAENIFORMES, TRIGLIDAE)
IN THE NORTH-CENTRAL ADRIATIC SEA**

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Vallisneri M., Tommasini S., Stagioni M., Manfredi C., Isajlović I., Montanini S. 2014. Distribution and some biological parameters of the red gurnard, *Chelidonichthys cuculus* (Actinopterygii, Scorpaeniformes, Triglidae) in the north-central Adriatic Sea. *Acta Ichthyol. Piscat.* 44 (3): 173–180.

Background. Red gurnard, *Chelidonichthys cuculus* (Linnaeus, 1758), is distributed in the Mediterranean Sea, Black Sea, and in the eastern Atlantic Ocean from Norway to Mauritania, although it is rarely found in the North Sea and on the Norwegian shelf. The aim of this work was to conduct a comprehensive study of red gurnard in the Adriatic Sea on selected aspects the population biology and some of its environmental implications.

Materials and methods. The sampling covered the entire subdivision GSA17 (north-central Adriatic Sea) and it was based on several bottom trawl surveys of MEDITS and GRUND projects from 2000 through 2009. Biometric parameters, as well as the spatial- and depth distributions were analyzed. The parameters such as the length–weight relation, sex, gonad maturity stage, and the stomach content were determined on a subsample of 539 specimens collected seasonally (summer and autumn–winter) within 2007–2009. The data were processed statistically.

Results. *Chelidonichthys cuculus* was mainly distributed in the central Adriatic. Juveniles showed a distribution in shallower waters while adults tended to migrate towards the Croatian coast at greater depths. The total length of the fish sampled ranged from 40 to 303 mm (TL). We assumed the length of 100 mm as a threshold separating juveniles from adults and the length range of 170–190 mm as the maturity size for females and males. Isometric- and allometric growth patterns were showed for the juveniles and the adults, respectively. The diet was based almost exclusively on crustaceans (IRI% > 98%) and it did not change upon reaching the sexual maturity. *Chelidonichthys cuculus* should be considered a specialist predator feeding mostly on necto-benthic lophogastrids (IRI% > 72%).

Conclusion. This work constitutes an attempt to summarize some environmental and biology aspects of the red gurnard in Adriatic population: differences in depth distribution and growth were found between juveniles and adults but not in diet. Males and females showed differences in growth parameters. Extending the studied area and collecting data of red gurnard in the main areas of production should help in better understanding the biology and dynamics of this species.

Keywords: red gurnard, *Aspitrigla cuculus*, distribution, biology, north-eastern Mediterranean, diet, growth

INTRODUCTION

Red gurnard, *Chelidonichthys cuculus* (Linnaeus, 1758)**, is one of the most abundant and commercially important triglid fish species in the Mediterranean Sea and therefore has been included in the list of target species of the MEDITS project (Mediterranean International Trawl Survey) (Bertrand et al. 2002) carried out in the Mediterranean to monitor commercially exploited demersal resources. ICES has identified red gurnard as a new MoU

(Memorandum of understanding) species and has recommended that a survey on its biological data should be conducted in order to define stock characteristics and assist in the development of management strategies for sustainable exploitation (Marriott et al. 2010, Anonymous 2006, 2012).

Red gurnard is a demersal fish species, with a pelagic phase during the early life (Tsimenides et al. 1992, Colloca et al. 1994). The distribution of *Chelidonichthys cuculus* in the Mediterranean Sea is restricted to the depth range of

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** Even though some authors have recently referred to this species as *Aspitrigla cuculus*, the editors decided to follow the nomenclature of FishBase and used *Chelidonichthys cuculus* as the valid name (Froese and Pauly 2014). According to FishBase the reference for *Chelidonichthys cuculus* as the accepted senior synonym is Richards and Saksena (1990).

100–220 m (Dieuzeide et al. 1955, Papaconstantinou 1983, Serena et al. 1990, Tsimenides et al. 1992). Juveniles tend to occur in shallower waters than the adults (Serena et al. 1990, Colloca et al. 1994). Due to the missing parts of the time-series data of red gurnard it is difficult to provide a preliminary analytical assessment (Anonymous 2012). Its biology is poorly understood in the Atlantic Ocean (Marriott et al. 2010), and in the northern Mediterranean Sea (Colloca et al. 2003, Vallisneri et al. 2010, 2012). A number of researchers studied the growth of this fish (Baron 1985a, Maisan et al. 1998, Colloca et al. 2003), its length–weight relations (Coull et al. 1989, Mendes et al. 2004, Olim and Borges 2004), reproduction (Baron 1985b), and the diet (Moreno-Amich 1992, Colloca et al. 1994, Terrats et al. 2000). The aim of this study was to analyse the distribution and selected biological traits of the red gurnard population in the north-central Adriatic Sea. Such data are needed for the proper stock assessment.

MATERIALS AND METHODS

Sampling. The presently reported study was based on the sampling operations of the MEDITS and GRUND projects (summer survey: MEDITS project, Mediterranean International Trawl Survey; winter survey: GRUND project, Gruppo Nazionale Demersali) in the north-central Adriatic Sea (north-eastern Mediterranean) from the Gulf of Trieste to the imaginary line joining the Gargano promontory (Italy) to the borderline between Croatia and Montenegro. The sampling depth distribution ranged from 10 to 500 m. The sampling gear was a bottom trawl made of four panels with the cod-end mesh size of 20 mm (MEDITS surveys, Fiorentini et al. 1999) and a commercial bottom trawl with the cod-end mesh size of 40 mm (GRUND surveys). The GRUND project covered Italian territorial waters and international waters while the MEDITS project covered the whole north-central Adriatic Sea, including Croatian territorial waters.

The analysis of the distribution of *Chelidonichthys cuculus* in terms of areas and depths was performed over the whole north-central Adriatic Sea using MEDITS data from 2000 through 2009, while other biological analyses were performed only on the samples collected from the Italian territorial waters and the international area using both MEDITS and GRUND data from 2007 through 2009. A total of 539 specimens of red gurnard, *C. cuculus*, were analysed. The fish captured were frozen on board to prevent digestion of their stomach contents, and finally taken to the laboratory.

Data analyses. Specimens were measured (TL, to the nearest 1 mm), weighed (W , to the nearest 0.01 g), and dissected. Sex and gonad maturity stages were verified by macroscopic examination of the gonads. The stages of maturation were classified according to Relini et al. (2008). Specimens were classified into two groups: juveniles with TL \leq 100 mm and adults with TL $>$ 100 mm. The cut-off size to define juveniles was calculated as the first modal component of length-frequency distribution, identified by means of the Battacharya's method (Battacharya 1967), plus standard deviation.

The abundance of *Chelidonichthys cuculus* was standardized to 1 km² by using the distance covered and the horizontal opening of the fishing gear. A map with miniature pie charts at the sampling sites was used to represent the geographic distribution of the abundance of both juveniles and adults. The size of the pie charts on the map is proportional to the standardized total abundance of the species. Based on the depth distribution of our data, the studied area was divided into five zones for assessment of the relation between the depth and the fish size (length). Minimum-, maximum-, and mean lengths were calculated by 50-m depth intervals and graphically represented.

Statistical analyses. The chi-squared test was used to compare differences between size classes and depth zones. The non-parametric Kruskal–Wallis test was performed to check differences in the fish depth preferences of juveniles and adult individuals. All the statistical inferences were based on the 0.05 significance level.

We tested both linear and means of non-linear regressions to describe the relation between fish size and fish weight and adopted relations which explained the highest proportion of the variance (highest value of r^2). Length–weight relation was calculated for each size class (juveniles and adults) and sex (females and males) by using the formula:

$$W = aTL^b$$

where W is the total weight of the fish [g], TL is the total length [mm], a is the intercept on the Y axis of the regression curve, and b is the regression coefficient. To test for possible significant differences between the estimated b -values and $b = 3$, the t -test was used and 95% confidence limits (CL) for parameters b were calculated (Froese 2006). Statistical procedures (Shapiro–Wilk test, to check if residuals look normal, and Fligner–Killeen test, to check assumption of homogeneity of variance) followed Zuur et al. (2010).

The maturity stage frequencies by size were computed. In order to estimate the size at which 50% of individuals were sexually mature, the percentage of mature individuals in 2-cm intervals was calculated for females and males at spawning seasons (from January to June) according to Tsikliras et al. (2010).

Subsamples of 390 stomach contents were taken and preserved in 70% ethanol solution to prevent further food digestion. Prey items were identified to the lowest possible taxonomic level, counted, and weighed to the nearest 0.1 mg after removal of surface water by blotting paper. The importance of prey categories was evaluated using the percentage frequency of occurrence ($F^0\%$), the percentage by number ($N^0\%$), and the percentage by weight ($W^0\%$). We used these values to calculate the index of relative importance (IRI) (Pinkas et al. 1971):

$$IRI = F^0\% \cdot (N^0\% + W^0\%)$$

as modified by Cortés (1997):

$$IRI\% = (IRI \cdot \sum IRI^{-1}) \cdot 100$$

Prey categories were sorted in decreasing order according to their IRI%.

All data analyses were performed with R software ver. 3.0.2 base (Anonymous 2014).

RESULTS

Area distribution. Red gurnard, *Chelidonichthys cuculus*, occurred in the 26.3% of trawl samples, through the investigated area, although showing a marked preference for the central Adriatic Sea (Fig. 1). Maximum abundances were recorded in the Croatian territorial waters hauls which showed a mean abundance of 847 individuals per 1 km² in the positive hauls; mean abundance in the Italian side was 202 individuals per 1 km² in the positive hauls. Juveniles represented the greatest fraction in the Italian area and in some hauls near the Croatian coast; adults represented the greatest fraction in almost all Croatian hauls.

Depth distribution. A highly significant test results (χ^2 , $P < 0.001$) reflected how strong the size-with-depth at different seasons patterns were.

The species was never sampled 0–50 m and occasionally between 50–100 m. The red gurnard occurred almost regularly in the depth range of 100–200 m. The distribution of size classes (juveniles and adults) was significantly different (Kruskal–Wallis test, $P < 0.001$). Juveniles were observed preferentially between 100–150 m in the Central Adriatic Sea (mean 109 ± 25.1 m), while adults between 100–200 m down to 250 m (mean 150 ± 55.5 m) (Figs. 1, 2; Table 1).

Size distribution. Table 2 reports the size range of the specimens classified as juveniles and adults. It also summarizes the data for the two sexes: females ranging from 102 to 303 mm TL and males from 105 to 277 mm TL.

Length–weight relations. The non-linear regression model was fitted to the length–weight values by size class (juveniles and adults) and by sexes (females and males) (Figs. 3 and 4). All these relations were highly significant ($P < 0.001$) and were able to explain over 94% of the variance. About size classes, adults exhibited positive allometric growth ($b = 3.266$; 95% CL of $b = 3.210$ – 3.321 ; $b \neq 3$ $P < 0.001$) while juveniles showed an isometric growth in fact the value of parameter b ($b = 3.008$; 95% CL of $b = 2.892$ – 3.125) in the length–weight relation was not statistically different from 3 (t -test, $P > 0.001$). In terms of the sexes, both females and males exhibited a positive allometric growth (for females: $b = 3.354$; 95% CL of $b = 3.246$ – 3.462 and for males: $b = 3.183$; 95% CL of $b = 3.100$ – 3.266 ; $b \neq 3$ $P < 0.001$).

Maturity stages distribution by size. The smallest mature males and females of *Chelidonichthys cuculus* measured around 100 mm. Between 130 and 150 mm TL a lot of stages were recorded for both sexes highlighting individual variability in gonad maturation. The length at sexual maturity was estimated for both sexes as the total length at which 50% of the fish were mature (Table 3). In fact, over 50% of the individuals were at stage 3 in the sample taken during the reproductive period (January–June). In both

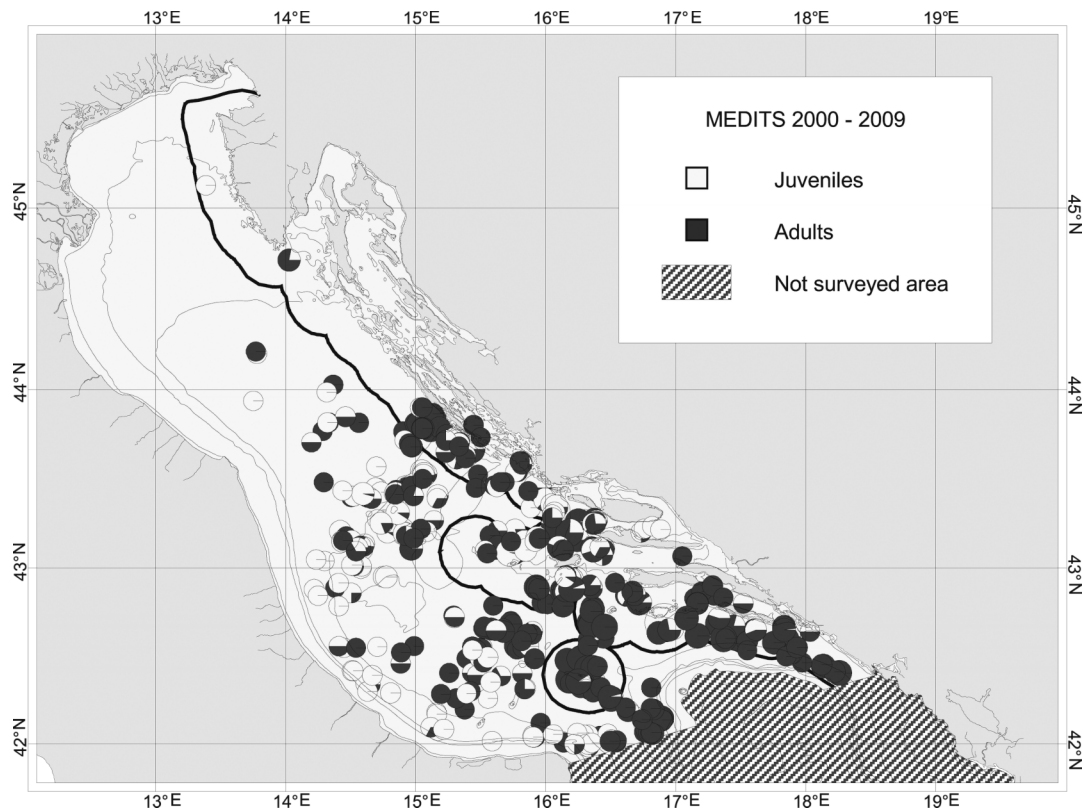


Fig. 1. Map showing the studied area and geographic distribution of the abundance of red gurnard, *Chelidonichthys cuculus*, in the Adriatic Sea by juveniles and adults (only the successful hauls are shown)

sexes, some specimens within the 130–170 mm range had already reached the spawning-ready stage.

Diet. Stomach contents were summarized in Table 4. A total of 17 different food items were found in the diet and classified into five main categories. Almost the entire diet of this species was based on malacostracan crustaceans (%*N* = 93.9; %*W* = 91.2; %*F* = 76.9; %*IRI* = 98.1) while teleost fishes, molluscs, and echinoderms were classified as accessory prey. As for the crustaceans, Lophogastrida (%*N* > 64; %*IRI* > 70) represented the most important prey taxa while Decapoda (%*N* > 24; %*IRI* > 18) were classified as secondary ones. *Lophogaster typicus* constituted the basic prey food for both juveniles (%*N* = 73.9; %*IRI* = 97.1) and adults (%*N* = 83.5; %*IRI* = 97.6) showing a quite exclusive preference for this species.

DISCUSSION

Interest in gurnards as commercial species has increased. As a consequence monitoring programmes targeting red gurnard for the stock assessment were recommended by ICES (Anonymous 2012), which should also contribute to a better understanding of the species biology.

Red gurnard, *Chelidonichthys cuculus*, in the north-central Adriatic Sea occurred at depths between 50–250 m. It was absent at depths lower than 50 m preferring the range of 100–200 m, according to the majority of published records. The depth distribution of this fish in the Atlantic was reported as 20 to 250 m (Wheeler 1969, Anonymous 2006), although with rare occurrences at depths lower than 20 m and most common presence from 30 to 60 m (Forest 2001). The juveniles, however, were distributed in more shallow waters than the adult individuals (Serena et al. 1990, Colloca et al. 1994). In the

Mediterranean Sea, red gurnard was reported over a similar depth range as in the Atlantic, with usual depth range of 40 to 300 m (Tsimenides et al. 1992, Terrats et al. 2000, Colloca et al. 2003, Massutí and Reñones 2005), although they were also recorded as deep as 800 m off the Cretan shelf (Kallianiotis et al. 2000). Preferred and tolerated temperatures of red gurnard are not known (Marriott et al. 2010) and whether the Atlantic population differs from Mediterranean population, preference for deeper water may be temperature related or linked to the absence of preferred habitat in the shallow inshore water (Ramsay et al. 1998, Rees 2004). Therefore, it will be necessary to

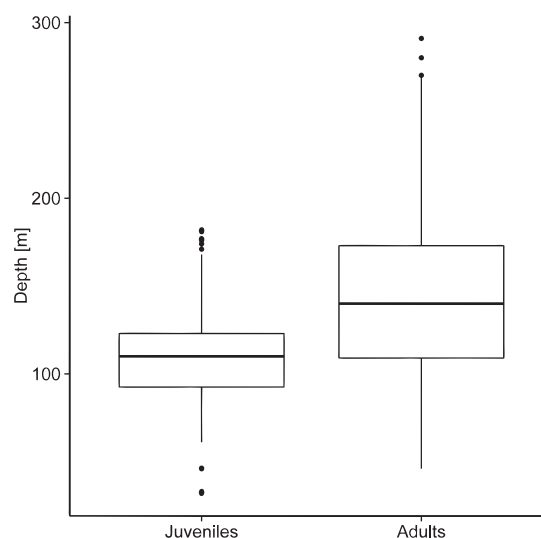


Fig. 2. Boxplot of depth distribution of red gurnard, *Chelidonichthys cuculus*, from the Adriatic Sea by size classes (MEDITS 2000–2009)

Table 1

Depth distribution of red gurnard, *Chelidonichthys cuculus*, from the Adriatic sea (MEDITS surveys 2000–2009)

Category	Depth [m]				
	0–50	50–100	100–150	150–200	200–300
Females	7	294	580	361	297
Males	7	203	472	272	203
Juveniles	12	407	1032	74	0
Adults	14	497	1052	633	500

In the depth ranges the start point is exclusive and the end point inclusive.

Table 2

Total length (TL) and depth range of red gurnard, *Chelidonichthys cuculus*, from the Adriatic Sea (MEDITS surveys 2000–2009)

Category	<i>n</i>	Total length [mm]			Depth [m]	
		Min	Max	Mean ± SD	Min	Max
Females	1539	102	303	168.93 ± 29.26	46	291
Males	1157	105	277	159.80 ± 22.63	46	291
Juveniles	1525	40	100	81.41 ± 10.96	32	182
Adults	2696	102	303	165.01 ± 27.00	46	291

n = number of specimens examined, SD = standard deviation.

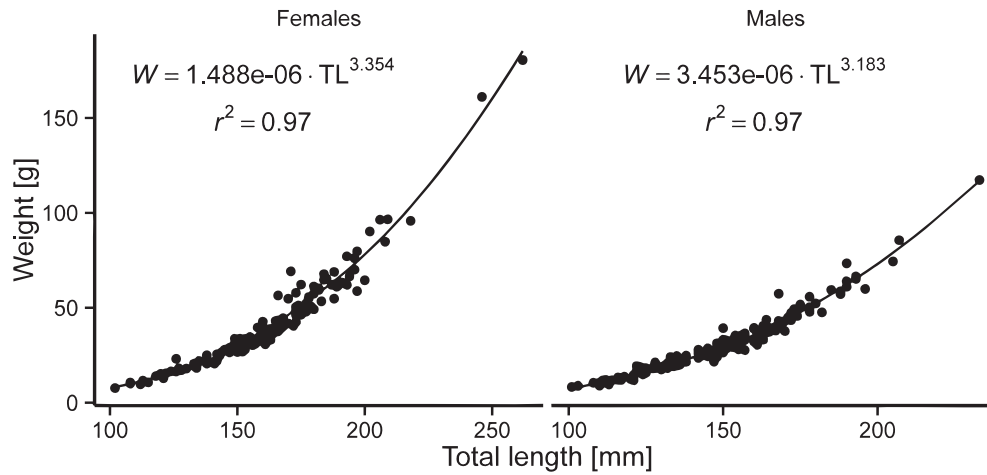


Fig. 3. Length–weight relations for red gurnard, *Chelidonichthys cuculus*, from the Adriatic Sea: non-linear regression plots for juveniles (left; $n = 168$) and adults (right; $n = 371$), respectively

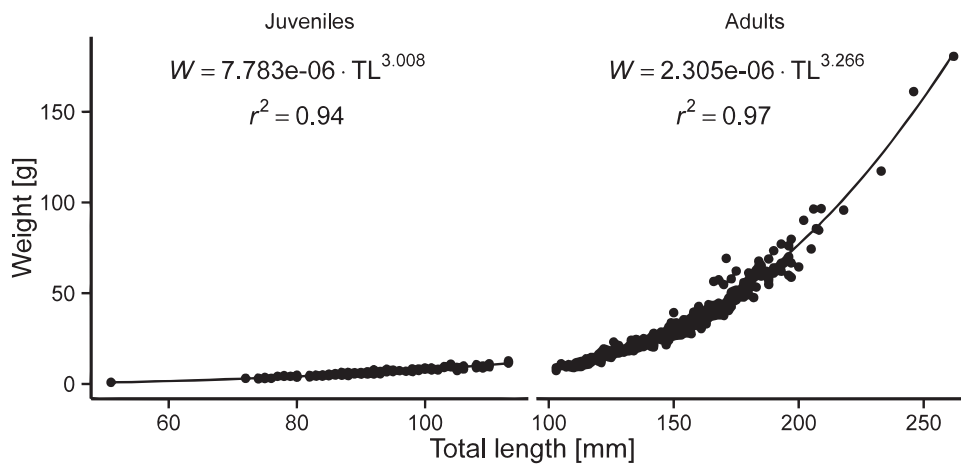


Fig. 4. Length–weight relations for red gurnard, *Chelidonichthys cuculus*, from the Adriatic Sea: non-linear regression plots for females (left; $n = 131$) and males (right; $n = 166$), respectively

Table 3

Percentage of specimens of red gurnard, *Chelidonichthys cuculus*, from the Adriatic Sea in individual stages of gonad maturity, in relation to the total length (MEDITS surveys 2007–2009)

TL [mm]	Maturity stage (females)							Maturity stage (males)						
	1	2A	2B	2C	3	4A	4B	1	2A	2B	2C	3	4A	4B
90–110	67	33	—	—	—	—	—	25	75	—	—	—	—	—
110–130	8	85	7	—	—	—	—	8	53	20	17	—	2	—
130–150	—	19	50	12	4	11	4	3	3	22	40	15	17	—
150–170	—	—	18	21	37	8	16	—	—	23	40	15	8	13
170–190	—	—	9	9	76	3	3	—	—	5	21	63	11	—
190–210	—	—	—	—	8	69	23	—	—	—	—	20	—	80
210–230	—	—	—	—	100	—	—	—	—	—	—	—	—	—
230–250	—	—	—	100	—	—	—	—	—	—	—	—	—	100
250–270	—	—	—	—	—	—	100	—	—	—	—	—	—	—

In the length ranges the start point is exclusive and the end point inclusive; TL = total length; Maturity stages (Relini et al. 2008): 1 = immature-virgin, 2A = virgin-developing, 2B = recovering, 2C = maturing, 3 = mature/spawner, 4A = spent, 4B = resting.

Table 4

Trophic spectrum of red gurnard, *Chelidonichthys cuculus*
(from the Adriatic Sea (MEDITS surveys 2007–2009))

Prey taxon	%N	%W	%F	%IRI
Arthropoda: Crustacea	93.88	91.15	76.92	98.07
<i>Lophogaster typicus</i>	82.54	69.44	37.95	97.75
<i>Goneplax rhomboides</i>	7.69	5.27	7.44	1.63
<i>Solenocera membranacea</i>	1.46	7	1.79	0.26
<i>Liocarcinus</i> sp.	1.87	2.04	2.05	0.14
<i>Alpheus glaber</i>	1.25	2.04	1.54	0.09
<i>Chlorotocus crassicornis</i>	0.62	1.76	0.51	0.02
<i>Galathea</i> sp.	1.04	0.44	0.77	0.02
<i>Liocarcinus depurator</i>	1.04	2.52	0.26	0.02
<i>Ebalia</i> sp.	0.42	0.52	0.51	0.01
<i>Plesionika</i> sp.	0.42	0.28	0.51	0.01
<i>Pirimela denticulata</i>	0.21	1.07	0.26	0.01
<i>Philocheras trispinosus</i>	0.21	0.76	0.26	<0.01
<i>Macropipus tuberculatus</i>	0.21	0.76	0.26	<0.01
<i>Liocarcinus corrugatus</i>	0.21	0.24	0.26	<0.01
<i>Galathea intermedia</i>	0.21	0.02	0.26	<0.01
Chordata: Actinopterygii	1	3.38	1.79	0.05
<i>Callionymus</i> sp.	0.42	5.41	0.51	0.05
Echinodermata	0.3	0.28	0.77	<0.01
<i>Cidaris cidaris</i>	0.21	0.41	0.26	<0.01
Mollusca	0.8	0.3	1.54	0.01
Unidentified remains	4.02	4.89	30.26	1.86

%N = numeric prey abundance [%], %W = wet weight prey abundance [%], %F = frequency of occurrence [%], %IRI = index of relative importance [%].

understand the abiotic and biotic factors controlling the distribution of red gurnard relating to area.

A positive allometric growth with similar length–weight relations for males and females, was demonstrated by Papaconstantinou (1983), Baron (1985a), Anonymous (2006), Marriott (2010), and by the presently reported study. However, Olim and Borges (2004) found differences in the *b* values for males and females. In relation to the size classes, the adult individuals showed a steeper slope of the regression indicating a higher growth rate. This greater variability in the length–weight relation depends on many factors that affect the weight of adults such as maturation of gonads or change of diet (Froese 2006).

Red gurnard females reached maturity at greater size than males, in the Atlantic Ocean (Baie de Douarnenez, Brittany, 48°N—Baron et al. 1985b; North West Wales, 53°N—Marriott et al. 2010) and in the Mediterranean Sea (Central Tyrrhenian Sea, 43°N—Colloca et al. 2003). As for the size at maturity, our data are comparable to those reported in the literature being related to the latitude (Vallisneri et al. 2012). The differences can be attributed to environmental changes (particularly temperature that stimulate sexual maturation), such as the photoperiod and prey availability, in addition to internal physiological factors.

Red gurnard, *Chelidonichthys cuculus*, feed mainly on Crustacea (mostly Lophogastrida) and less on Decapoda, according to many published reports (Colloca et al. 1994

for the Tyrrhenian Sea, Terrats et al. 2000 for the Aegean Sea). However Labarta (1976) noted Decapoda as the main food item of red gurnard off the Spanish coast of the Atlantic Ocean, while Mysidacea (including Lophogastrida and Mysida) were found to be of minor importance. Feeding habits did not change when the fish approached the size of sexual maturity although individuals showed a bathymetrical pattern linked to growth. Our data were in agreement with Colloca et al. (2010) that described red gurnard prey mostly on the lophogastrid, *Lophogaster typicus*, showing a specialist trophic behaviour overlapping in both food and depth preferences. Our data did not agree with the ones reported by Moreno-Amich (1992): around size of sexual maturity, Decapoda increased in respect to Mysidacea and fish represented the most important prey after the crustaceans.

Further investigations are needed to progress on stocks boundaries such as population studies and next management regulations. Overall official catch statistics reported to ICES have shown a decreasing trend over the period 2001–2010 from 6500 to 4000 t, however, there is currently no technical measure specifically applied to red gurnard or other gurnard species (Anonymous 2012).

In conclusion, this study contributes to the general knowledge of a poorly known population biology and environmental of red gurnard in north-eastern Mediterranean.

ACKNOWLEDGEMENTS

Sampling was funded through MEDITS and GRUND projects. We thank Professor Corrado Piccinetti for his assistance in bottom trawl surveys and for his valuable suggestions.

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Received: 9 December 2013

Accepted: 1 June 2014

Published electronically: 15 October 2014

Body size, sexual maturity and diet in *Chelidonichthys lucerna* (Osteichthyes: Triglidae) from the Adriatic Sea, north eastern Mediterranean

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A total of 1,114 tub gurnard (*Chelidonichthys lucerna* L., 1758) were caught by trawl surveys carried out between May 2005 and March 2007 in the Adriatic Sea (north eastern Mediterranean). Females ranged from 113 to 415 mm TL and males from 128 to 299 mm TL. At 50% maturity, females matured at a fork length of 270 mm, while males matured at 220 mm. The tub gurnards prey mainly crustaceans and fishes. Molluscs are also consumed. A length around 180 mm is a critical size that coincides with the slope to great depths, the migration along the Italian coast towards the Croatian coast, the start of sexual maturity and the change of their main food, from crustaceans to fish.

Key words: body size, sexual maturity, feeding habits, *C. lucerna*, Adriatic Sea

INTRODUCTION

ICES has identified the tub gurnard, *Chelidonichthys lucerna* (Linnaeus, 1758) as a potential commercial species and a new MOU (Memorandum Of Understanding) species and has recommended that monitoring programmes should be conducted to derive information on biological parameters for stock assessment purposes (ICES, 2006). The tub gurnard lives in the Mediterranean Sea, Black Sea and eastern Atlantic from Norway to Senegal. Along the Italian coast the species generally occurs at up to 200 m depth and it is the most important gurnard species for Italian fisheries. Many researchers during recent years investigated its growth and reproduction in the eastern Mediterranean

(PAPACONSTANTINO, 1984-Greece; ABDALLAH & FALTAS, 1998-Egypt; ISMEN *et al.*, 2004-Turkey; ERYILMAZ & MERIC, 2005-Turkey; UCKUN ILHAN & TOGULGA, 2007-Turkey; CICEK *et al.*, 2008-Turkey; BOUDAYA *et al.*, 2008-Tunisia).

Concerning length-frequency distribution, generally tub gurnard females reach a length greater than males, for example: males 8.3- 21.2 cm, females 8.0-30.3 cm (ISMEN *et al.* 2004); males 12.0-37.9 cm, females 12.0-41.9 cm (ERYILMAZ & MERIC, 2005); males 14.1-29.9 cm, females 12.7-34.4 cm (UCKUN ILHAN & TOGULGA, 2007) and males 6.5-29.3 cm, females 6.1-30.3 cm (CICEK *et al.*, 2008).

Concerning reproduction, generally (at 50% maturity) tub gurnard males mature at a smaller size than females, for example males 18.0 cm,

females 20.0 cm (ISMEN *et al.*, 2004); males 18.50 cm, females 18.97 (ERYILMAZ & MERIC, 2005); males 17.7 cm, females 19.0 (UCKUN ILHAN & TOGULGA, 2007).

However, there is a lack of knowledge on the life cycle of this species and its ecological characteristics along the Italian coast of the north eastern Mediterranean. Most of the studies provided information on nursery areas and the distribution of juveniles concentrating in shallow waters, mainly in estuarine waters, where food is abundant (COLLOCA *et al.* 1994 - Tyrrhenian Sea; SERENA *et al.*, 1998 -Tyrrhenian Sea).

A few studies examined some of its biological characteristics in the Adriatic Sea. Regarding feeding habits, tub gurnard changes diet during growth with the capture of bigger-sized prey and the replacement of food categories or bathymetric migration (FROGLIA, 1976 - middle Adriatic Sea). Early larval development in the laboratory was described by DULČIĆ *et al.*, (2001). The

length–weight relationships varied significantly according to juvenile and adult specimens (CERIOLOLA *et al.*, 2004 -southern Adriatic Sea; VALLISNERI *et al.*, 2010 - northern-middle Adriatic Sea).

The aim of the present study is to contribute to knowledge of the tub gurnard, that is a reference species of the Mediterranean Sea, regarding its size distribution, size at maturity and diet in the coastal waters of the Adriatic Sea.

MATERIAL AND METHODS

A total of 1,114 specimens of tub gurnard (*Chelidonichthys lucerna* L., 1758) were collected from May 2005 until March 2007 in the Adriatic Sea (north eastern Mediterranean) from the Gulf of Trieste (45°40' N 13°37' E) to the Tremiti Islands (42°0,8' N 15°16' E) (Fig.1) at depths ranging from 11 to 257 m. Samples were collected during seasonal oceanographic bottom trawl surveys (winter survey: GRUND project,

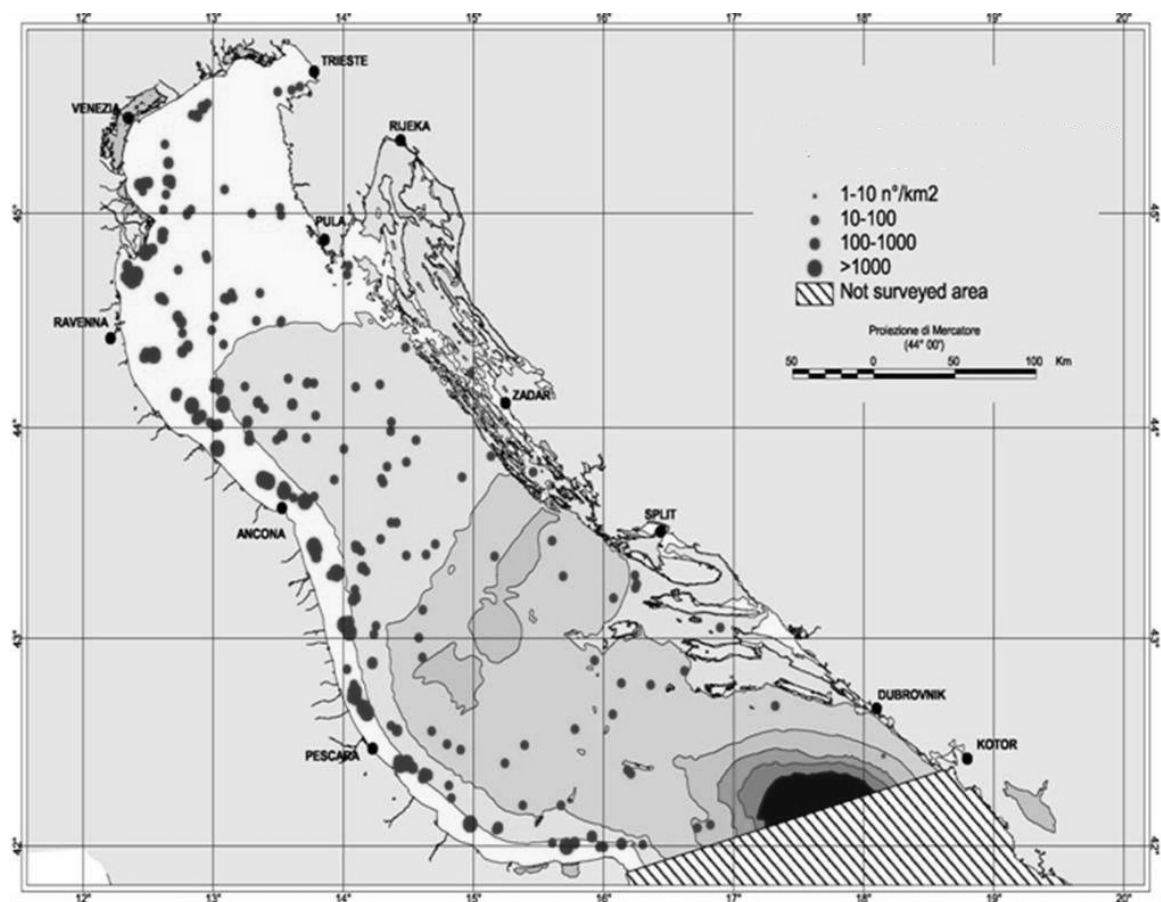


Fig. 1. Map of sampling areas in the Adriatic Sea

GRUppe Nazionale Demersali; summer survey: MEDITS project, MEDiterranean International Trawl Survey) using a special bottom trawl (GOC 73) designed for experimental fishing for scientific purposes (RELINI *et al.*, 2008). To increase the catch of demersal species, the gear was characterized by a high vertical opening (about 2.5 m), greater than the most common professional gear used in this area, and a mesh codend size of 20 mm (stretched mesh) (FIORENTINI *et al.*, 1999). Catches were frozen to prevent digestion of their stomach contents, and subsequently taken to the laboratory.

All specimens were measured (total length, TL, to the nearest 1 mm) and weighed with 0.1 g precision. The length-weight relationship was determined by the equation: $W = aL^b$, where W is the total weight of the fish (g), L is the total length (mm), a is the intercept on the Y-axis of the regression curve and b is the regression coefficient.

The sex and maturity stages were determined by macroscopic examination of the gonads following a scale according to RELINI *et al.* (2008). The specimens were classified as juveniles (J) and adults: females (F) or males (M). The percentage of mature individuals in 5 cm intervals was calculated for both sexes. A logistic ogive was fitted to the data in order to estimate the size at which 50% of individuals were sexually mature.

Stomachs were preserved in 70% ethanol solution, while prey were identified to the lowest possible taxonomic level, counted and weighed to the nearest 0.1 mg after removal of surface water by blotting paper. The importance of prey was evaluated using the percentage frequency of occurrence (%O), the percentage by number (%N) and the percentage by weight (%W). We used these values to calculate the index of relative importance (IRI) of PINKAS *et al.*, (1971) expressed as $IRI = \%O(\%N + \%W)$ and modified by HACUNDA (1981) expressed as $IRI\% = (IRI / \sum IRI) \times 100$.

Diet pattern according to tub gurnard size classes was evaluated by cluster analysis (cluster methods: complete) of square root transformed numeric prey abundance at species level, using

the Bray-Curtis similarity index (CLARKE & WARWICK, 1994). This method was computed to test the correlation between predator length and number of ingested food categories.

Multivariate analyses by PCA (Principal Component Analysis) plot were performed between predator size ranges and prey classes in order to evaluate environmental and ontogenetic patterns. All data analyses were performed with R software ver. 2.9.2 base and Vegan package (R DEVELOPMENT CORE TEAM, 2009).

RESULTS

The length–frequency distribution of all samples ranged from 63 to 415 mm (mean 208.8 ± 58.9 mm) and articulates on two principal cohorts: the first includes small samples more frequent in the summer and next to the coast, while the second includes individuals of average and large size, more frequent in winter and to great depths (Pearson's Chi-squared test: $X^2 = 226.061$, $df = 4$, $p\text{-value} < 2.2e-16$) (Fig. 2; Table 1). Total body weight ranged from 2.2 to 650.9 g (mean 110.9 ± 79.1).

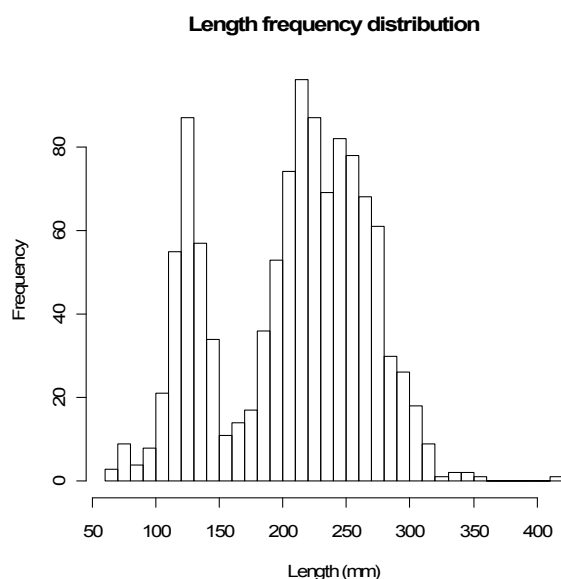


Fig. 2. Length-frequency distribution for *C. lucerna* juveniles ($n=234$) and adults ($n=880$)

TL of females ($n=484$) ranged from 113 to 415 mm (mean 237.1 ± 47.5 mm) and W from 15.6 to 650.9 g (mean 147 ± 81.1 g).

Table 1. Distribution by depth

Depth classes	Juveniles	Females	Males
(10,25) m	199	167	113
(25,50) m	29	144	104
(50,250) m	6	173	179

TL of males (n=396) ranged from 128 to 342 mm (mean 225.9 ± 32.5 mm) and W from 32.7 to 321.8 g (mean 120.9 ± 53.9 g).

TL of juveniles (n=234) ranged from 63 to 180 mm (mean 121.6 ± 17.6 mm) and W from 2.2 to 54.3 g (mean 19.3 ± 7.1 g).

The regression of total wet body weight (W) as a function of total length (TL) was statistically significant ($p < 0.001$). For all size range (juveniles and adults: females and males), weight increased proportionally to length (Fig. 3).

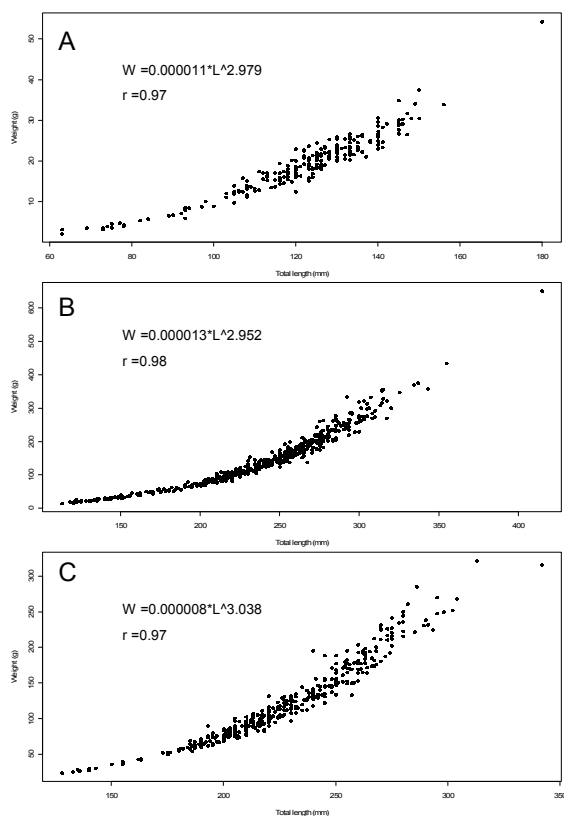


Fig. 3. Length-weight relationship between juveniles (n=234), females (n=484) and males (n=396) for *C. lucerna*

The smallest mature males and female measured around 180 mm. 50% of males were mature at 238 mm TL and 50% of females were mature at 255 mm TL respectively (Fig. 4).

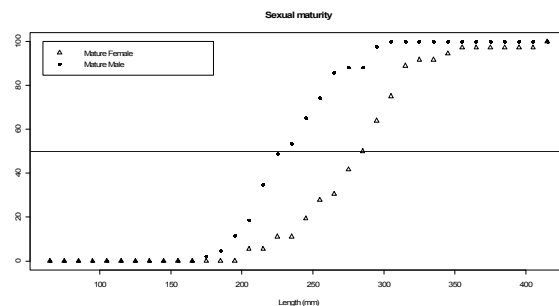


Fig. 4. Relationship between the percentage of mature *C. lucerna* and total length for females and males

With respect to diet, crustaceans had the highest index of relative importance as the main prey items (%IRI=92) and occurred in 58% of stomachs with food. These were followed by fish (%IRI=7), which were the other important prey group, while molluscs and polychaetes were minor components (Table 2). Cluster analysis of %N (numeric prey abundance percent) values according to class size showed tub gurnard to be mostly associated with fish and crustacean preys. Substantial differences in the diet were observed between juveniles (≤ 180 mm) and adults (> 180 mm) when crustaceans decreased and fish increased (Fig. 5).

Taxa	N(%)	W(%)	O(%)	IRI(%)
Crustacea	89.69	58.01	7.47	92.24
Teleostei	6.72	39.74	1.92	7.45
Bivalvia	1.13	0.23	0.25	0.003
Gastropoda	0.3	0.22	0.12	0.001
Cephalopoda	0.14	0.1	0.04	0
Polychaeta	0.03	0.01	0.01	0

Table 2. Trophic spectrum. N(%): numeric prey abundance percent, W(%): wet weight prey abundance percent, O (%): frequency of occurrence, IRI(%): percentual index of relative importance

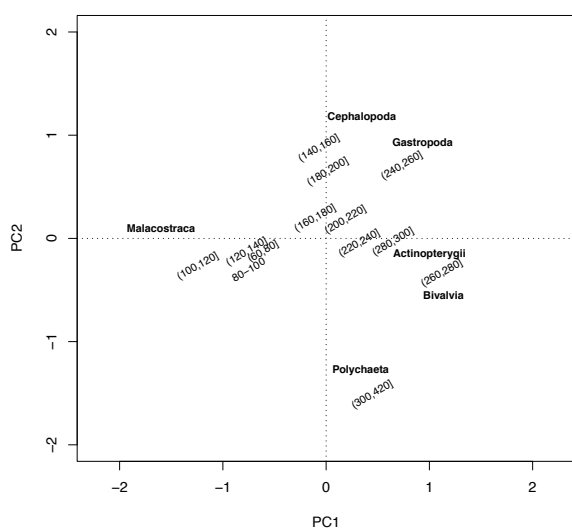


Fig. 5. Multivariate analyses plot of numeric prey abundance by predator size classes

DISCUSSION

The length–frequency distribution for the southern Adriatic Sea ranged on two principal cohorts, according to CERIOLA *et al.*, (2004).

A relationship between size and depth has been shown; in fact juveniles are concentrated in shallow waters probably because food is abundant, according to the literature (FROGLIA, 1976; PAPAConstantinou, 1984; BARON, 1985; SERANGELI *et al.*, 1985; COLLOCA *et al.*, 1994; BOUDAYA *et al.*, 2008). *C. lucerna* females reach greater lengths than males, according to the literature on other areas (SERENA *et al.*, 1998 - Tuscany coast; ISMEN *et al.*, 2004 - Iskenderun Bay; ERYILMAZ & MERIC, 2005 - Sea of Marmara; BOUDAYA *et al.*, 2008 - Gulf of Gabès).

Tub gurnard mature at a smaller size for males than females according to some authors (PAPAConstantinou, 1984; BOUDAYA *et al.*, 2008). This phenomenon is attributed to the fact that body size is a less important factor for male fitness, while for females a large size at maturity probably implies less fitness costs, as large eggs, large fecundity and access to the best spawning sites (BOUDAYA *et al.*, 2008). Regarding size at maturity, our data are comparable to those reported in the literature as being related to area. The differences can be attributed to ecological conditions, particularly temperature, that

stimulate sexual maturation (UCKUN ILHAN & TOGULGA, 2007) and the methods used.

Mediterranean gurnards share a common feeding pattern based on crustaceans and other epibenthic infauna (COLLOCA *et al.*, 1994; MORTE *et al.*, 1997; LABROPOULOU & MACHIAS, 1998; BOUDAYA *et al.*, 2008). According to COLLOCA *et al.* (1994) and BOUDAYA *et al.* (2008), tub gurnards change their diet with size. Smaller individuals feed upon benthic crustaceans. Tub gurnards prey increasingly on Decapoda Reptantia and small fish as their body sizes increase.

A length of around 180 mm represents a critical size for *Chelidonichthys lucerna* in this area, and coincides with the slope to great depths, the migration from the Italian coast towards the Croatian coast, the start of sexual maturity and the change of their main food from crustaceans to fish. A rapid change in the feeding strategy of Mediterranean gurnard species generally coincides with the size of first maturity (COLLOCA *et al.*, 1994).

Control of fishing activity is achieved by enforcement of the current system and covers restrictions on species, fish sizes, mesh sizes, locations, etc. The available data suggest that the minimum fishing size should be limited for the Mediterranean tub gurnard, according to ISMEN *et al.* (2004).

In conclusion, updated biological parameters should be produced in order to define stock characteristics and assist in the development of management strategies for sustainable exploitation. The results of this study help to provide such information for tub gurnard in the Adriatic Sea.

ACKNOWLEDGEMENTS

Sampling was funded through the MEDITS and GRUND projects. We thank Professor Corrado PICCINETTI for his assistance in bottom trawl surveys and for his valuable suggestions.

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Received: 23 October 2009

Accepted: 15 November 2010

Veličina, spolna zrelost i ishrana lastavice balavice, *Chelidonichthys lucerna* (Osteichthyes: Triglidae), u Jadranskom moru,

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SAŽETAK

Uhvaćeno je ukupno 1.114 primjeraka lastavice balavice (*Chelidonichthys lucerna* L., 1758) tijekom kočarenja provedenih između svibnja 2005. i ožujka 2007. u Jadranskom moru (sjeverno istočni Mediteran). Veličina ženki je bila u rasponu od 13 do 415 mm ukupne duljine (TL), dok je ukupna veličina mužjaka iznosila od 128 do 299 mm (TL). Dužina pri kojoj 50% populacije dosiže spolnu zrelost, kod ženki iznosi 270 mm, a kod mužjaka 220 mm. Plijen lastavice se pretežito sastoji od rakova i riba. Također su konzumirani i mekušci.

Dužina jedinki od oko 180 mm jest kritična u tom smislu jer se podudara sa prelaskom jedinki na život u većim dubinama, početkom migracije uzduž talijanske obale pa prema hrvatskoj obali, početkom spolne zrelosti te sa promjenom u njihovoj glavnoj hrani koja prelazi sa rakova na ribe.

Ključne riječi: veličina, spolna zrelost, hranidbene navike, *C. lucerna*, Jadransko more



NOTE SU DISTRIBUZIONE E “AREE DI NURSERY” DI DUE SPECIE DI TRIGLIDI (SCORPAENIFORMES) NEL NORD-CENTRO ADRIATICO

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Il ruolo degli habitat costieri come *nurseries* del novellame di specie ittiche è fondamentale in relazione alla conservazione ed alla gestione delle risorse alieutiche. Ancora oggi il concetto di nursery è ambiguo ed è necessario considerare la combinazione di vari fattori, quali densità, accrescimento, sopravvivenza del novellame e migrazione verso gli habitat degli adulti, per determinare se un habitat è utilizzabile come *nursery* per il reclutamento dei giovanili. A tale scopo è stata condotta un'analisi su distribuzione e aree di *nurseries* del capone cocchio, *Aspitrigla cuculus* (L.) e del capone ubriaco, *Trigloporus lastoviza* (Bonnaterre, 1788) in Adriatico (GSA 17) fra il 1996 e il 2008. I risultati preliminari mostrano che l'area di distribuzione di *A. cuculus* copre tutto l'Adriatico centrale (ad eccezione delle acque profonde della fossa di Pomo/Jabuka) e le zone costiere occidentale e orientale dell'Adriatico; i giovanili sono distribuiti principalmente in mare aperto nell'Adriatico centrale; l'area di *nursery* che mostra il maggiore indice di persistenza ($PI > 0,75$) risulta sul fondo molle a nord della fossa di Pomo/Jabuka tra 100 e 200 m di profondità. Si conferma quindi che l'area a ridosso e all'interno della fossa di Pomo/Jabuka rappresenta una delle principali aree di nursery per molte specie demersali, indicandone la notevole influenza sulle attività di pesca. I risultati preliminari relativi a *T. lastoviza* mostrano che è distribuita principalmente nella parte mediana ed orientale dell'Adriatico settentrionale e nella zona del canale fra Spalato e Dubrovnik, generalmente fino a 100 m di profondità; i giovanili sono concentrati soprattutto lungo la costa nord-orientale; l'area di *nursery* che mostra il maggiore indice di persistenza ($PI > 0,75$) è stata individuata al largo dell'isola di Pag.

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DEPTH DISTRIBUTION OF GURNARD SPECIES (SCORPAENIFORMES: TRIGLIDAE) FROM GSA 17

DISTRIBUZIONE BATRIMETRICA DEI TRIGLIDI DEL NORD-CENTRO ADRIATICO

Abstract - The depth distributions of three gurnard species, *Chelidonichthys cuculus*, *Eutrigla gurnardus* and *Trigloporus lastoviza*, were analysed using MEDITS data in Adriatic Sea. Abundance were related to depth stratum showing species specific differences: different bathymetric distribution were highlighted for recruits and spawners of *C. cuculus* while no different pattern were found for *E. gurnardus* and *T. lastoviza*.

Key-words: *Triglidae*, Adriatic Sea, depth distribution.

Introduction - Red gurnard (*Chelidonichthys cuculus* Linnaeus, 1758), grey gurnard (*Eutrigla gurnardus* L., 1758) and streaked gurnard (*Trigloporus lastoviza* Bonnaterre, 1788) are three Triglidae species occurring in Mediterranean and included in the list of target species of the MEDITS project (Relini *et al.*, 2008). Despite these species are the object of many studies about growth, sexual maturity, feeding habits and morphological and behavioural peculiarities for the Adriatic area (northeastern Mediterranean), there is still a lack of knowledge on the factors affecting the distribution of populations. The goal of this study is to estimate bathymetric distribution of these species and to evaluate the difference in depth distribution within species between recruits and spawners.

Materials and methods - Data were collected in the North-Central Adriatic Sea (GSA17) during MEDITS bottom trawl surveys carried out from 2002 to 2012. Catches data were standardized per km² in order to compute abundance indices (N/km²). Abundance indices were analysed as mean value of all sampled hauls per bathymetric stratum (25 meters depth stratum breaks) for whole population, recruits (first cohort identified by means of Bhattacharya's method) and spawners (L₅₀ from maturity ogive, Vallisneri *et al.*, 2012). Non parametric Spearman's correlation rank test was used to test the differences in bathymetric distribution among years and to compare the depth distribution of recruits and spawners.

Results and conclusions - No different distributions by species were found among years, all annual bathymetric distributions being significantly correlated to each other. For each species, threshold length for recruits and spawners identification and depth distribution were summarized in Tab. 1. About *C. cuculus* (catches depth range: 32-292 m) the trend of abundance of recruits by depth strata was different from that of spawners ($r = -0.15$, $p = 0.66$). Recruits were found at shallower depth (mostly between 76-150 m) while adults were observed at greater depth (mostly deeper than 150 m) (Fig. 1a). For *E. gurnardus* (catches depth range: 14-266 m) no differences about trend of abundance along depth were found between recruits and spawners, their depth abundances varying concordantly ($r = 0.94$, $p < 0.001$; greatest abundance between 51-

150 m) (Fig. 1b). For *T. lastoviza* (catches depth range: 17-158 m) no differences in bathymetric distribution were found between recruits and spawners, their depth abundance being strictly correlated ($r=1$, $p<0.001$; greatest abundance between 26-100 m) (Fig. 1c). In conclusion bathymetric distribution showed intra- and inter-specific pattern linked to biological and ecological behaviour of gurnard species, highlighting for *C. cuculus* preference for deeper water according to Tsimenides *et al.* (1992) and Marriot *et al.* (2010). *C. cuculus* recruits and spawners show preference for areas at different depth, as also observed in other seas (Serena *et al.*, 1990).

Tab. 1 - Threshold length, bathymetric range and n. of specimens analysed of recruits and spawners.
Dati biometrici e intervallo di profondità ripartiti per reclute e riproduttori.

Gurnard species	TL (mm) range	N° samples	Min depth (m)	Max depth (m)	Depth of peak density (m)
<i>C. cuculus</i> recruits	≤ 110	1533	32	182	76 - 150
<i>C. cuculus</i> spawners	≥ 160	1226	58	292	>150
<i>E. gurnardus</i> recruits	≤ 100	1679	16	266	51 - 150
<i>E. gurnardus</i> spawners	≥ 135	1934	16	219	51 - 150
<i>T. lastoviza</i> recruits	≤ 110	1579	17	130	26-100
<i>T. lastoviza</i> spawners	≥ 140	1071	19	142	26-100

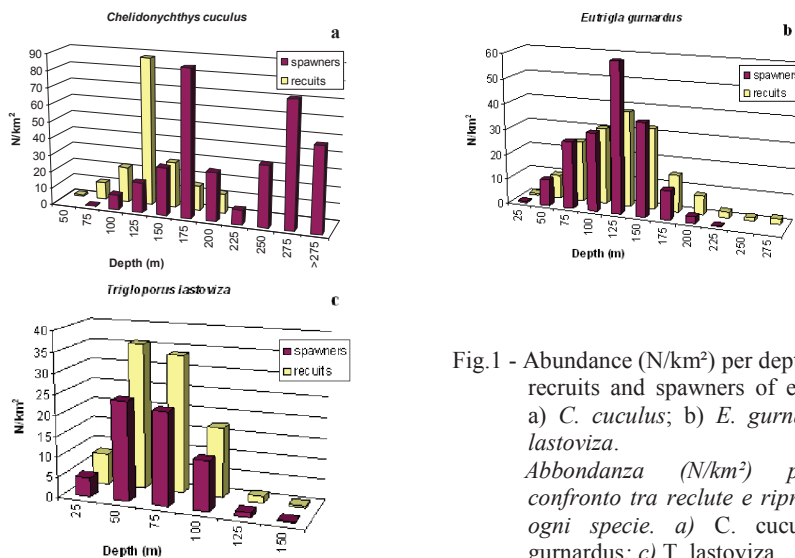


Fig. 1 - Abundance (N/km^2) per depth stratum of recruits and spawners of each species. a) *C. cuculus*; b) *E. gurnardus*; c) *T. lastoviza*.
Abbondanza (N/km^2) per strato, confronto tra reclute e riproduttori per ogni specie. a) C. cuculus; b) E. gurnardus; c) T. lastoviza.

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Short communication

Size at maturity of triglid fishes in the Adriatic Sea, northeastern Mediterranean

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Introduction

Aspitrigla cuculus (red gurnard), *Chelidonichthys lucerna* (tub gurnard) and *Eutrigla gurnardus* (grey gurnard) (Scorpaeniformes, Triglidae) occur in the Mediterranean Sea and in the eastern Atlantic. These gurnard species were included in the list of target species from the Coordination of the International Bottom Trawl Survey in the Mediterranean Sea (MEDIT project) (Relini et al., 2008). Under an European Union grant since 1994 this project monitors the abundance and distribution of Mediterranean commercial demersal resources.

Despite the abundance and importance of these triglids as an economic resource, knowledge on their basic biology, stock composition and population dynamics are lacking (Olim and Borges, 2006; Marriott et al., 2010), particularly in Italian seas (Colloca et al., 2003; Vallisneri et al., 2010). Knowledge on the reproductive features of fish stocks, such as size at maturity, is important for estimation of the spawning biomass in stock assessment models. Thus the objective of this study was to estimate the size at first maturity, one of the key parameters related to the reproductive strategy of red gurnard, tub gurnard and grey gurnard in the coastal waters of the Adriatic Sea.

Materials and methods

Samples were collected during seasonal bottom trawl surveys carried out at the same times of the year (between May–August and October–March) from 2005 to 2009 along the Italian coasts of the Adriatic Sea at depths of 12–252 m (northeastern Mediterranean) in the Gulf of Trieste (45°40'N 13°37'E) to the line joining the Gargano–Dubrovnik (42°08'N 15°16'E).

A total of 1602 specimens were caught and analysed. Total length (TL, in mm) and weight (W, 0.1 g) were recorded. Sex and maturity stages were determined by macroscopic examination of the gonads (eight maturity stages were distinguishable for females and males) according to the manual elaborated within the framework of an international project to harmonize the samplings by bottom trawling of the demersal resources in

the Mediterranean Sea (MEDIT project); spawning begins in 2c and 3 gonad maturity stages (maturing and mature/spawners) (Relini et al., 2008: web site).

A total of 295 *Aspitrigla cuculus* individuals (130 females, 165 males), 975 *Chelidonichthys lucerna* (545 females, 430 males) and 332 *Eutrigla gurnardus* (195 females, 137 males) were analysed.

The percentage of mature individuals in 10 mm class intervals was calculated for both sexes. The cumulative frequency curve and maturity ogive plots were used to estimate the size at which 50% of individuals were sexually mature.

Statistical analysis of the collected data was carried out using R software (R Development Core Team, 2010).

Results

Biometric data of *A. cuculus*, *C. lucerna*, and *E. gurnardus* in the Adriatic Sea are given in Table 1.

Size distribution and percentage of mature female and male *A. cuculus* are presented in Fig. 1. Mature females ranged from 135 to 246 mm; mature males ranged from 116 to 196 mm. About 50% of females were mature at 167 mm TL and 50% of males were mature at 150 mm TL.

Size distribution and percentage of mature female and male *C. lucerna* are presented in Fig. 2. Mature females ranged from 201 to 415 mm; mature males ranged from 180 to 306 mm. About 50% of females were mature at 243 mm TL and 50% of males were mature at 221 mm TL.

Size distribution and percentage of mature female and male *E. gurnardus* are presented in Fig. 3. Mature females ranged from 110 to 253 mm; mature males ranged from 107 to 172 mm. About 50% of females were mature at 150 mm TL and 50% of males were mature at 122 mm TL.

Discussion

Gurnards are demersal fish with a pelagic phase during their early life history stages. Changes of habitat occur during the demersal life of the species, which have been related to the

Table 1
Biometric data

Species	No. female	TL range (mm) female	No. male	TL range (mm) male	Depth range (m)
<i>Aspitrigla cuculus</i>	130	108–262	165	103–233	60.7–252
<i>Chelidonichthys lucerna</i>	545	148–415	430	155–342	12.1–217.5
<i>Eutrigla gurnardus</i>	195	100–253	137	93–172	30.9–245

TL, total length.

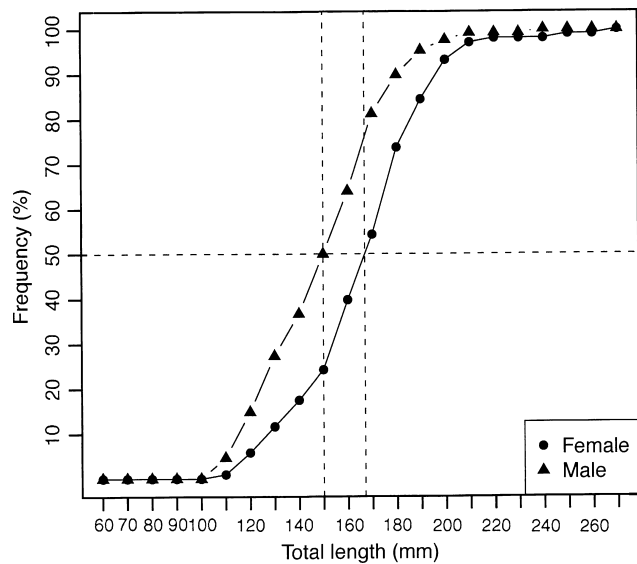


Fig. 1. Size distribution of *A. cuculus* samples caught 2005–2009 and percentage of mature fish to determine size at first maturity; n = 295 (130 females, 165 males)

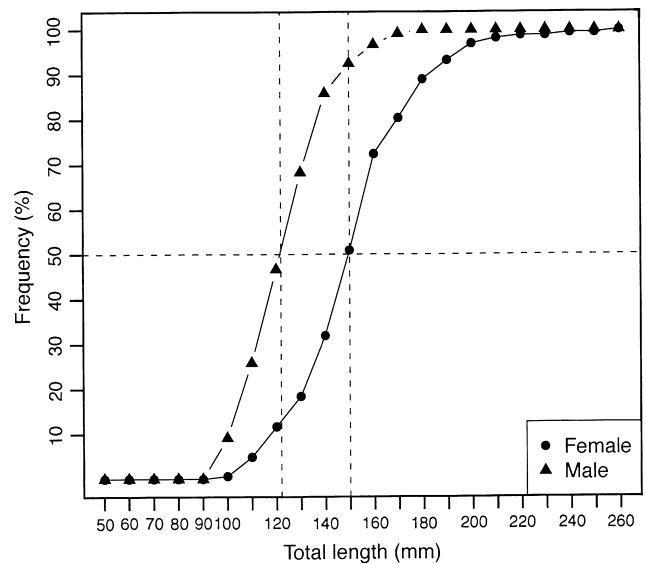


Fig. 3. Size distribution of *E. gurnardus* samples caught 2005–2009 and percentage of mature fish to determine size at first maturity; n = 332 (195 females, 137 males)

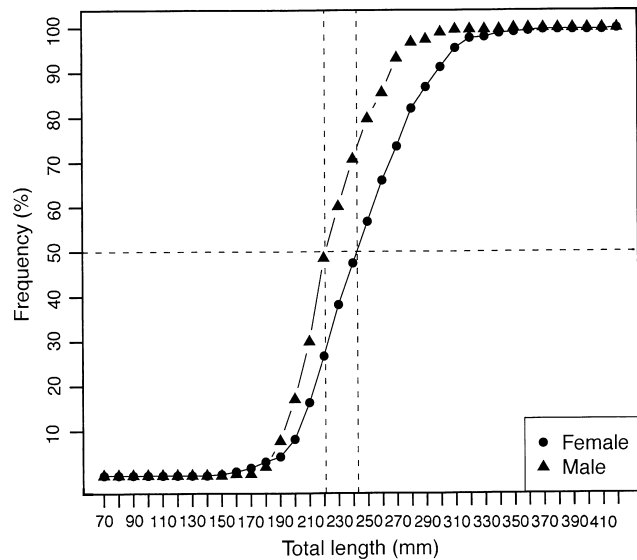


Fig. 2. Size distribution of *C. lucerna* samples caught 2005–2009 and percentage of mature fish to determine size at first maturity; n = 975 (545 females, 430 males)

growth and spawning processes (Papaconstantinou, 1984; Colloca et al., 2003). A rapid change in the feeding strategy of Mediterranean gurnard species generally coincides with the size of first maturity (Colloca et al., 1994). According to the literature, the triglid species considered here showed a critical size relation coinciding with the start of sexual maturity, the tendency to migrate to greater depths, a change of diet from crustaceans to fish and an increase in the variety of food items eaten (Montanini et al., 2008, 2009). Such behaviour probably depends on the predator changing its energy requirements in relation to fish size and dietary protein levels influencing the size of the fish at first maturity (Al Hafedh et al., 1999).

For comparative purposes, Table 2 shows data for various stocks throughout northwestern Europe and the Mediterranean according to latitude. Results presented here show that males attain sexual maturity at smaller sizes than females, just as other stocks do in different areas (Papaconstantinou, 1984; Baron, 1985; McPhail et al., 2001; Boudaya et al., 2008). This phenomenon is usually attributed to the fact that reaching a larger size at maturity has selective advantages for females as they produce larger eggs with higher survival rates, have a larger fecundity, and have access to the best spawning sites.

Table 2

First sexual maturity length of *A. cuculus*, *C. lucerna*, *E. gurnardus* in different areas according to latitude (bold values refer to this paper)

	Female	Male	Location	Authors	Latitude
<i>Aspitrigla cuculus</i>	28.1	26.3	North West Wales (Atlantic Ocean)	Marriott et al. (2010)	53°
	28.4	27.0	Douarnenez Bay (Brittany, Atlantic Ocean)	Baron (1985)	48°
	17.5	/	Central Tyrrhenian Sea (NW Mediterranean)	Colloca et al. (2003)	42°
<i>Chelidonichthys lucerna</i>	16.7	15.0	Adriatic Sea (NE Mediterranean)	This study	43°
	40.1	35.5	Douarnenez Bay (France, Atlantic Ocean)	Baron (1985)	48°
	31.7	26.0	Thermaikos Gulf (NE Mediterranean)	Papaconstantinou (1984)	40°
	24.3	22.1	Adriatic Sea (NE Mediterranean)	This study	43°
	20.0	18.0	Iskenderun Bay (E Mediterranean)	Ismen et al. (2004)	36°
	19.0	18.5	Sea of Marmara (E Mediterranean)	Eryilmaz and Meric (2005)	40°
	19.0	17.7	Izmir Bay (Aegean Sea, E Mediterranean)	Uckun and Togulga (2007)	38°
<i>Eutrigla gurnardus</i>	21.6	19.2	Gulf of Gabès (Tunisia, SE Mediterranean)	Boudaya et al. (2008)	34°
	17.0	15.6	Egypt (S Mediterranean)	Abdallah and Faltas (1998)	30°
	24.0	18.0	North Sea (Atlantic Ocean)	Muus and Nielsen (1999)	59°
	15.0	12.2	Adriatic Sea (NE Mediterranean)	This study	43°

The opposite situation, whereby females are smaller and mature earlier than males, is usually found in species having a social structure and where strong competition for access to females takes place among the males (Boudaya et al., 2008).

Our size at maturity data are comparable to those reported in the literature and show a tendency towards a geographical cline, particularly for *C. lucerna*. These differences are usually attributable to various ecological and hydrographical conditions, particularly temperature, which stimulates sexual maturation (Abdallah and Faltas, 1998; Muus and Nielsen, 1999; Ismen et al., 2004; Eryilmaz and Meric, 2005; Uckun and Togulga, 2007). For Mediterranean fishes, the differences seem to be more related to the seasonal variation in feeding intensity (Colloca et al., 2003).

In conclusion, this study increases our knowledge of size at maturity of commercial species in the Adriatic Sea, an important parameter for the estimation of the spawning biomass in stock assessment models.

Acknowledgements

We thank Professor Corrado Piccinetti for his assistance in the bottom trawl surveys and for his valuable suggestions in the drafting of the paper.

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Technical contribution

Length–weight relationships for the family Triglidae in the Adriatic Sea, northeastern Mediterranean

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Summary

Length–weight relationships were estimated for the first time for seven species of gurnards (Triglidae) based on data collected over the past 27 years in the Adriatic Sea (northeast Mediterranean). Results show significantly different relationships between juveniles and adults (females and males combined) for *Aspitrigla cuculus*, *Eutrigla gurnardus*, *Chelidonichthys lucerna* and *Lepidotrigla cavillone*. Isometric growth of juveniles is indicated for four species, allometric growth of adults for all species.

Introduction

Length–weight relationships are needed for fisheries management and ecological studies. In this study, the parameters of

the length–weight relationships are reported for Triglidae caught in the north and middle Adriatic Sea (northeast Mediterranean) between 1982 to 2008, with special emphasis on sexes and life-stages.

Material and methods

The 26,953 samples analysed from 1982 to 2008, were collected during fishing surveys off the north-middle Adriatic Sea, from the Gulf of Trieste (45°40'N 13°37' E) to the Tremiti Islands (42°08'N 15°16'E). In the laboratory, total lengths (L) were measured to the nearest mm and weights (W) determined with a digital balance to an accuracy of 0.1 g. Macroscopic analysis of gonads was performed to determine the sex, with

Table 1

Descriptive statistics and estimated parameters of total length–weight relationships for seven species of triglids in the Adriatic Sea, northeastern Mediterranean

Specie	Sex	N	l	L	P	P	a	95% CL (a)	b	95% CL (b)	r ²
<i>Aspitrigla gurnardus</i>	J	3786	4.9	20.2	1.2	86.2	0.0085*	0.0085–0.0092	2.98*	2.96–3.00	0.97
	F	1648	7.2	30.3	3.5	263	0.0065	0.0062–0.0070	3.12	3.10–3.14	0.98
	M	1594	8.5	27.7	5	218	0.0067	0.0062–0.0072	3.10	3.07–3.13	0.97
	A	3242	7.2	30.3	3.5	263	0.0064*	0.0061–0.0067	3.12*	3.11–3.14	0.98
<i>Eutrigla gurnardus</i>	J	5017	4.4	16.3	0.6	38	0.0097*	0.0093–0.0101	2.93*	2.92–2.96	0.95
	F	5292	6.5	32.2	2.2	239.8	0.0086	0.0083–0.0090	3.01	2.99–3.02	0.97
	M	3723	8.3	24.3	4.1	125.8	0.0083	0.0077–0.0089	3.01	2.98–3.04	0.93
	A	9015	6.5	32.2	2.2	239.8	0.0077*	0.0075–0.0080	3.04*	3.03–3.06	0.96
<i>Chelidonichthys lastoviza</i>	J	180	6	18	2.3	67	0.0131	0.0104–0.0135	2.96	2.86–3.06	0.95
	F	826	9.5	27.5	9.4	269	0.0143	0.0127–0.0161	2.94	2.90–2.98	0.96
	M	696	9.1	25.9	9.9	178.9	0.0153	0.0135–0.0174	2.91	2.86–2.95	0.96
	A	1522	9.1	27.5	9.4	269	0.0144	0.0132–0.0157	2.93	2.90–2.97	0.96
<i>Chelidonichthys lucerna</i>	J	696	3	27.8	0.3	215.4	0.0068*	0.0060–0.0078	3.13*	3.08–3.18	0.95
	F	962	10.5	48	13.6	1250	0.0092	0.0086–0.0098	3.02	3.00–3.18	0.99
	M	772	10	34.2	13	321.3	0.0096	0.0088–0.0106	3.00	2.97–3.03	0.98
	A	1734	10	48	13	1250	0.0093*	0.0088–0.0098	3.01*	3.00–3.03	0.98
<i>Lepidotrigla cavillone</i>	J	219	4.2	13.2	0.9	32	0.0104*	0.0081–0.0134	3.05*	2.94–3.16	0.93
	F	676	7.6	15.1	5	44	0.0062	0.0052–0.0074	3.29	3.22–3.37	0.92
	M	423	7.4	14.3	4.8	35.6	0.0090	0.0069–0.0117	3.13	3.01–3.24	0.88
	A	1099	7.4	15.1	4.8	44	0.0070*	0.0061–0.0081	3.24*	3.17–3.30	0.90
<i>Lepidotrigla dieuzeidei</i>	J	20	7.2	12	4.2	16	0.0112	0.0040–0.0381	2.99	2.46–3.52	0.89
	F	91	7.4	13.7	4.3	33	0.0054	0.0038–0.0078	3.32	3.17–3.48	0.95
	M	52	8.1	12.5	5.3	24	0.0060	0.0037–0.0096	3.27	3.06–3.46	0.96
	A	143	7.4	13.7	4.3	33	0.0055	0.0041–0.0073	3.31	3.19–3.43	0.95
<i>Trigla lyra</i>	J	222	4.5	27	1.0	143	0.0150	0.0130–0.0172	2.79	2.73–2.85	0.98
	F	25	13	37	19.9	395.5	0.0116	0.0067–0.0199	2.88	2.70–3.06	0.98
	M	33	12	35	22.5	307	0.0248	0.0140–0.0442	2.63	2.44–2.82	0.96
	A	58	12	37	19.9	395.5	0.0177	0.0119–0.0265	2.74	2.60–2.87	0.97

N = number of specimens; l = minimum length (mm); L = maximum length (mm); p = minimum weight (g); P = maximum weight (g); a = intercept of the relationship; 95% CL (a) = confidence limits of a; b = slope of the relationship; * = Significant differences of a or b values (P < 0.05) between undetermined (J) and adult s (A = F + M); 95% CL (b) = confidence limits of b; r² = coefficient of determination.

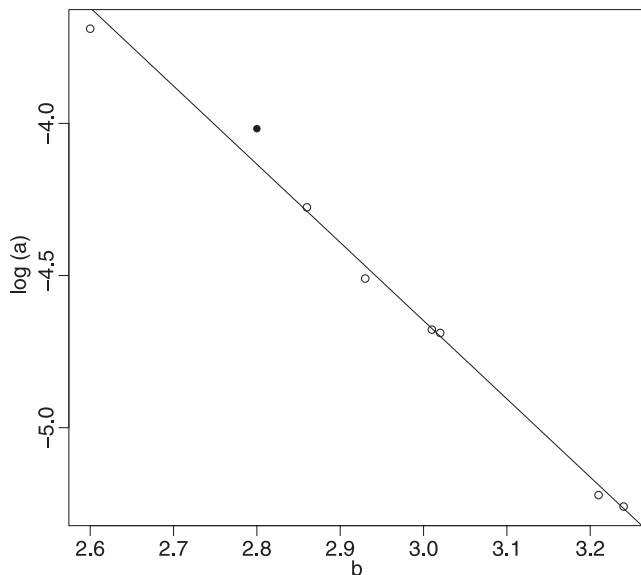


Fig. 1. Plot of $\log a$ over b for eight length–weight relationships of *Chelidonichthys lucerna*

specimens classified as females (F), males (M), or juveniles (J). The L–W relationship was determined by the equation: $\log W = \log(a) + b \log(L)$, where W is the total weight of the fish (g), L is the total length (cm), a is the intercept on the Y-axis of the regression curve and b is the regression coefficient. In order to verify if b was significantly different from the isometric value ($b = 3$), the Student's t -test ($H_0: b = 3$) with a confidence level of $\pm 95\%$ ($\alpha = 0.05$) and thus for sexes or life-stages was employed (Sokal and Rohlf, 1987). Geographic cline was tested by $\log(a)$ over b plot (Froese, 2006).

Table 2
Total length–weight relationships of Triglidae in different locations

Species	Location	N	l	L	p	P	a	b	SE_b	r^2	Author
<i>A. cuc</i>	Portugal, Atlantic O.	75	85	158	6.5	51.1	0.007	3.22	0.29	0.94	Olim and Borges, 2006
	Portugal, Atlantic O.	156	180	405	18	810	0.0035	3.33	0.064	0.95	Rosa et al., 2006
	Italy, Adriatic Sea	3242	72	303	3.5	263	4.8E-06	3.12	0.009	0.98	This study
<i>E. gur</i>	Portugal, Atlantic O.	84	111	182	12.5	73.9	0.019	2.81	0.29	0.87	Olim and Borges, 2006
	Italy, Adriatic Sea	9015	65	322	2.2	239.8	7.0E-06	3.04	0.006	0.96	This study
<i>C. las</i>	Portugal, Atlantic O.	45	94	274	9	222.1	0.007	3.12	0.53	0.98	Olim and Borges, 2006
	Italy, Adriatic Sea	1522	91	275	9.4	269	1.7E-05	2.93	0.016	0.96	This study
<i>C. luc</i>	Spain, Atlantic O.	150	60	270			0.025	2.60		0.95	Mata et al., 2008
	Portugal, Atlantic O.	75	140	344	35	368	0.0180	2.80	0.059	0.97	Santos et al., 2002
	Portugal, Atlantic O.	21	75	277	4.1	177.9	0.011	2.93	0.51	0.98	Olim and Borges, 2006
	Italy, Tyrrhenian Sea	538					0.0139	2.86		0.99	Serena et al., 1998
	Italy, Adriatic Sea	1734	100	480	13	1250	0.0093	3.01	0.009	0.98	This study
	Turkey, Sea of Marmara Edremit Bay, Aegean Sea	224 262						0.0092 0.0054	3.02 3.21		0.99 0.99
<i>L. cav</i>	Turkey, Aegean Sea	546	120	344			0.0052	3.24		0.99	Uckun and Togulga, 2007
	Portugal, Atlantic O.	550	82	165	7.3	61.7	0.010	3.07	0.12	0.90	Olim and Borges, 2006
	Italy, Tyrrhenian Sea	500	30	170			0.0086	3.11		0.97	Voliani et al., 2000
<i>L. die</i>	Italy, Adriatic Sea	1099	74	151	4.8	44	4.1E-06	3.24	0.032	0.90	This study
	Portugal, Atlantic O.	255	68	162	3.2	51	0.009	3.11	0.13	0.95	Olim and Borges, 2006
	Italy, Tyrrhenian Sea	321	50	190			0.0085	3.12		0.95	Voliani et al., 2000
<i>T. lyr</i>	Italy, Adriatic Sea	143	74	137	4.3	33	2.7E-06	3.31	0.062	0.95	This study
	Portugal, Atlantic O.	40	70	240	2.9	112.1	0.005	3.17	0.21	0.95	Olim and Borges, 2006
	Portugal, Atlantic O. Italy, Adriatic Sea	15 58	136 120	380 370	30 19.9	522.7 395.5	0.0217 3.2E-05	2.73 2.74	0.097 0.067	0.98 0.97	Santos et al., 2002 This study

N = number of specimens; l = minimum length (mm); L = maximum length (mm); p = minimum weight (g); P = maximum weight; SE_b = standard error of b; r^2 = coefficient of determination.

Results and discussion

Length–weight relationship parameters are shown in Table 1. Confidence limits showed significant differences of a and b values between juveniles and adults for *Aspitrigla cuculus*, *Eutrigla gurnardus*, *Chelidonichthys lucerna* and *Lepidotrigla cavillone*. In none of the species is there a significant difference in exponent a and b between the sexes (confidence limits overlap). In terms of growth type, results revealed in both sexes: isometric growth for *E. gurnardus*; positive allometric growth ($b > 3$) for *A. cuculus*, *L. cavillone*, *Lepidotrigla dieuzeidei*; negative allometric growth ($b < 3$) for *Chelidonichthys lastoviza*. There is no evidence of geographic cline for *C. lucerna* (Fig. 1; Table 2).

Parameters of the length–weight relationships for *C. lucerna* may vary significantly according to juveniles and adult specimens (Orlov and Binohlan, 2009), sex (Rojas-Herrera et al., 2009) and food (Stergiou and Moutopoulos, 2001; Santos et al., 2002; Olim and Borges, 2006).

Acknowledgements

We thank Professor Corrado Piccinetti for his assistance in fishing cruises and for his valuable suggestions in the drafting of the paper.

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RELAZIONE LUNGHEZZA-PESO DI 7 SPECIE DELLA FAMIGLIA TRIGLIDAE DELL'ALTO-MEDIO ADRIATICO

LENGTH-WEIGHT RELATIONSHIPS FOR SEVEN SPECIES OF THE FAMILY TRIGLIDAE FROM THE MIDDLE AND NORTH ADRIATIC SEA

Abstract - Gurnards or triglids are marine bottom fishes that live in temperate and tropical seas. This work presents the reference on length-weight relationships of five genera and seven species, poorly discussed for the Mediterranean Sea. Samples were collected during MEDITS and GRUND trawl survey between 1982-2008 in the North-Middle Adriatic Sea. The parameters of a and b of the equation $W=aL^b$ were estimated.

Key-words: length-weight relationships, Triglidae, Middle and North Adriatic Sea.

Introduzione - I triglidi (Scorpaeniformes, Teleostei) del mar Adriatico sono rappresentati da 5 generi e da 7 specie: *Aspitrigla cuculus* (*A. Cuc*), *Eutrigla gurnardus* (*E. Gur*), *C. lastoviza* (*C. Las*), *C. lucerna* (*C. luc*), *Lepidotrigla cavillone* (*L. cav*), *L. dieuzeidei* (*L. die*), *Trigla lyra* (*T. Lyr*). La relazione lunghezza-peso nelle specie ittiche si dimostra fondamentale per la valutazione dello stato ecologico delle comunità marine, ciò nonostante scarseggiano tali conoscenze sugli stocks mediterranei (Serena *et al.*, 1990-Tirreno; Stergiou e Moutopoulos, 2001-Grecia) e atlantici (Santos *et al.*, 2002; Olim e Borges, 2006-Portogallo). Scopo del lavoro è la costruzione di un quadro sinottico di confronto della relazione lunghezza-peso dei triglidi del Nord-Medio Adriatico.

Materiali e metodi - Lungo le coste dell'Alto-Medio Adriatico fra il golfo di Trieste e la congiungente Gargano-Dubrovnik, nel corso di 27 anni di campagne di pesca a strascico MEDITS e GRUND 1982-2008 sono stati pescati esemplari appartenenti a 7 specie di triglidi a profondità comprese tra 10 e 260 m. Sul totale di 26.953 esemplari sono state condotte le consuete analisi biometriche suddividendo gli esemplari in femmine (F), maschi (M) e indeterminati (I). È stata costruita la relazione lunghezza-peso secondo l'equazione $W=aL^b$, dove W è il peso (g), L la lunghezza totale (mm), a l'intercetta della regressione e b è il coefficiente di regressione. I parametri a e b della relazione lunghezza-peso sono stati stimati mediante l'analisi della regressione lineare basata sul logaritmo: $\log(W) = \log(a) + b \log(L)$. Il valore di b è stato testato mediante t -test a livello di 0.05 (secondo i fattori specie, sesso) per verificare se differisce significativamente da 3. Per testare le differenze significative fra sessi è stata usata l'analisi della covarianza (ANCOVA). È stato calcolato il fattore di condizione K mediante la formula: $K = (W/L^b) \times 100$ (Le Cren, 1951), dove W è il peso totale, L la lunghezza totale, b il coefficiente di allometria della relazione.

Risultati - Sono stati stimati i parametri biometrici di 7 specie di triglidi divisi per sesso. In particolare, sono stati analizzati e confrontati i parametri di statistica descrittiva ed i parametri relativi alla relazione lunghezza-peso (a , b , r^2) (Tab. 1).

Tutte le regressioni sono altamente significative ($P < 0,01$).

Nell'areale studiato, si segnalano: dimorfismo sessuale in *L. cavillone* (rapporto lunghezza-peso maggiore per le femmine); allometria positiva ($b > 3$) in entrambi i sessi di *A. cuculus*, *L. cavillone*, *L. dieuzeidei*; allometria negativa ($b < 3$) in entrambi i sessi di *C. lastoviza*. Il valore elevato di K per i maschi di *T. lyra* è attribuibile al numero ridotto di campioni ed al particolare accrescimento cefalico.

Tab. 1 - Relazione lunghezza-peso dei triglidi dell'Adriatico centro settentrionale. (l: lunghezza minima, L: lunghezza massima, lm: lunghezza media, p: peso minimo, P: peso massimo, pm: peso medio, SE_a: errore standard di a, SE_b: errore standard di b).

Length-weight relationships for triglids from the Middle and North Adriatic Sea (l: minimum length, L: maximum length, lm: mean length, p: minimum weight, P: maximum weight, pm: mean weight, SE_a: standard error of a, SE_b: standard error of b).

Specie	Sex	N	l	L	lm	p	P	pm	a	SE_a	b	SE_b	r ²	K
<i>A. cuc</i>	I	3786	49	202	100	1.2	86.2	9.5	9.2E-06	1.041	2.98	0.009	0.97	0.93
<i>A. cuc</i>	F	1648	72	303	160	3.5	263	43.2	4.9E-06	1.059	3.12	0.011	0.98	1.01
<i>A. cuc</i>	M	1594	85	277	148	5	218	31.1	5.3E-06	1.073	3.10	0.014	0.97	0.97
<i>E. gur</i>	I	5017	44	163	96	0.6	38	8.1	1.1E-05	1.045	2.93	0.009	0.95	1.13
<i>E. gur</i>	F	5292	65	322	145	2.2	239.8	30.6	8.5E-06	1.039	3.01	0.008	0.97	0.85
<i>E. gur</i>	M	3723	83	243	127	4.1	125.8	18.6	8.1E-06	1.069	3.01	0.014	0.93	0.81
<i>C. las</i>	I	180	60	180	109	2.3	67	17.2	1.4E-05	1.261	2.96	0.050	0.95	1.45
<i>C. las</i>	F	826	95	275	159	9.4	269	51.7	1.7E-05	1.118	2.94	0.024	0.96	1.65
<i>C. las</i>	M	696	91	259	153	9.9	178.9	45.0	1.9E-05	1.127	2.91	0.022	0.96	1.91
<i>C. luc</i>	I	696	30	278	127	0.3	215.4	22.1	5.0E-06	1.137	3.13	0.026	0.95	0.51
<i>C. luc</i>	F	962	105	480	222	13.6	1250	130.5	8.7E-06	1.059	3.02	0.011	0.99	0.87
<i>C. luc</i>	M	772	100	342	216	13	321.3	109.2	9.6E-06	1.090	3.00	0.016	0.98	0.97
<i>L. cav</i>	I	219	42	132	95	0.9	32	11.2	9.2E-06	1.293	3.05	0.056	0.93	0.93
<i>L. cav</i>	F	676	76	151	103	5	44	14.1	3.2E-06	1.192	3.29	0.038	0.92	0.32
<i>L. cav</i>	M	423	74	143	104	4.8	35.6	14.1	6.7E-06	1.304	3.13	0.057	0.88	0.68
<i>L. die</i>	I	20	72	120	101	4.2	16	11.7	1.2E-05	3.200	2.99	0.252	0.89	1.16
<i>L. die</i>	F	91	74	137	105	4.3	33	14.4	2.6E-06	1.429	3.32	0.077	0.95	0.26
<i>L. die</i>	M	52	81	125	104	5.3	24	13.0	3.2E-06	1.590	3.27	0.099	0.96	0.33
<i>T. lyr</i>	I	222	45	270	122	1.0	143	22.8	2.4E-05	1.146	2.79	0.029	0.98	2.48
<i>T. lyr</i>	F	25	130	370	201	19.9	395.5	86.4	1.5E-05	1.595	2.88	0.089	0.98	1.54
<i>T. lyr</i>	M	33	120	350	204	22.5	307	84.5	5.8E-05	1.648	2.63	0.095	0.96	5.89

Conclusioni - Si conferma l'importanza dello studio delle relazioni lunghezza-peso per valutarne le caratteristiche (in base al sesso, alla taglia, all'areale, alla stagione) e lo stato dei pesci, sia all'interno delle singole specie che fra specie diverse. I risultati ottenuti, pur con differenze correlabili all'area, sono nel complesso confrontabili a quelli osservati in altre aree mediterranee (Stergiou e Moutopoulos, 2001) e in Atlantico (Santos *et al.*, 2002; Olim e Borges, 2006).

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NOTE SULLA BIOLOGIA DI *CHELIDONICHTHYS LUCERNUS* (TELEOSTEI: TRIGLIDAE) NELL'ALTO-MEDIO ADRIATICO

NOTES ON THE BIOLOGY OF *CHELIDONICHTHYS LUCERNUS* (TELEOSTEI: TRIGLIDAE) IN THE NORTHERN-MIDDLE ADRIATIC SEA

Abstract – A total of 1.114 specimens of *Chelidonichthys lucernus* (*tub gurnard*) were collected in the Northern-Middle Adriatic sea during trawl surveys carried out between May 2005 and March 2007. Length-frequency distribution, length at first sexual maturity, spawning season, stomachs contents were investigated.

Key-words: *tub gurnard*, *Chelidonichthys lucernus*, growth, reproduction, feeding habits, Adriatic sea.

Introduzione - *Chelidonichthys lucernus* (L., 1758), denominata volgarmente gallinella o cappone, è una specie demersale ampiamente distribuita in tutto il Mediterraneo su fondali sabbiosi entro i 200 m di profondità. Il ciclo vitale è stato poco indagato, pur rappresentando la specie di triglide più importante per la pesca nei mari italiani (Serena *et al.*, 1998; Relini *et al.*, 1999; Ceriola *et al.*, 2004). Obiettivo del lavoro consiste nell'ampliamento delle conoscenze relativamente ad Alto e Medio Adriatico attraverso la stima dei principali parametri biologici quali accrescimento, riproduzione e dieta.

Materiali e metodi - Lungo le coste dell'Alto-Medio Adriatico fra il golfo di Trieste e la congiungente Gargano - Dubrovnik, nel corso di campagne di pesca a strascico MEDITS e GRUND 2005-2007 (circa 500 campionamenti di cui 228 positivi) si sono raccolti campioni di *C. lucernus* a profondità comprese tra 10 e 260 m. Sul totale di 1.114 esemplari sono state condotte le analisi biometriche: lunghezza totale (mm), peso totale (g), sesso, maturità gonadica macroscopica secondo la scala a 7 stadi (Ungaro, 2006). Gli stomaci sono stati immediatamente rimossi e ne è stato valutato il grado di riempimento; le prede, identificate fino al livello di specie quando possibile, sono state contate e pesate. I dati sono stati opportunamente analizzati graficamente e statisticamente, mediante la valutazione di: frequenza di distribuzione della lunghezza; taglia di prima maturità sessuale (al 50% degli individui); stagione riproduttiva; contenuto stomacale quali-quantitativo.

Risultati - La distribuzione della frequenza di lunghezza si articola su due principali coorti in un range di 63-415 mm: la prima coorte comprende le giovani reclute (mediana 128 mm) più frequenti in estate e vicino costa; la seconda, individui adulti di media e grossa taglia (mediana 238 mm), più frequenti d'inverno ed a profondità maggiori, in accordo con Serena *et al.* (1998) nel Tirreno. Le femmine raggiungono taglie maggiori (415 mm contro 342 mm dei maschi), in accordo con la letteratura (Ismen *et al.*, 2004; Ilhan e Togulga, 2007-Turchia). Il profilo di variazione relativo alla maturità sessuale mostra una dicotomia tra i sessi attorno a 180 mm ed il raggiungimento della taglia di maturità (al 50% degli individui) a circa 220 mm per i maschi e 270 mm per le femmine. Il risultato è in accordo con quello ottenuto nel Mediterraneo sud est (Ismen *et al.*, 2004; Ilhan e Togulga, 2007-Turchia), circa il raggiungimento della

maturità a taglie minori nei maschi, mentre si differenzia nella taglia di prima maturità, più alta in Adriatico. Il periodo riproduttivo appare compreso fra Dicembre e Febbraio, risultando sovrapponibile rispetto a quello riscontrato in Turchia dagli Autori suddetti. Tali differenze sono correlabili al gradiente geografico ed alle relative condizioni ambientali del Mediterraneo. L'alimento preferito a tutte le taglie è costituito da Crustacea (89,5 % in numero e 58% in peso) e Teleostea (6,9% in numero e 40,2% in peso). La dieta si modifica durante la crescita, in particolare a ridosso dei 180 mm, sia quantitativamente, con la cattura di prede di dimensioni crescenti e la conseguente riduzione del numero, che qualitativamente con l'aumento della componente ittica, in accordo con la letteratura (Serena *et al.*, 1998-Tirreno; Ceriola *et al.*, 2004-Sud Adriatico).

Conclusioni - Lo studio della biologia di *Chelidonichthys lucernus* in Alto-Medio Adriatico ha evidenziato che a circa 180 mm di taglia, hanno luogo: 1) la differenziazione sessuale (valutata macroscopicamente): 2) la discesa a maggiori profondità e l'allontanamento dalle coste italiane verso quelle croate e 3) il cambiamento nella composizione della dieta.

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Chapter III. Diet, trophic interactions, and potential ecological impact on the ecosystem

Scientific papers are reported below the summary of chapter III as follows:

STAGIONI M., **MONTANINI S.**, VALLISNERI M. (2012). Feeding of tub gurnard *Chelidonichthys lucerna* (Scorpaeniformes: Triglidae) in the north-east Mediterranean. *J. Mar. Biol. Ass. U. K.*, 92 (3): 605-612.

VALLISNERI M., **MONTANINI S.**, STAGIONI M., TOMMASINI S. (2013). Feeding habits of *Aspitrigla cuculus* (Linnaeus, 1758) in northern-central Adriatic Sea. *Biol. Mar. Mediterr.*, 20 (1): 206-207.

MONTANINI S., STAGIONI M., BENASSI FRANCIOSI C., ANIBALDI A., VALLISNERI M. (2013). Prime indagini sulle abitudini alimentari di *Trigloporus lastoviza* (Scorpaeniformes: Triglidae) in Adriatico. *Atti Soc. Nat. Mat. Modena*, 144: p. 162. 74° Congresso UZI, Modena, 30 settembre-3 ottobre 2013.

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MONTANINI S., STAGIONI M., VALLISNERI M. (2010). Diet of the grey gurnard, *Eutrigla gurnardus* in the Adriatic Sea, north-eastern Mediterranean. *Cybium*, 34 (4): 367-372.

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SUMMARY OF CHAPTER III.

Generally the diet of studied gurnards (target species *C. cuculus*, *C. lucerna*, *E. gurnardus* and *T. lastoviza*) were in line with feeding habits of other coastal benthic population in the Mediterranean, characterized by large amount of different prey items. Malacostraca decapods were the most occurred prey items according to literature (Terrats et al. 2000; Ben Jrad et al. 2010) while other prey items were recorded as accessory ones. Generally gurnard species also showed an opportunistic predator behaviour classified as bottom feeder fish with a broad niche width. This feeding behaviour was showed for other gurnards which using free rays of pectoral fin as feelers over the sea bottom (Wheeler 1969). About the methodology of the investigations, samples were dissected and stomachs were removed and preserved in 70% ethanol for stomach content analysis. Diet was expressed as percentage by number (%N), weight (%W), frequency of occurrence (%F), index of relative importance (%IRI). Prey-specific abundance index (%PSA) plotted against occurrence of prey species was computed to study feeding strategy according to Amundsen et al., 1996. Levins index was employed to measure diet breadth (Levins 1968). Seasonally fullness index were calculated and tested by chi-squared test ($p < 0.05$). Multivariate analysis (principal component analysis (PCA) and non metric multidimensional scaling (nMDS) plot) and cluster analysis (based on Bray-Curtis index) were carried out on prey numeric and weight abundance classes to discovered possible feeding patterns. Diet overlap was tested using Morisita's index. None of the gurnards examined exhibited different food types, fed mainly on Crustacea at all size class level, but appear to have distinct feeding behaviour. *C. lucerna*, *E. gurnardus* and *T. lastoviza* were opportunistic predators that had a more diverse diet and fed on epibenthic, benthopelagic and necto-benthic preys while *A. cuculus* may be considered as a specialist feeder, fed almost exclusively on necto-benthic invertebrates. In all the species studied, diet was influenced by predator “critical sizes” marking off juvenile from adult individuals (120 TL mm for *E. gurnardus*; 180 TL mm for *C. lucerna*). On the basis of these sizes, the feeding habits of adults were seen to be similar to those of juveniles in all the species; in fact, the epibenthic and necto-benthic invertebrates were the dominant group for all

sizes considered. The index of relative importance of Crustacea reached high values ranging from 88.35 to 99.52. For *A. cuculus*, *Lophogaster typicus* (Lophogastrida) was the most abundant and preferred prey as shown by its almost exclusive presence both in the diet of juveniles and adults of red gurnard (%IRI = 97.08 - %IRI = 97.81, respectively). Crustacea *Philocheras* sp. (Decapoda, Macrura-Natantia) was the dominant prey (%IRI = 79.99) of juveniles of *C. lucerna*, while adults of this species were found to predominantly feed on *Goneplax rhomboides* (Decapoda, Brachyura) (%IRI = 80.63). Seventeen different species of Teleostei were found in the diet of adults of tub gurnard and were considered secondary preys (%Cn = 16.86). Anchovy *Engraulis encrasicolus* and gobiid *Gobius niger* gave a quite good contribution in terms of weight (%Cw = 12.98; %Cw = 10.29). Pelagic preys as *Engraulis encrasicolus* could probably resulted such as active predation or such as discarded food although Olaso et al. (2002) indicates that *C. lucerna* doesn't show scavenging behaviour. For *E. gurnardus*, *L. typicus* is the preferred prey for both ontogenetic groups (%IRI = 64.31 - %IRI = 47.46, respectively). *Liocarcinus* sp. (Decapoda, Brachyura), *Solenocera membranacea* (Decapoda, Macrura-Natantia) and *Acanthomysis longicornis* (Mysida) occurred quite frequently in the diet of juveniles of grey gurnards, being subsequently replaced by *G. rhomboides* in the diet of the adults of this species. *E. gurnardus* is also confirmed, for the Adriatic area, a predator that potentially compete with other fish species, in agreement with the literature (Floeter et al. 2005; Montanini et al. 2008). In fact, in the North Sea, grey gurnard predation is a very critical process showing a significant top-down effect on whiting (*Merlangius merlangus*) and potentially also on cod recruitment (Floeter and Temming 2005). For all species, ontogenetic dietary shift showed that the number of prey items in the gut increased with increasing predator body size but it didn't always corresponded to a higher diversity index. This study provided to show that dietary overlap was higher generally at intra-specific level, a finding that highlights strong trophic intraspecific competition, while food resource partitioning occurred mainly at the interspecific level. At least the main variables affecting trophic niche breadth of Adriatic gurnards were deemed to be predator size range, prey item and depth distribution. Intra-specific feeding

competition was observed between juvenile and adult individuals of *A. cuculus* and *E. gurnardus*. In fact Morisita index values are high in both species ($C = 0.991$ for *A. cuculus*, and $C = 0.843$ for *E. gurnardus*). Vice versa, very low intra-specific competition levels were recorded for *C. lucerna* ($C = 0.186$). Intraspecific multidimensional analysis does not show up any difference between juveniles and adults. Inter-specific niche overlap appears quite relevant only between juveniles and adults of *A. cuculus* and *E. gurnardus* (C range = $0.596 - 0.696$). Low levels of inter-specific competition show the trophic niche of *C. lucerna* to be different from those of the other two gurnards (C range = $0.249 - 0.589$). Inter-specific multidimensional analysis (nMDS) highlights three different groups within the same species: juveniles and adults of each species were grouped together, reflecting greater intra- (juvenile/adult) than inter-specific competition. This trend was further borne out by the non metrical multidimensional analysis of the size-depth relationship; in fact, same-species clusters were tendentially found also by this type of analysis.

Feeding of tub gurnard *Chelidonichthys lucerna* (Scorpaeniformes: Triglidae) in the north-east Mediterranean

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The stomach contents of 1096 specimens of Chelidonichthys lucerna were examined in order to analyse their diet composition according to fish size, sex, depth and season. Sampling was carried out from May 2005 to March 2007 during several bottom trawl surveys in the Adriatic Sea (north-east Mediterranean). Feeding activity was more intense in juveniles than in adults. The most important prey was Crustacea (mainly Decapoda: Brachyura, such as Goneplax rhomboides, Liocarcinus spp., Philocheras spp.) and Teleostei (mainly European anchovy Engraulis encrasicolus and black goby Gobius niger). There was no difference between male and female diet. Feeding habits varied with size, with fish dominating the stomach contents of larger specimens. Finally, fish increased in winter and crustaceans in summer. Chelidonichthys lucerna shows a generalist and opportunistic foraging behaviour, preying mainly epibenthic and nectobenthic organisms.

Keywords: *Chelidonichthys lucerna*, tub gurnard, feeding activity, Mediterranean Sea

Submitted 2 November 2010; accepted 11 April 2011; first published online 31 May 2011

INTRODUCTION

Chelidonichthys lucerna (Linnaeus, 1758) occurs in the Mediterranean Sea, Black Sea and eastern Atlantic from Norway to Senegal. It is a nectobenthic fish mainly distributed on the soft bottoms of the continental shelf and is more abundant in coastal areas (Serena *et al.*, 1998). Commercial Triglidae catches have greatly diminished worldwide in recent years, dropping from about 38,000 tonnes in 2000 to 11,000 in 2004 (FAO, 2007), a negative trend that underscores the need for adopting suitable fishery management policies. Despite its importance for the Mediterranean fishery and the increasingly intense fishing pressure (FAO, 2007), information on its feeding habits are few and sparse (Atlantic Ocean: Nouvel, 1950; Costa, 1988; Mediterranean: Reys, 1960; Faltas, 1996; Morte *et al.*, 1997), particularly in Italian Seas (central Adriatic Sea: Froglià, 1976; Tyrrhenian Sea: Colloca *et al.*, 1994).

With growth, the species changes its diet both in terms of prey size and type of prey, a fact that has also been attributed to its bathymetric migratory behaviour (Colloca *et al.*, 1994; Morte *et al.*, 1997). Smaller individuals feed upon benthic Crustacea, mainly Mysidacea (North Sea: Hostens & Mees, 1999), Amphipoda and Decapoda Natantia (*Philocheras monacanthus*). As they grow larger, *C. lucerna* increasingly preys on decapods (mainly Portunidae and Crangonidae) and teleosts (mainly Callionymidae) (Froglià, 1976; Colloca *et al.*, 1994; Stagioni *et al.*, 2007).

Determining fish diet and knowing trophic behaviour is necessary information for fishery resource management and

for assessing fishing activity impact on the ecosystem. Acknowledging ecological interactions, such as predation, is essential for an ecosystem approach to fisheries (Bascompte *et al.*, 2005; Cury *et al.*, 2005). The inadequacy of traditional single-species models is amplified by the lack of knowledge as to long-term inter-species relationships. A ‘multi-species fisheries assessment model’ requires biological data on fish for a better understanding of how species relate to one another (Adriamed Project, 2001; Eldredge, 2002; Stergiou & Karpouzi, 2002; DCR Medits Working Group, 2007).

The aim of this paper was to assess the diet of tub gurnard in the north-east Mediterranean Sea, introduced from 2006 in the list of ‘reference species from the Coordination of the International bottom trawl survey in the Mediterranean Sea (Medits)’ (Relini *et al.*, 2008), in order to increase the knowledge about the trophic biology of this scarcely studied species (comparing the diet among sex, size groups, depth and season).

MATERIALS AND METHODS

Sampling oceanographic surveys and samples processing

A total of 1096 specimens of tub gurnard were collected seasonally (winter and summer) during several international bottom trawl surveys (MEDITS project) using as sampling gear a bottom trawl made of four panels (Relini *et al.*, 2008). The surveys were carried out between May 2005 and March 2007 at depths ranging from 10 to 260 m in the north-east Mediterranean (Adriatic Sea from the Gulf of

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Trieste (13°37'E and 45°40'N) to the Tremiti Islands (15°16'E and 42°08'N) (Figure 1).

In the laboratory, total length (TL) was measured to the nearest mm and specimens were weighed (W) to the nearest 0.1 g. Macroscopic analysis of gonads was performed to determine sex, with specimens classified as females, males, juveniles and not determined (sex not distinguished). The stomachs were immediately removed and preserved in 70% ethanol solution for stomach content analysis. Preys were identified to the lowest possible taxonomic level, counted, and weighed to the nearest 0.1 mg after removal of surface water using blotting paper.

Data analysis

Stomach contents, according to size-class, sex, season (of international bottom trawl survey in the Mediterranean Sea: winter and summer) and water depth, were investigated by qualitative and quantitative analyses. Size-classes were defined based on the results of cluster analysis of diet composition, using the Bray–Curtis similarity index (Clarke & Warwick, 1994) that assessed differences between predator length and number (%N) of ingested food categories.

The diet was described as follows:

%N = the number of individuals of each prey type expressed as a percentage of the total number of prey items from all stomachs;

%W = the weight of individuals of each prey type expressed as a percentage of the total weight of prey items from all stomachs;

%F = the number of stomachs in which a food type occurred expressed as a percentage of the total number of stomachs that contained prey;

%IRI = the index of relative importance expressed as a percentage where IRI (Pinkas *et al.*, 1971), is expressed as: $IRI = (\%N + \%W) \%F$ where %N is the percentage of numerical composition, %W is the percentage in weight, %F is the percentage of frequency of occurrence.

Prey-specific abundance (PSA) is defined as the percentage of a prey taxon comprising all prey items in only those predators in which the actual prey occurs, or in mathematical terms (Amundsen *et al.*, 1996):

$PSA = \frac{P_i}{\sum S_i / \sum S_{t_i}}$ where P_i is the prey-specific abundance of prey i , S_i the stomach content (number) comprising prey i , S_{t_i} the total stomach content of only those predator specimens with prey i in their stomachs.

The Shannon index (H') was employed to measure trophic diversity as: $H' = -\sum N_i \ln(N_i)$.

Multivariate analyses were performed on prey classes in order to evaluate environmental and ontogenetic patterns: non-metric multidimensional scaling (nMDS) plot, analysis of similarity (ANOSIM) and multiple response permutation procedure (MRPP) were calculated on numerical abundance Bray–Curtis dissimilarity matrix.

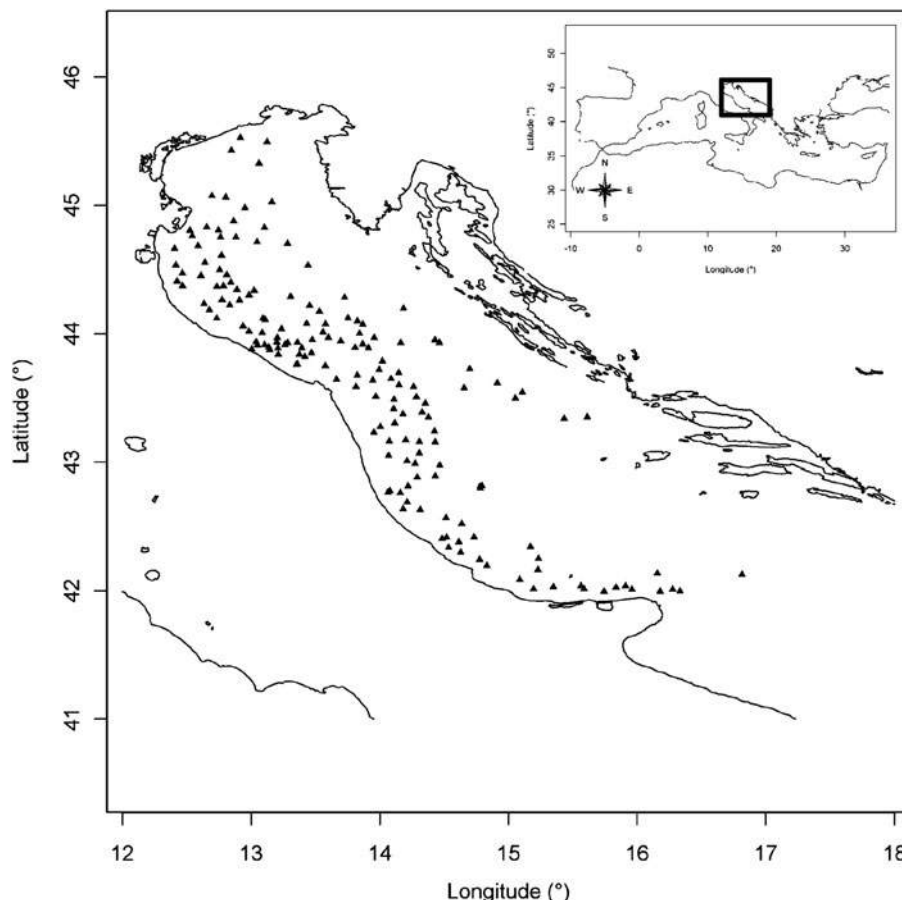


Fig. 1. Map of sampling sites in northern-central Adriatic Sea.

Feeding trends were performed with R software version 2.10 base and Vegan package (R Development Core Team, 2010).

RESULTS

The TL of the 1096 tub gurnard examined ranged from 63 to 415 mm (mean size = 207 mm; median size = 218 mm). According to sex determination, 466 females, 373 males, 235 juveniles and 22 not determined were recorded.

Overall diet description

Qualitative analysis permitted to identify 55 food items belonging to 6 main taxa (Table 1). Tub gurnard diet chiefly consisted of Crustacea (especially Decapoda) and Teleostei. In quantity terms, Crustacea (%N = 89.7, %W = 58) were the most abundant prey taxon followed by Teleostei (%N = 6.7, %W = 39.7). Other preys such as Mollusca (%N = 1.6, %W = 0.6) and Anellida: Polychaeta (%N = 0.03, %W = 0.01) were occasionally recorded. In terms of number and weight abundance (%N and %W) and of relative importance (%IRI), the most important preys were: *Goneplax rhomboides* (%N = 14.2, %W = 29.5, %IRI = 50.3), *Liocarcinus depurator* (%N = 6, %W = 20.3, %IRI = 10.5) and *Liocarcinus* spp. (%N = 13, %W = 24.8, %IRI = 5) for Decapoda: Reptantia; *Philocheras* spp. (%N = 59.5, %W = 2.5, %IRI = 26.5) and *Philocheras bispinosus* (%N = 15.1, %W = 1, %IRI = 3) for Decapoda: Natantia; *Engraulis encrasicolus* (%N = 0.6, %W = 11.9, %IRI = 1.1) and *Gobius niger* (%N = 0.6, %W = 8.8, %IRI = 0.8) for fish.

In terms of PSA, which is a food preference index, the most important preys were found to be *Corystes cassivelaunus* and *Munida* spp. for crustaceans, and *Merlangius merlangius*, *Callionymus risso*, *Merluccius merluccius*, *Pomatoschistus minutus*, *Trisopterus minutus*, *Cepola macrophthalma* and *Deltentosteus quadrimaculatus* for fish.

Diet variation with sex

No significant difference was found between feeding habits and sex (Crustacea F-M: $\chi^2 = 0.0011$, $df = 1$, $P = 0.974$; Teleostei: F-M: $\chi^2 = 3.6085$, $df = 1$, $P = 0.05749$). No trophic diversity between sex was found ($H' = 0.219$ for both). Multivariate analyses showed no difference: no pattern in nMDS plot (Figure 6), ANOSIM $R = -0.0018$ $P = 0.635$, MRPP $A = 0.0013$ $P = 0.069$.

Diet variation with fish size-class

Diet variation as a function of length was appreciable. Cluster analysis based on similarity in the diet showed a first dichotomy that discriminated two main groups with a total length superior or lower than 180 mm (Figures 2 & 3).

Juveniles (≤ 180 mm) were more related to Crustacea, while adults were more related to (> 180 mm) to fish. Trophic diversity was lower in juveniles ($H' = 0.098$) than adults ($H' = 0.229$).

Multivariate analyses showed difference between juveniles and adults (Figure 7), ANOSIM $R = 0.1039$ $P = 0.001$, MRPP $A = 0.0499$ $P = 0.001$.

Table 1. Diet composition of *Chelidonichthys lucerna*. %N, percentage in number; %W, percentage in weight; %F, frequency of occurrence; %IRI, percentage of index of relative importance of prey items; PSA, % prey taxon over all preys in all predators.

Prey taxa	%N	%W	%F	%IRI	PSA
Crustacea	89.69	58.01	7.47	92.24	72.51
<i>Philocheras</i> spp.	44.38	1.53	14.52	26.51	23.98
<i>Philocheras bispinosus</i>	15.10	0.96	4.75	3.04	33.50
<i>Goneplax rhomboides</i>	14.16	29.52	28.95	50.3	60.41
<i>Liocarcinus</i> spp.	7.09	4.47	10.95	5.04	59.10
<i>Processa</i> spp.	6.15	1.80	5.26	1.66	17.52
<i>Liocarcinus depurator</i>	5.96	20.32	10.02	10.47	73.70
<i>Alpheus glaber</i>	0.83	1.01	3.23	0.24	19.63
<i>Solenocera membranacea</i>	0.62	1.51	1.95	0.17	47.18
<i>Squilla mantis</i>	0.43	2.95	1.78	0.24	54.32
<i>Macropodia</i> spp.	0.28	0.16	0.34	0.01	26.02
<i>Jaxea nocturna</i>	0.21	0.51	0.93	0.03	16.05
<i>Liocarcinus maculatus</i>	0.23	0.43	0.68	0.02	33.92
<i>Corystes cassivelaunus</i>	0.11	1.79	0.51	0.04	81.96
<i>Upogebia</i> spp.	0.08	0.28	0.34	<0.01	16.83
<i>Lophogaster typicus</i>	0.08	0.02	0.34	<0.01	6.42
<i>Liocarcinus vernalis</i>	0.08	0.09	0.17	<0.01	41.35
<i>Sicyonia carinata</i>	0.06	0.09	0.17	<0.01	30.56
<i>Pontophilus spinosus</i>	0.06	0.09	0.17	<0.01	26.84
<i>Parapenaeus longirostris</i>	0.04	0.15	0.17	<0.01	17.35
<i>Brachynotus</i> spp.	0.04	0.05	0.17	<0.01	11.90
<i>Chlorotocus crassicornis</i>	0.04	0.05	0.08	<0.01	51.53
<i>Liocarcinus pusillus</i>	0.02	0.02	0.08	<0.01	16.68
<i>Pisidia longimana</i>	0.02	0.02	0.08	<0.01	10.67
<i>Ebalia granulosa</i>	0.02	<0.01	0.08	<0.01	52.46
<i>Processa modica</i>	0.02	<0.01	0.08	<0.01	1.29
<i>Munida</i> spp.	0.02	<0.01	0.08	<0.01	100.00
<i>Meliceratus kerathurus</i>	0.02	<0.01	0.08	<0.01	2.99
Teleostei	6.72	39.74	1.92	7.45	74.65
<i>Engraulis encrasicolus</i>	0.60	11.91	2.29	1.14	81.89
<i>Gobius niger</i>	0.60	8.77	2.04	0.76	86.93
<i>Lesueurigobius friesii</i>	0.49	1.57	1.53	0.13	52.88
<i>Cepola macrophthalma</i>	0.13	2.01	0.59	0.05	98.84
<i>Pachygrapsus marmoratus</i>	0.41	0.32	1.10	0.03	46.96
<i>Lesueurigobius suerii</i>	0.26	0.86	0.68	0.03	38.63
<i>Trisopterus minutus</i>	0.08	1.80	0.17	0.01	99.78
<i>Arnoglossus laterna</i>	0.08	1.00	0.25	0.01	73.34
<i>Gobius</i> spp.	0.08	0.52	0.34	0.01	27.40
<i>Serranus hepatus</i>	0.04	0.79	0.17	0.01	87.19
<i>Callionymus maculatus</i>	0.08	0.35	0.25	<0.01	56.29
<i>Gaidropsarus biscayensis</i>	0.08	0.18	0.34	<0.01	40.43
<i>Merlangius merlangus</i>	0.02	0.93	0.08	<0.01	100.00
<i>Deltentosteus quadrimaculatus</i>	0.04	0.15	0.17	<0.01	92.81
<i>Callionymus</i> spp.	0.04	0.13	0.17	<0.01	84.73
<i>Callionymus risso</i>	0.04	0.11	0.17	<0.01	100.00
<i>Merluccius merluccius</i>	0.02	0.24	0.08	<0.01	100.00
<i>Microchirus variegatus</i>	0.02	0.03	0.08	<0.01	79.17
<i>Pomatoschistus minutus</i>	0.02	0.01	0.08	<0.01	100.00
Mollusca	1.57	0.55	0.41	0.04	30.13
<i>Corbula gibba</i>	0.34	0.19	1.44	0.03	4.69
<i>Turritella communis</i>	0.21	0.22	0.93	0.02	9.49
<i>Anadara demiri</i>	0.04	0.01	0.17	<0.01	2.94
<i>Nassarius</i> spp.	0.06	0.01	0.25	<0.01	0.48
<i>Arca tetragona</i>	0.04	0.02	0.17	<0.01	3.69
<i>Tellina</i> spp.	0.04	0.01	0.17	<0.01	3.01
<i>Epitonium</i> spp.	0.02	0.03	0.08	<0.01	10.65
Anellida	0.03	0.01	0.01	<0.01	4.92
<i>Aphrodita aculeata</i>	0.02	0.01	0.08	<0.01	3.80
<i>Sternaspis scutata</i>	0.02	0.01	0.08	<0.01	9.67
Not determined	1.99	1.7	0.92	0.28	17.38

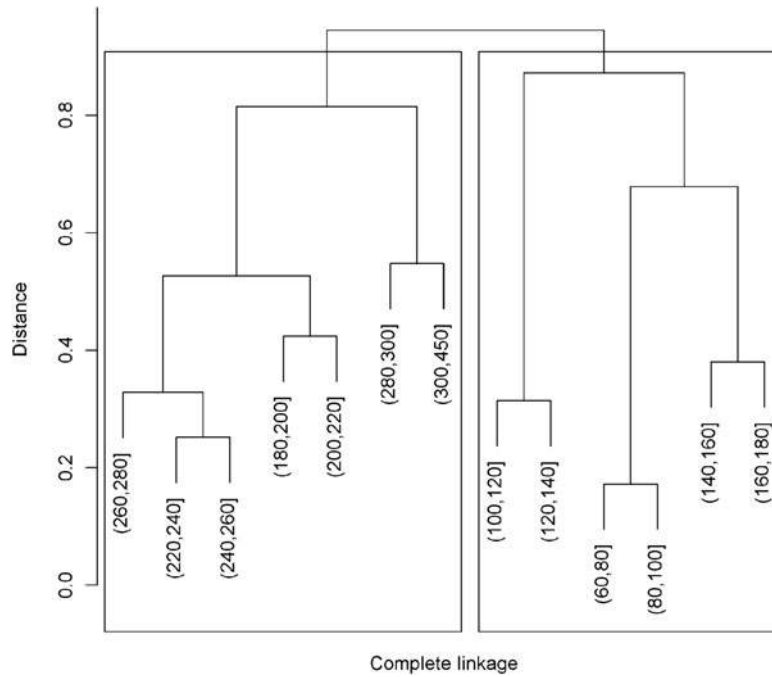


Fig. 2. Cluster analysis results based on the feeding habits of *Chelidonichthys lucerna* per size-class of predators (mm).

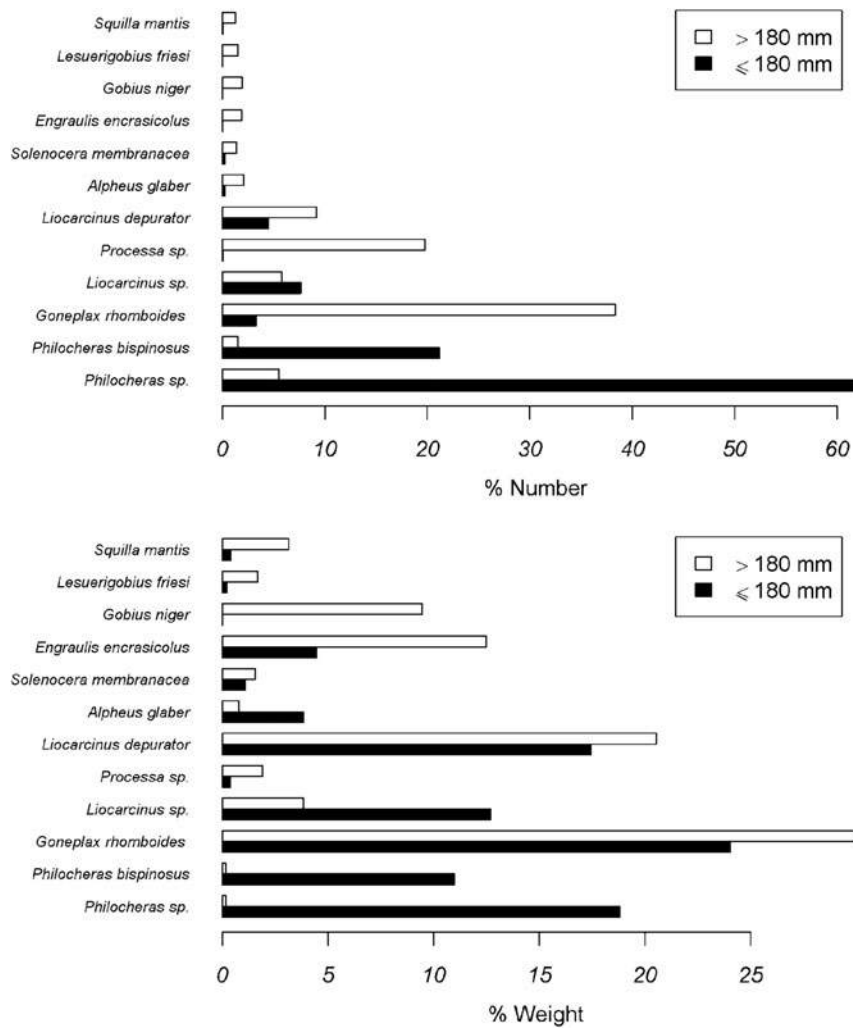


Fig. 3. Class size-wise numeric and gravimetric prey abundance percentage of the most abundant prey item.

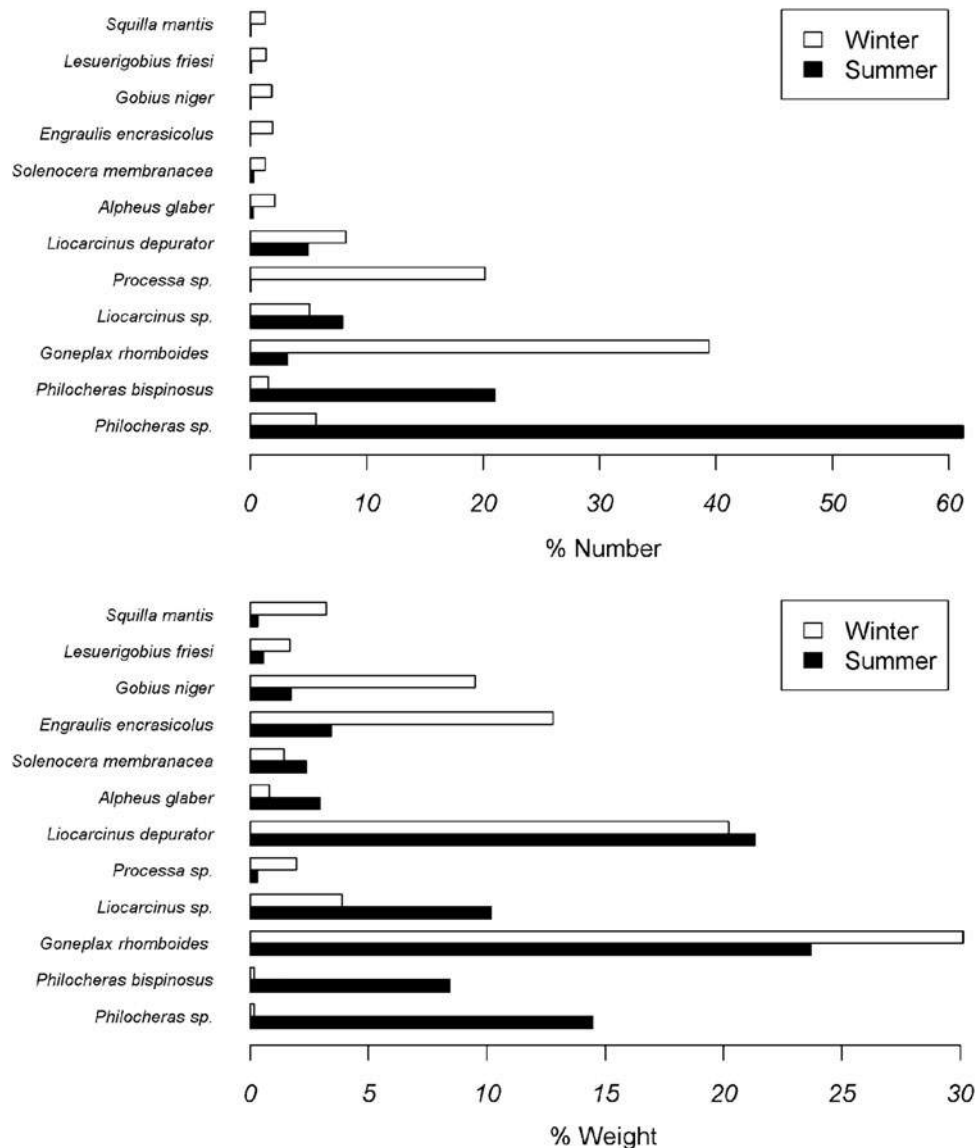


Fig. 4. Season-wise numeric and gravimetric prey abundance percentage of the most abundant prey item.

Diet variation with season

Seasonal diet showed an increase of fish in winter (*E. encrasicolus* and *G. niger*) and Crustacea in summer (*Philocheas* sp.). However, some Crustacea such as *L. depurator* and *G. rhomboides* were found in high abundance in both seasons (Figure 4).

Trophic diversity was lower in summer ($H' = 0.099$) than winter ($H' = 0.229$).

Multivariate analyses showed difference between winter and summer (Figure 8), ANOSIM $R = 0.1064$ $P = 0.001$, MRPP $A = 0.0509$ $P = 0.001$.

Diet variation with depth

Three depth strata (I: 10–25 m; II: 25–50 m; III: 50–260 m) were considered. No noticeable differences were found between large taxonomic groups ($H'I = 0.187$; $H'II = 0.205$; $H'III = 0.170$), but there were differences in a specific level. For instance, *G. niger* prevailed in first and second depth

strata, while *E. encrasicolus* prevailed in second and third strata (Figure 5).

Multivariate analyses showed difference between strata (Figure 9), ANOSIM $R = 0.0767$ $P = 0.001$, MRPP $A = 0.0465$ $P = 0.001$.

DISCUSSION

The diet of *C. lucerna* in the study area mainly consisted of epibenthic crustacea such as *Goneplax rhomboides*, *Liocarcinus* spp. and *Philocheas* spp. There were also necto-benthic preys, mainly fish while pelagic preys such as *Engraulis encrasicolus* could probably result from being active predation or discarded food, although Olaso *et al.* (2002) indicates that *C. lucerna* does not show scavenging behaviour.

Crustacea and Teleostei were the two principal prey categories, while Mollusca and Polychaeta were found in low

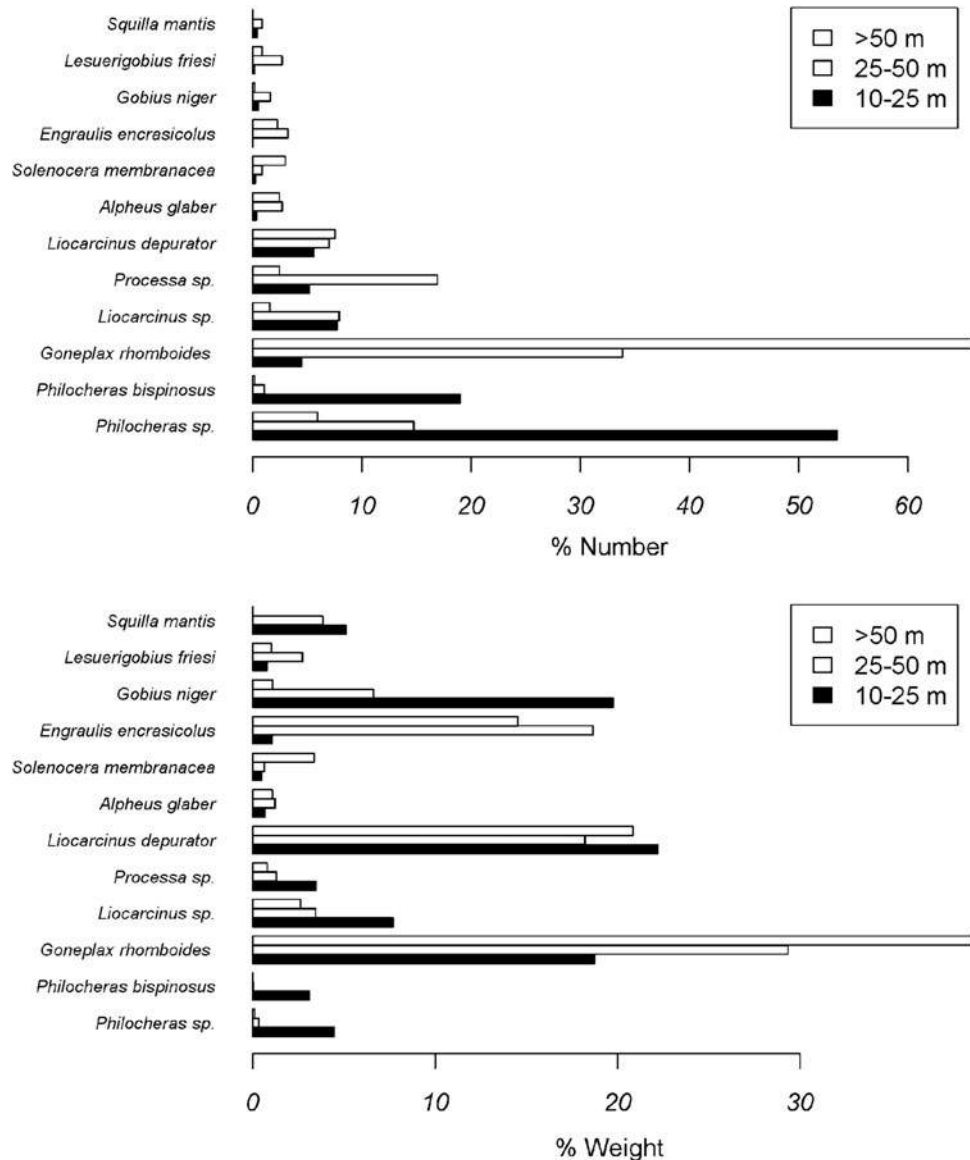


Fig. 5. Depth-wise numeric and gravimetric prey abundance percentage of the most abundant prey item.

numbers. The results of this study are in accordance with previous studies (Atlantic Ocean: Nouvel, 1950; Costa, 1988; Hostens & Mees, 1999; Mediterranean Sea: Reys, 1960 (Gulf of Lions); Frogli, 1976 (juveniles, central Adriatic Sea); Colloca *et al.*, 1994 (Tyrrhenian Sea); Faltas, 1996 (Egyptian waters); Morte *et al.*, 1997 (Spain)).

The feeding of the species changes substantially as a function of size. It is in fact greater, as measured by amount of prey in the stomach, in juveniles, during summer and at shallow depths. Crustacea were found to be the main prey regardless of predator size. They were however found to prevail in the diet of juveniles, while in the stomachs of adult specimens, Crustacea were almost equal, weight-wise, to fish, a fact that has been previously reported for this species in the study area (Frogli, 1976; Colloca *et al.*, 1994). Particularly, the predator length of 180 mm was found to correspond to predator growth 'critical size', that is to the onset of the start of sexual maturity in the Adriatic Sea (Montanini *et al.*, 2008; Vallisneri *et al.*, in press). Variation observed between juveniles and adults could be attributed mainly to differences in

gonad maturity and in the biological cycle, that requires greater energy. So, this critical size was related to the start of sexual maturity, the tendency to migrate to greater depths (generally muddy substrate), a change of diet from crustaceans to fish and an increase of variety of food items eaten (Montanini *et al.*, 2008).

Variation observed between seasons and depths reflected the spawning period of this species (Tsikliras *et al.*, 2010): juveniles were more abundant during summer along the Italian coast, adults in winter at greater depth.

Based on the results presented here, it may be said that *C. lucerna* is an 'opportunistic predator', since its feeding habits are not species-specific. Its trophic spectrum is in fact very wide, being characterized by a high degree of biodiversity and correlated with changes in feeding habits during growth. Stergiou & Karpouzi (2002), who identified Triglidae as 'omnivores with preference for animal material', are not in agreement with the present study, where only only nectobenthic fish and epibenthic invertebrates were found in its diet.

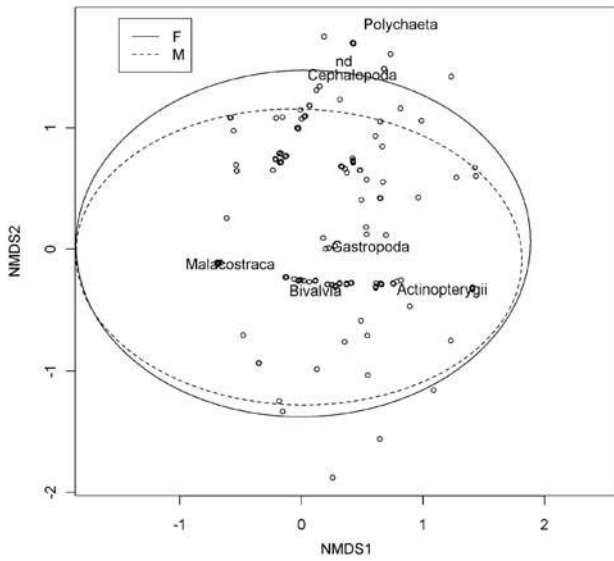


Fig. 6. Non-metric multidimensional scaling plot between sexes (females and males) with superimposed 95% confidence ellipses.

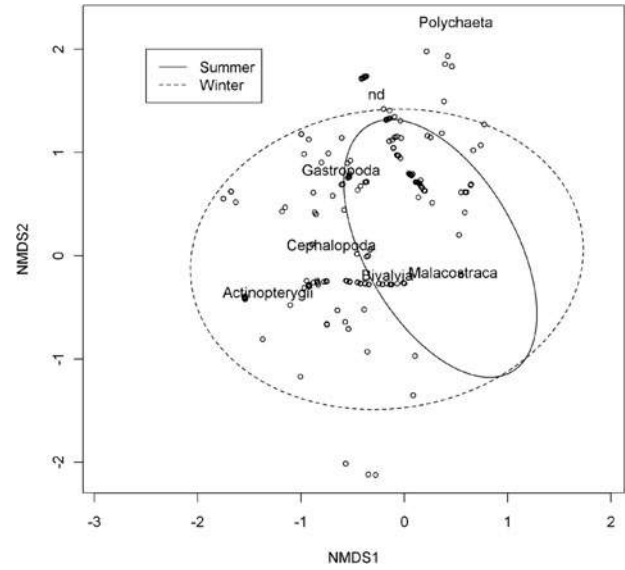


Fig. 8. Non-metric multidimensional scaling plot between seasons (winter and summer) with superimposed 95% confidence ellipses.

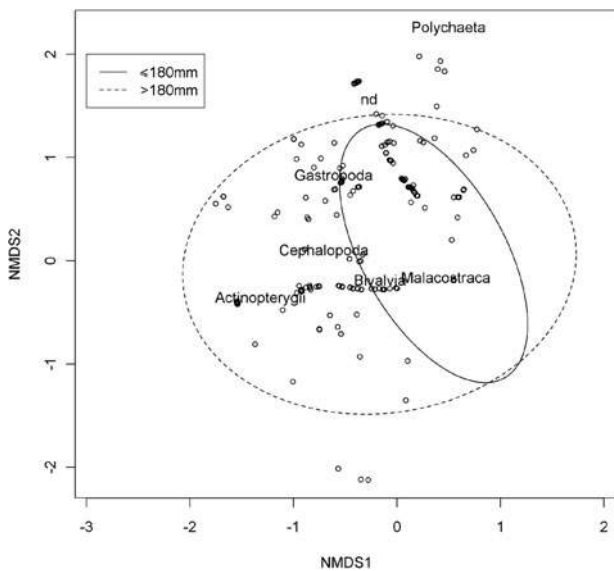


Fig. 7. Non-metric multidimensional scaling plot between two size-classes (Class I ≤ 180 ; Class II > 180) with superimposed 95% confidence ellipses.

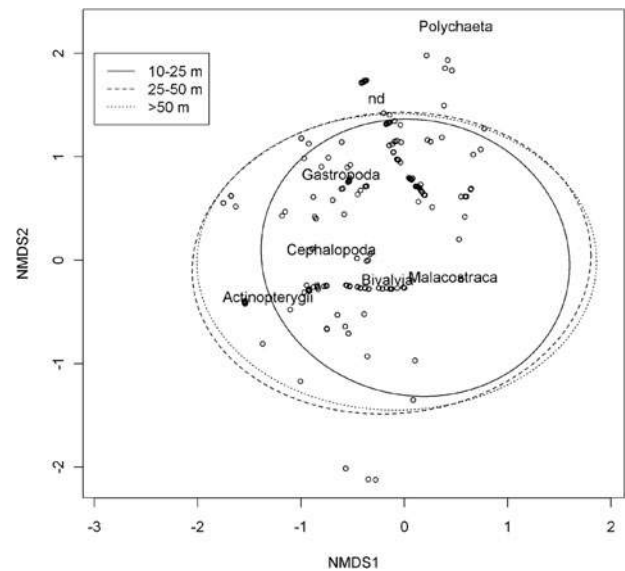


Fig. 9. Non-metric multidimensional scaling plot among three depth strata (I: 10–25 m; II: 25–50 m; III: 50–260 m) with superimposed 95% confidence ellipses.

Diet composition is considered to reflect the biocenosis typical of the area (e.g. Colloca *et al.*, 1994; Serena *et al.*, 1998; Costa & Cabral, 1999; Link, 2004; Morte *et al.*, 1997). For instance, *E. encrasicolus* plays an essential role in adult diet and is found in large quantities on the same relatively shallow beds (<100 m) of the western Adriatic Sea (Piccinetti & Piccinetti Manfrin, 1971), which is also the favourite habitat of *C. lucerna*.

Moreover, it is necessary to consider the possible impact among *C. lucerna* and other commercial fish and Crustacea (such as mantis prawn and shrimps). It in fact preys intensively on these species with marked effects on food webs, and this alone justifies the importance of a 'multispecific approach' in terms of analyses and management.

Despite the considerable increase in fishing activity, the inadequacy of traditional single-species models is aggravated by the lack of long-term routine fishery data and scarce scientific information (Stergiou & Karpouzi, 2002; Cury *et al.*, 2005; Vallisneri *et al.*, 2010). Food webs depict the feeding relationships (who eat whom) in communities, but for the large number of species and interactions, our knowledge of real food webs is limited (Bascompte *et al.*, 2005).

In conclusion, the goal of feeding behaviour studies for fishery management applications is to improve knowledge as to fish population biology and ecology in view of an ecosystem-based management of commercially important stocks.

ACKNOWLEDGEMENTS

Sampling was performed under the MIPAAF project ‘Trophic demersal population structure’. We thank Professor Corrado Piccinetti for his assistance in bottom trawl surveys and for his valuable suggestions in the drafting of the paper and Dr Emanuela Mazzoni for his precious help regarding stomach contents sampling.

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FEEDING HABITS OF *ASPITRIGLA CUCULUS* (LINNAEUS, 1758) IN NORTHERN-CENTRAL ADRIATIC SEA

ABITUDINI ALIMENTARI DI ASPITRIGLA CUCULUS (LINNAEUS, 1758) IN NORD-CENTRO ADRIATICO

Abstract - The stomach contents of red gurnard specimens collected in the Northern-Central Adriatic Sea have been analyzed to determine diet according to fish size and depth. Crustaceans, particularly *Lophogaster typicus* (Lophogastrida) and Decapoda represented the basic food for all sizes (%IRI=99.12 for juveniles; %IRI=97.92 for adults). The diet of adults was similar to those of juveniles and it does not change with depth, indicating trophic specialization and preference for prey with a wide distribution.

Key-words: diet, Adriatic Sea, *Aspitrigla cuculus*.

Introduction - Notwithstanding the ecological and economic interest about *A. cuculus* in the Mediterranean, many aspects of its biology are poorly understood (Vallisneri *et al.*, 2012). Moreover, the feeding activity and possible ontogenesis-related diet changes, though essential for stock assessment models, are almost unknown (Terrats *et al.*, 2000), particularly in Italian Sea (Colloca *et al.*, 1994). Along the Italian coasts it lives down to 400 m with the highest density between 100 and 200 m, while it lacks to under 50 m.

Materials and methods - A total of 390 specimens of *A. cuculus* were collected in the Northern-Central Adriatic Sea by bottom trawl surveys (2005-2009). Specimens were measured to the nearest mm (total length) and weighed to the nearest 0.1 g. Sex and gonad maturity levels were defined according to the code of sexual maturity used in the European MEDITS project (MEDITS, 2007). Fish lengths were classified into two size groups: juveniles and adults according to the maturity scale. Samples were dissected and stomachs were removed and preserved in 70% ethanol for analysis of stomach content. Diet was expressed as percentage by number, by weight, by index of relative importance (Tab. 1). Depth distribution was analysed by Pearson's Chi-squared test in order to test the significance of the relationship between size groups and depth. Ontogenetic diet change was explored using "Morosita index" values (Pusey and Bradshaw, 1996) that investigate intra-specific niche overlap among size classes.

Results - Juveniles (n°118) showed maximum size of 113 mm TL and depth range between 55.25-176.5 m. Adults (n°272) showed maximum size of 262 mm and migrated to greater depth respect to juveniles (p<0.001), between 82.3-246 m. Diet of *A. cuculus* was classified into four main categories: Crustacea, Teleostei, Mollusca and Echinodermata (Tab. 1). Almost the entire diet of this species based on crustaceans (%Cn = 93.88; %Cw = 91.15; %IRI = 98.26) while other taxa were classified as accessory prey. *Lophogaster typicus* (Lophogastrida) was the most abundant and preferred prey as showed by its almost exclusive presence both in the diet of juveniles and adults; decapods were the secondary prey. The feeding habits of adults were seen

to be similar to those of juveniles. For the ontogenetic groups (juveniles and adults) intraspecific overlap were recorded: "Morosita index" values was extremely high: C = 0.991.

Tab. 1-Diet analysis by *A. cuculus* in the Northern-Central Adriatic Sea. %Cn=percentage by number; %Cw=percentage by weight; IRI%=percentage of index of relative importance.
Analisi della dieta in A. cuculus del nord-centro Adriatico. %Cn=numero percentuale; %Cw=peso percentuale; IRI%=percentuale dell'indice di importanza relativa.

TAXA	ASPITRIGLA CUCULUS					
	JUVENILES			ADULTS		
	%Cn	%Cw	%IRI	%Cn	%Cw	%IRI
CRUSTACEA	97.62	96.35	99.12	92.61	90.51	97.92
TELEOSTEI	0.40	0.22	<0.01	1.21	3.77	0.04
MOLLUSCA	–	–	–	1.08	0.34	0.03
ECHINODERMATA	0.40	0.02	<0.01	0.27	0.32	<0.01
UNIDENTIFIED REMAINS	1.59	3.42	0.87	4.84	5.07	2.00

Conclusions - The dominant role which the crustaceans play in the diet of *A. cuculus* is confirmed by literature, nevertheless, the main species are different in different places (Colloca *et al.*, 1994). Diet doesn't vary significantly around the size of sexual maturity, around 110 mm (Vallisneri *et al.*, 2012), although the samples show a migration pattern related to growth. Data aren't according to Moreno-Amich (1992), because around size of sexual maturity Decapoda increase respect to Mysida and Lophogastrida, and fish represent the most important prey after the crustaceans. In the Adriatic Sea, *A. cuculus* appears to be a "specialist predator" that feeds almost exclusively on necto-benthic crustaceans in agreement with data from other Mediterranean areas (Colloca *et al.*, 1994, Tyrrhenian; Terrats *et al.*, 2000-Aegean Sea). The diet of *A. cuculus* is not to vary with depth, indicating trophic specialization and preference for prey with a wide distribution (eg. *Lophogaster typicus*).

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PRIME INDAGINI SULLE ABITUDINI ALIMENTARI DI *Trigloporus lastoviza* (SCORPAENIFORMES: TRIGLIDAE) IN ADRIATICO

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Trigloporus lastoviza (Bonnaterre, 1788), volgarmente nota come “gallinella o capone ubriaco”, in Adriatico è distribuita principalmente a nord, nella zona centroorientale e nell’area compresa tra Spalato e Dubrovnik, fino a 100 m di profondità, in acque poco profonde, potendo quindi disporre di una maggiore diversità di prede rispetto ad altri triglidi (per es. *Aspitrigla cuculus*) che vivono a profondità maggiori. È stato analizzato il contenuto stomacale di 138 esemplari adulti di *T. lastoviza* prelevati in Adriatico durante 4 campagne di pesca a strascico, 2 invernali e 2 estive condotte tra il 2007 e il 2012. Su tutti gli esemplari sono stati registrati i parametri biometrici (LT, mm), peso corporeo (P, g), sesso (valutato sulla base dell’analisi macroscopica delle gonadi), sono state identificate le prede fino al livello tassonomico più basso possibile, successivamente pesate, contate, fotografate, mediante sistema computerizzato di immagini. I dati sono stati informatizzati con database relazionale “Microsoft Access” e analizzati mediante pacchetto statistico “R ver. 3.0.1”. Sono stati calcolati i principali indici alimentari, al fine d’individuare la tipologia qualiquantitativa del regime alimentare di *T. lastoviza*. I risultati ottenuti mostrano che la dieta verte essenzialmente su crostacei (IRI% = 95,8%), per lo più decapodi. *Galathea intermedia* e *Liocarcinus* sp. rappresentano le specie prevalenti. Nonostante il fatto che la popolazione bentonica costiera del Mediterraneo nordorientale sia caratterizzata da elevata diversità specifica, questa non si riflette nel contenuto stomacale di *T. lastoviza*, indicando con queste indagini preliminari, che la specie tende a comportarsi da “predatore selettivo”, nutrendosi preferibilmente di pochi organismi specifici, in accordo con la letteratura relativa ad altre aree geografiche. Concludendo, si conferma l’importanza degli studi relativi alla biologia trofica ed ai rapporti predapredatore, essenziali per quantificare i ruoli ecologici dei diversi componenti delle comunità marine.

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DIET AND FEEDING STRATEGY OF STREAKED GURNARD *TRIGLOPORUS LASTOVIZA* IN NORTHERN-CENTRAL ADRIATIC SEA

ABITUDINI ALIMENTARI DI TRIGLOPORUS LASTOVIZA IN ADRIATICO CENTRO SETTENTRIONALE

Abstract - The stomach contents of 136 samples of streaked gurnard, *Trigloporus lastoviza* (Osteichthyes: Scorpaeniformes) carried out in the Northern-Central Adriatic Sea, have been analyzed to describe the diet, feeding strategy and niche width components. Results suggested that the species was an opportunistic bottom feeder with a relative broad niche, preying primarily on benthic crustaceans.

Key-words: *Trigloporus lastoviza*, Adriatic Sea, diet, feeding strategy, niche breadth.

Introduction - The streaked gurnard *Trigloporus lastoviza* (Bonnaterre, 1788) is included in the list of target species of MEDITS project since 1998. It occurs in North-Central Adriatic Sea where it's mainly distributed in the central and eastern part of the North Adriatic and in the channel area from Split to Dubrovnik, generally down to 100 m. Few biological data are available on Adriatic sea (Vallisneri *et al.*, 2010). The diet of the species was investigated in the Southern-Eastern Mediterranean (Greece, Terrats *et al.*, 2000) and in Southern Mediterranean (Gulf of Tunis, Ben Jrad *et al.*, 2010); it was mainly composed by crustaceans, mostly mysids and decapods. The aim of the present study was to contribute to the knowledge of the feeding habits of this species in order to understand its ecological role in the demersal communities.

Materials and methods - 136 samples of streaked gurnard were collected in the Northern-Central Adriatic Sea by four seasonal bottom trawl surveys (GRUND-winter 2007-2008 and MEDITS-summer 2008 and 2012). Specimens were measured to the nearest mm (TL, total length), weighed to the nearest 0.1 g and sex was determined by gonadic macroscopic evaluation according to the code of sexual maturity for fish (Relini *et al.*, 2008). Samples were dissected and stomachs were removed and preserved in 70% ethanol for stomach content analysis. The contribution of each prey item was expressed as percentage by number (%N), weight (%W), frequency of occurrence (%F), index of relative importance (%IRI). Prey-specific abundance index (%PSA) plotted against occurrence of prey species was computed to study feeding strategy according to Amundsen *et al.* (1996). Levins index was employed to measure diet breadth (Levins, 1968). Seasonally fullness index was calculated and tested by chi-squared test ($p < 0.05$). Multivariate analysis (principal component analysis (PCA); non metric multidimensional scaling (nMDS) plot) and cluster analysis (based on Bray-Curtis index) were carried out on prey numeric and weight abundance classes to discovered possible feeding patterns.

Results - The size of the 136 streaked gurnards examined ranged from 108 to 221 mm TL. According to sex determination all samples were adult specimens and 61 females, 39 males and 36 not determined (sex not evaluated) were recorded. A total of 52 different food items were found in the diet and classified into seven main categories. Almost the entire diet of this species based on crustaceans malacostraca

(%N=93.7; %W=98.6; %F=94.1; %IRI=99.7) while other taxa were classified as accessory prey (Tab. 1). The feeding strategy diagram showed that most of the specimens of gurnards preferred different prey species and only a small fraction of predators feed on single or few species. The niche width computed on preys category number (n=52) reached high value B=8.99, displaying relative wide niche breadth. About fullness index, no differences were observed between winter and summer (X-squared=4.486, df=3, p-value=0.214). PCA and nMDS multivariate statistics as well Cluster analysis didn't show feeding patterns linked to season, sex and class size.

Tab. 1 - Diet of *T. lastoviza*. %N=percentage by number; %W=percentage by weight; %F=frequency of occurrence; %IRI=index of prey relative importance; %PSA=prey specific abundance.

Dieta di T. lastoviza. %N=numero percentuale; %W=peso percentuale; %F=frequenza di ritrovamento; %IRI=indice di importanza relativa; %PSA (abbondanza specifica).

TAXA	%N	%W	%F	%IRI	%PSA
Malacostraca	93.68	98.58	94.12	99.71	25.43
Ostracoda	0.39	0.12	0.74	<0.01	6.1
Bivalvia	2.14	0.28	11.03	0.15	1.92
Cephalopoda	1.46	0.64	7.35	0.09	4.5
Gastropoda	0.68	0.19	3.68	0.02	2.35
Teleostei	0.87	0.12	5.88	0.03	2.31
Bryozoa	0.29	0.06	1.47	<0.01	1.49
Algae: Phaeophyceae	0.1	0.01	5.88	<0.01	0.95
Foraminifera	0.29	<0.01	2.21	<0.01	0.03
Cnidaria: Hydrozoa	0.1	<0.01	0.74	<0.01	0.04

Conclusions - The diet of streaked gurnard was in line with feeding habits of other coastal benthic populations in the Mediterranean, characterized by large amount of different prey items. Malacostraca decapods were the most frequent prey items according to literature (Terrats *et al.*, 2000; Ben Jrad *et al.*, 2010) while other prey items were recorded as accessory ones. The species showed an opportunistic predator behaviour classified as bottom feeder fish with a broad niche width. This feeding behaviour was showed for other gurnards which using free rays of pectoral fin as feelers over the sea bottom (Wheeler, 1969).

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**ETÀ, ACCRESCIMENTO, MATURITÀ SESSUALE ED ECOLOGIA
TROFICA DI TRE SPECIE ITTICHE 'BERSAGLIO'
(SCORPAENIFORMES: TRIGLIDAE) DEL MARE ADRIATICO**

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Lo studio dei triglidi assume particolare importanza nella gestione delle risorse demersali perché poco noti, come evidenziato dal protocollo d'intesa tra Commissione europea e ICES. Scopo del lavoro è la valutazione della bio-ecologia delle popolazioni adriatiche, al fine di analizzarne i rapporti intra ed interspecifici in relazione alla rete trofica.

530 esemplari di *Aspitrigla cuculus*, 1444 di *Chelidonichthys lucerna*, 679 di *Eutrigla gurnardus* sono stati campionati stagionalmente mediante campagne oceanografiche tra il 2005-2009 registrandone i parametri biometrici standardizzati e lo stadio di maturità sessuale. Sono stati utilizzati subcampioni di stomaci per comparazioni trofiche e di otoliti per determinare l'età.

Il passaggio giovanile-adulto avviene intorno al primo anno di età, la maturità sessuale al secondo con differenze di taglia correlate a sesso e specie. Prede preferite sono i crostacei con differenze relative a taglia e specie. Gli adulti pur evidenziando uno spettro trofico maggiore rispetto ai giovanili mostrano specializzazioni individuali.

I triglidi analizzati raggiungono una 'taglia critica' alla fine del primo anno in concomitanza con: passaggio stadio giovanile - stadio adulto, inizio dicotomia tra sessi, allontanamento dalla costa, colonizzazione di nicchie trofiche differenti. Si segnala l'importanza della relazione taglia-profondità e l'ampiezza di nicchia dei triglidi nella distribuzione delle risorse trofiche.

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3 **Diet overlap among gurnard species in the north-eastern Mediterranean**

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15 **Running headline:** FEEDING ECOLOGY OF MEDITERRANEAN GURNARDS

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27 **Abstract**

28 The diet of three commercial gurnard species (*Aspitrigla cuculus*, *Chelidonichthys lucerna*
29 and *Eutrigla gurnardus*) was compared during ontogeny in the study area of Adriatic Sea
30 (northeastern Mediterranean) for evaluate prey-resource partitioning between these species. A
31 total of 1.818 specimens (390 *A. cuculus*, 973 *C. lucerna*, 455 *E. gurnardus*) collected by
32 bottom trawling were analysed. These species fed mainly Crustacea both juveniles and adults
33 but showed distinct feeding behaviour. *C. lucerna* and *E. gurnardus* were opportunistic
34 predators, showing a diverse diet based on epi-benthic, benthopelagic and necto-benthic
35 preys, while *A. cuculus* may be considered as a specialist feeder, feeding almost exclusively
36 necto-benthic invertebrates. The Morisita's index calculated for critical size classes (juveniles
37 and adults) among these gurnards showed differences. At inter-specific level, a possible diet
38 competition showed between *A. cuculus* and *E. gurnardus* ($C > 0.65$) only. At intra-specific
39 level, high diet overlap showed between juveniles and adults of *A. cuculus* ($C = 0.98$) and
40 between juveniles and adults of *E. gurnardus* ($C > 0.84$). In contrast, *C. lucerna* don't
41 competed with increasing body size ($C < 0.20$) showing a change from crustaceans to
42 piscivorous diet. The possibility that *A. cuculus* and *E. gurnardus* may be compete for the same
43 prey resources while *C. lucerna* show food resource partitioning, was discussed. Better
44 understanding of the ecology of these coexisting predators should lead to improved
45 conservation and improved fisheries management for all three predator species.

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48 Key words: red gurnard; tub gurnard; grey gurnard; feeding habits; niche overlap; Adriatic
49 Sea.

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INTRODUCTION

54 Stomach content and trophic interactions among species are important biological and
55 ecological parameters and need to be studied more for improve fishery management and
56 protection. Many of the ecological issues discussed by Stergiou & Karpouzi (2002) permitted
57 to study resource partitioning as well as prey selection competition within and between
58 species and predator-prey size relationships. Such information regarding feeding habits and
59 behaviour is of operational relevance for fishery management research and may represent an
60 important contribution for overcoming the inadequacies of traditional single-species models.
61 The shortcomings of such models being further aggravated by the lack of long-term routine
62 fishery data and the poor level of scientific information, despite the considerable increase in
63 fishing activity in recent times (Stergiou *et al.*, 1997). Furthermore, such information may be
64 useful for an ecosystem-based management of the Adriatic Sea (north-eastern Mediterranean)
65 in fact studying stomach content permits the analysis of niche overlap, increases
66 understanding of biological interactions in animal communities and examines how these
67 communities respond to human exploitation. The Northern and Central Adriatic Sea
68 constitutes the widest continental shelf in the Mediterranean Sea and is of great value for the
69 Italian and, more generally, the European fishing industry (Coll *et al.*, 2007). While the
70 continental shelf in almost all the Mediterranean Sea is of limited extension, the northern part
71 of the Adriatic basin lies entirely on the shelf and is characterized by very shallow waters with
72 an average depth of 35 metres. The central part of this basin gently slopes down to a depth of
73 100 m, with distinctive morphological features represented by two small bottom depressions
74 (the so-called “Pomo” or “Jabuka” Pits) reaching a maximum depth of 250 m (Zavatarelli &
75 Pinardi, 2003).

76 Given such an environment, fish stomach content analysis may be a useful indirect method
77 for drawing information as to benthic communities on continental shelves (Link, 2004).
78 Moreover, diet composition data is also important in the study of ontogenetic diet shift, which

79 occurs in most fish. Such dietary changes may depend on the interaction of changing external
80 factors (e.g. feeding habits, food availability, predation risk) and internal conditions (e.g.
81 anatomical structures, behaviour, physiological demands) (Luczkovich *et al.*, 1995). For a
82 large variety of species, size-specific shifts in food types are often related to shifts in habitat
83 (Werner & Gilliam, 1984) associated with food availability, resulting in changes in both their
84 behaviour and migration pattern. Ontogenetic dietary shifts may also depend on changes in
85 mouth size and anatomy (Wainwright & Richard, 1995).

86 Gurnards (Scorpaeniformes, Triglidae) are demersal fishes that inhabit the continental and
87 insular shelves of tropical and temperate seas to depths of 500 m, and are found on sandy,
88 muddy or rubble substrates (Fischer *et al.*, 1987). There are seven different species in the
89 north-middle Adriatic Sea (13°-15°E, 42°-45°N) showing differences related to biometric
90 features and ecological ones such as diet, spawning period and depth distribution (Tsimenides
91 *et al.*, 1992; Colloca *et al.*, 1994; Vallisneri *et al.*, 2010). During their early life cycle they go
92 through a pelagic phase, while during their demersal stage changes in their ecologic behaviour
93 are associated with the onset of sexual maturity (Vallisneri *et al.*, 2012), the tendency to
94 migrate to greater depths, and a change in diet from crustaceans to fishes with a wider trophic
95 spectrum (Colloca *et al.*, 1994; Montanini *et al.*, 2010). As is known, gurnards use the free
96 radii of their pectoral fins to search for prey on sedimentary beds (Whitehead *et al.*, 1986).

97 Red gurnard (*Aspitrigla cuculus* Linnaeus, 1758), tub gurnard (*Chelidonichthys lucerna* L.,
98 1758) and grey gurnard (*Eutrigla gurnardus* L., 1758) have been included in the list of
99 reference species of the MEDITS project that are studied for biological parameters (MEDITS,
100 2007). *C. lucerna* is the most landed and commercially important gurnard species for Italian
101 fishery, in particular in the Adriatic Sea (Relini *et al.*, 1999). This species as well as *A.*
102 *cuculus* and *E. gurnardus* have been classified as new MOU (Memorandum of
103 Understanding) species by the International Council for the Exploration of the North Sea
104 (ICES). The Council has recommended monitoring programs to be undertaken for these

105 species in order to gain information as to biological parameters for stock assessment purposes
106 (ICES, 2006). Subsequent to the decline in traditionally exploited fish stock, Triglidae have
107 come to be considered new landable species by trawlers (Boudaya *et al.*, 2008 – South
108 Mediterranean) and emergent key predators (as is the case with *Eutrigla gurnardus* in the
109 North Sea reported by Floeter & Temming (2005) and Weinert *et al.* (2010)).

110 Regarding stomach contents some studies have focused on the diet of gurnards in the
111 Adriatic Sea (Valiani, 1934; Frogliola, 1976; Montanini *et al.*, 2010; Stagioni *et al.*, 2011). No
112 study, however, has taken diet in terms of niche overlap, resource partitioning and ontogenetic
113 diet shift, into account as a possible important contributing factor to the segregation of
114 gurnard species in the studied area.

115 For all the above reasons, then, the study of gurnard ecology has become of considerable
116 importance for correct fishery management. The aim of the present study, therefore, was: (i)
117 to examine the feeding habits and trophic interactions of the three gurnards considered at both
118 the intra- and the inter-specific level; (ii) to investigate the occurrence of any ontogenetic diet
119 shift by comparing the diets of two different size groups, i.e. juveniles and adults, in relation
120 to their different depth distribution.

121

122 **MATERIAL AND METHOD**

123 Data from a total of 1818 stomachs belonging to 3 gurnard species collected at depths
124 of between 12 and 252 metres was examined. The sampling site was in the GFCM-GSA 17
125 area (Northern and Central Adriatic Sea) and covered a surface area of 59,400 sq.kms from
126 the Gulf of Trieste (45°40' N 13°37' E) to the Tremiti Islands (42°08' N 15°16' E) (Fig. 1).
127 Samples were taken on a seasonal basis (summer and autumn-winter) between 2005 and 2009
128 as part of several national and international oceanographic bottom trawl surveys, namely
129 MEDITS (MEDiterranean International Trawl Survey – summer surveys) and GRUND
130 (Assessment of Italian Demersal Resources – autumn/winter surveys). The sampling gear

131 included an experimental bottom trawl featuring four panels and a mesh cod-end size of 20
132 mm (stretched mesh) for the MEDITS while a 40 mm cod-end was used for the GRUND
133 surveys (Fiorentini *et al.*, 1999). Hauls were positioned according to a random stratified
134 sampling scheme; strata were defined according to depth and the number of hauls in each
135 stratum was proportional to the surface of the stratum. As far as possible, hauls were made in
136 the same positions each year.

137 A total of 390 individuals of *A. cuculus*, 973 of *C. lucerna* and 455 of *E. gurnardus*
138 were collected. Specimens from each trawl were measured to the nearest mm (total length =
139 TL) and weighed to the nearest 0.1 g. Sex (female, male, undetermined and not determined)
140 and gonad maturity levels were defined according to the code of sexual maturity used in the
141 European MEDITS project (MEDITS, 2007) and recorded. Maturity levels were classified
142 into eight different classes: 0 undetermined, 1 virgin, 2a virgin-developing, 2b recovering, 2c
143 maturing, 3 mature-spawner, 4a spent and 4b resting. Fish lengths were classified into two
144 size groups according to the above maturity scale: juveniles (individuals corresponding to
145 stages 0 and 1) and adults (individuals corresponding to stages from 2a to 4b). Juvenile to
146 adult transition size was marked off at 110 mm for red gurnard, 180 mm for tub gurnard and
147 100 mm for grey gurnard. Data is summarized in Table I. Depth distribution of the specimens
148 belonging to the three species was analysed by Pearson's Chi-squared test in order to test the
149 significance of the relationship between size classes (juveniles and adults) and depth (3 depth
150 strata considered: 0-50 m; 50-100 m; 100- 250 m). Differences in species niche depth were
151 compared using a Kruskal-Wallis one-way analysis of variance on ranks and a *post hoc* all
152 pairwise multiple comparison procedure using Dunn's method.

153 Samples were dissected and stomachs were removed and preserved in 70% ethanol for
154 analysis of stomach content. Gut contents were analysed with a stereoscopic microscope
155 identifying each prey to the lowest taxonomic level possible by using specialised keys. Prey
156 types were counted (Np) and weighed (Wp) after removal of excess moisture. Diets were

157 expressed as percentage abundance (Cn%) by prey item for each dietary category, percentage
158 by weight (Cw%) and percentage by frequency of occurrence (F%) being understood as the
159 proportion of non-empty stomachs containing a given prey item or category. According to
160 N'Da (1992), prey items were considered dominant when Cn% > 50%; secondary prey with
161 values of Cn% ranging from 10% - 50%; accidental prey when Cn% < 10%. The main food
162 items were identified using the index of relative importance:

163 $IRI_i = F\% (Cn\% + Cw\%)$ and the modified form

164 $IRI\% = (IRI_i / \sum IRI_i) \times 100$

165 These indexes were calculated for each prey category and used in diet comparisons (Pinkas *et*
166 *al.*, 1971; Cortés, 1997). Data was sorted on the basis of the decreasing value of IRI%.
167 Ontogenetic diet change analysis was carried out on two size classes: juveniles and adults.
168 The presence of an ontogenetic shift in the diet was explored using a hierarchical cluster
169 analysis based on numerical abundance Bray–Curtis dissimilarity (Clarke & Warwick, 1994).
170 Data was transformed by Wisconsin double standardization (square root transformation) and
171 then analysed by non metric multidimensional scaling (nMDS) in relation to size class and
172 depth. Regression fit goodness (Stress *S*) was determined on the basis of the sum of squared
173 differences between ordination-based distances and predicted distances. Low Stress *S* values
174 indicated goodness of fit. For the analysis of trophic niche breadth, the normalized version of
175 the Levins (B_i) index (Hulbert, 1978) was used:

176 $B_i = 1/n - 1 \{ (1/\sum p_{ij}^2) - 1 \}$,

177 where p_{ij} is the proportion of prey *j* in the diet of predator *i*, and *n* is the number of prey
178 categories. B_i was expressed on a scale from 0 to 1.0 (with a low value indicating a specialist
179 predator with a diet dominated by few prey items, and a high value a generalist predator) and
180 calculated for each size class.

181 Species diversity in prey number was calculated using the Shannon-Wiener index (H') and the
182 evenness measure of the Shannon-Wiener function (J') (Colwell & Futuyma, 1979). The
183 Shannon-Wiener formula was expressed as:

$$184 \quad H' = - \sum p_j \log p_j,$$

185 where p_j is the proportion of individuals found in or using resource j . A high H' value
186 indicates feeding on a higher spectrum width of prey-items. As Shannon-Wiener measures
187 may range from 0 to ∞ , an evenness measure (J') was calculated so as to standardize H' on a
188 0 to 1 scale:

$$189 \quad J' = H' / \log n,$$

190 where n is the total number of possible resource states. A high J' indicates a specimen feeding
191 on a relatively larger number of a few main prey types (Shuozeng, 1995).

192 The simplified Morisita index (C_H) proposed by Horn (1966) was used to investigate intra-
193 and inter-specific niche overlap among size classes and species. This index ranges from 0 (no
194 resource used in common) to 1.0 (full overlap), with values > 0.6 being considered to be
195 indicative of a biologically significant overlap (Pusey & Bradshaw, 1996). Morisita's measure
196 was expressed as:

$$197 \quad C_H = 2 \sum p_{ij} p_{ik} / \sum p_{ij}^2 + \sum p_{ik}^2 \quad ,$$

198 where p_{ij} is the corresponding proportion of resource i in the total resource used by species j
199 and p_{ik} is the corresponding proportion of resource i in the total resource used by species k .

200 Multivariate analysis was conducted using the R software version 2.13.2 base and Vegan
201 package for community ecology.

202

203

RESULTS

204 Significant differences were found as to distribution by depth (Pearson's Chi-squared
205 test with Yates' continuity correction: χ -squared = 84.9339, df = 1, p-value < 0.001). More
206 precisely, these significant differences were found by comparing all possible size class pairs

207 according to the *post-hoc* Dunn test (p -value < 0.001) in species niche depth (Kruskal-Wallis
208 test; p -value < 0.001) both among species (inter-specific differences) and between juveniles
209 and adults within the same species (intra-specific differences).

210 Specimens of *A. cuculus* were generally encountered at greater depths, while *C. lucerna*
211 was more common at shallow depths near the coast. Juveniles were found at shallower waters
212 than adults in all three gurnard species as adults were seen to consistently migrate to deeper
213 depths (Fig. 2). Sixteen food items were identified in the diet of *A. cuculus* and classified into
214 four main categories: Crustacea, Teleostei, Mollusca and Echinodermata (Table II). Almost
215 the entire diet of this species was observed to be based on crustaceans (%Cn = 93.88; %Cw =
216 91.15; %IRI = 98.26) while other taxa were classified as accessory prey. Sixty different preys
217 belonging to five main groups (Crustacea, Teleostei, Mollusca, Anellida and Bryozoa) were
218 identified in the stomachs of *C. lucerna* (Table II). Crustaceans were the most abundant in
219 number and weight with a high relative importance index value (%Cn = 90.0; %Cw = 58.11;
220 %IRI = 96.7). Teleostei were considered secondary prey in the diet of tub gurnard, with a
221 sensible contribution in terms of weight and frequency of occurrence (%Cw = 40.12; %F =
222 31.35). Molluscs, annelids and bryozoa were recorded as accidental prey. Thirty-four
223 categories of prey items were identified in the stomachs of *E. gurnardus* and were classified
224 into five main groups: Crustacea, Teleostei, Mollusca, Nematoda and Echinodermata (Table
225 II). As for the other two gurnard species, and even for grey gurnard, diet was found to be
226 essentially made up of crustaceans (%Cn = 93.65; %W = 61.25; %IRI = 98.5). Examination
227 of the collected specimens did not reveal any sign of regurgitation.

228 In all the three species studied, diet was influenced by predator “critical sizes” marking off
229 juvenile from adult individuals. On the basis of these sizes, the feeding habits of adults were
230 seen to be similar to those of juveniles in all three species; in fact, the epibenthic and necto-
231 benthic invertebrates were the dominant group for all sizes considered. The index of relative
232 importance of Crustacea reached high values ranging from 88.35 to 99.52.

233 For *A. cuculus*, *Lophogaster typicus* (Lophogastrida) was the most abundant and preferred
234 prey as shown by its almost exclusive presence both in the diet of juveniles and adults of red
235 gurnard (%IRI = 97.08 - %IRI = 97.81, respectively) (Table III). Crustacea *Philocheras* sp.
236 (Decapoda, Macrura-Natantia) was the dominant prey (%IRI = 79.99) of juveniles of *C.*
237 *lucerna*, while adults of this species were found to predominantly feed on *Goneplax*
238 *rhomboides* (Decapoda, Brachyura) (%IRI = 80.63). Seventeen different species of Teleostei
239 were found in the diet of adults of tub gurnard and were considered secondary preys (%Cn =
240 16.86). Anchovy *Engraulis encrasicolus* and gobid *Gobius niger* gave a quite good
241 contribution in terms of weight (%Cw = 12.98; %Cw = 10.29) (Table III). For *E. gurnardus*,
242 *L. typicus* is the preferred prey for both ontogenetic groups (%IRI = 64.31 - %IRI = 47.46,
243 respectively). *Liocarcinus* sp. (Decapoda, Brachyura), *Solenocera membranacea* (Decapoda,
244 Macrura-Natantia) and *Acanthomysis longicornis* (Mysida) occurred quite frequently in the
245 diet of juveniles of grey gurnards, being subsequently replaced by *G. rhomboides* in the diet
246 of the adults of this species (Table III).

247 The highest values of species diversity, $H' = 2.24$, and an evenness index of $J' = 0.79$, were
248 found for the juveniles of *E. gurnardus*. These values were quite similar to those found for the
249 adults of *C. lucerna* and *E. gurnardus*, that is $H' = 2.26$ and $J' = 0.56$ and $H' = 2.09$ and $J' =$
250 0.62 , respectively. For the other ontogenetic groups (juveniles and adults of *A. cuculus* and
251 juveniles of *C. lucerna*) low species diversity values were recorded ($H' < 1.50$; $J' < 0.60$). As
252 to niche breadth, Bi values were found to be low for all the three gurnard species considered,
253 ranging from 0.03 to 0.36 (Table IV). Such values point to a specialized feeding behaviour
254 trend.

255 Intra-specific feeding competition was observed between juvenile and adult individuals of
256 *A. cuculus* and *E. gurnardus*. In fact, as may be seen in Table V, Morisita index values are
257 high in both species ($C = 0.991$ for *A. cuculus*, and $C = 0.843$ for *E. gurnardus*). Vice versa,
258 very low intra-specific competition levels were recorded for *C. lucerna* ($C = 0.186$).

259 Intraspecific multidimensional analysis does not show up any difference between juveniles
260 and adults. The nMDS plots in Figure 3a,b,c show a very closed centroid defining the
261 structure with the minimum total base-pair distance to all prey-species in the set between the
262 two size classes considered. Stress S values are < 0.05 . Inter-specific niche overlap appears
263 quite relevant only between juveniles and adults of *A. cuculus* and *E. gurnardus* (C range =
264 0.596 – 0.696). Low levels of inter-specific competition show the trophic niche of *C. lucerna*
265 to be different from those of the other two gurnards (C range = 0.249 – 0.589) (Table V).
266 Inter-specific multidimensional analysis (nMDS) highlights three different groups within the
267 same species: juveniles and adults of each species were grouped together, reflecting greater
268 intra- (juvenile/adult) than inter-specific competition (Fig. 4). This trend was further borne out
269 by the non metrical multidimensional analysis of the size-depth relationship; in fact, same-
270 species clusters were tendentially found also by this type of analysis (Fig. 5).

271

272

DISCUSSION

273 Our study highlights the importance of investigating dietary habits of increasingly
274 exploited and poorly known species, such as gurnards from the Adriatic Sea, for the purpose
275 of quantifying their ecological role in the marine community. In particular, the study focuses
276 on the role of fish body size and depth distribution in ontogenetic dietary shift and food
277 resource partitioning of these demersal species. Before analyzing gurnards' spatial
278 distribution and feeding habits, the critical size differentiating juvenile from adult individuals
279 was established in order to identify any difference in feeding patterns during growth. Our
280 findings are in line with those of studies conducted in other Mediterranean areas where length
281 upon sexual maturity onset was found to correspond to a change in diet (Moreno-Amich,
282 1992; Colloca *et al.*, 1994; Boudaya *et al.*, 2008).

283

284 As to the present study in the Adriatic Sea, lengths upon onset of sexual maturity were
found to be 110 mm for *A. cuculus*; 180 mm for *C. lucerna*; 100 for *E. gurnardus*. At these

285 critical sizes, attained within the first year, individuals of all three species were seen to
286 migrate to greater depths and to change their dietary habits, increasing in particular the variety
287 of food items eaten (Vallisneri *et al.*, 2012).

288 Spatial distribution for all three gurnard species considered, namely *Aspitrigla cuculus*,
289 *Chelidonichthys lucerna* and *Eutrigla gurnardus*, exhibited a depth range similar to that
290 reported for other areas of the Mediterranean Sea, reaching as far down as 250 metres and
291 being limited to the continental shelf and shelf break (Kartas, 1971; Matallanas, 1979;
292 Papacostantinou, 1983; Colloca *et al.*, 1994; Serena *et al.*, 1998; Terrats *et al.*, 2000;
293 Katsanevakis & Maravelias, 2009). The three species cohabit the same area but differ in terms
294 of bathymetrical distribution. Inter-specifically, the narrowest bathymetric distribution,
295 mostly above 100 metres, was reported for *A. cuculus* while greatest relative abundance was
296 recorded for *C. lucerna* at between 0 and 50 metres. Intra-specifically, a species-specific
297 distribution pattern associated with nursery-recruitment patterns and individual size growth
298 was found for each gurnard species in accordance with Colloca *et al.* (1994). A spatial
299 separation between juveniles and adults was observed for the three gurnard species, with
300 adults tending to be sampled at greater depths than juveniles. Although significant in all cases,
301 juvenile-adult spatial separation was found to be most pronounced in the case of *C. lucerna*.
302 In fact, our findings in this regards are in line with those of other studies where this size class
303 and depth relationship for tub gurnard has also been reported, with younger individuals being
304 more frequently found in shallow waters and adults more dispersed towards off shore sites,
305 and consistent nursery area being identified along the coast (Frogliia, 1976; Papacostantinou,
306 1983; Tsimenides *et al.*, 1992; Colloca *et al.*, 1994; Serena *et al.*, 1998; Boudaya *et al.*, 2008).

307 The trophic niches occupied are different in spatial distribution but not in terms of prey
308 item composition. In fact, gurnards feed mainly on Crustacea, this prey group being both
309 inter- and intra-specifically predominant for the two maturity levels considered (juveniles and
310 adults). The stomachs of the larger individuals contained most of the prey taxa also fed on by

311 the smaller individuals, although in different proportions. This finding is in line with
312 Labradopoulou & Machias (1998) according to whom dietary differences reflect changes in
313 food preferences depending on size, given that larger individuals are capable of capturing
314 larger and more elusive preys. Other prey taxa represent secondary or accessory prey, being
315 important at some period in the gurnards' ontogenetic development. Our results are in
316 agreement with those of many other authors (Frogliola, 1976; Papacostantinou, 1983; Moreno-
317 Amich, 1992; Colloca *et al.*, 1994; Lopez-Lopez *et al.*, 2011). Inter-specifically, although all
318 the three species considered were seen to exhibit a crustacivorous feeding behaviour, on the
319 basis of prey species composition and major food item relative importance index they appear
320 to have distinct feeding preferences, a finding in line with Colloca *et al.* (1994), Morte *et al.*
321 (1997), Lopez-Lopez *et al.* (2011). In our study, multivariate analysis that distinguishes the
322 three species according to type of prey while also taking into consideration different depth
323 strata, further confirms this finding. In fact, *A. cuculus* was found to feed almost exclusively
324 on necto-benthic invertebrates; *C. lucerna* mostly on epibenthic crustacea and necto-benthic
325 teleost fish; *E. gurnardus* on a mixed diet of epibenthic, benthic-pelagic and necto-benthic
326 preys.

327 For all species examined, ontogenetic dietary shift showed that the number of prey items in
328 the gut increased with increasing predator body size. Ontogenetic diet shifts are often
329 explained as a reflection of the changing abilities of fish; essentially, as fish grow they
330 become more proficient at handling larger prey that are more profitable. Increasing prey size
331 usually leads to taxonomic changes in the diet. Migratory patterns associated with
332 reproduction and feeding patterns have also been reported for gurnards, and the occupation of
333 new niches may result in differences in diet (Papacostantinou, 1983; Colloca *et al.*, 1994).
334 The demersal migration undertaken by adults allows them to feed on a larger variety of prey
335 species (more than twice the number found in the diet of juveniles). The higher number of
336 different items consumed by adult as compared to juvenile individuals does not, however,

337 correspond to a higher diversity index. In fact, this index value is high only in the case of *C.*
338 *lucerna* while in the case of *E. gurnardus* and *A. cuculus* diversity values are greater in
339 juveniles, a finding that highlights species-specific differences. This result suggests that
340 individual specialization may occur within a predator population, lending support to Bolnick
341 *et al.*'s conclusion (2007) that the "more generalized the population, the higher the level of
342 individual specialization", as also reported in the case of the opportunistic feeding behaviour
343 observed for *E. gurnardus* from the North Sea (Weinert *et al.*, 2010). The low niche-breadth
344 index value shows that juvenile and adult gurnard diet is dominated by a limited variety of
345 prey items; however, the feeding behaviour in both size-classes of *C. lucerna* and *E.*
346 *gurnardus* differs from that of *A. cuculus* as the former were found to feed more on
347 epibenthic, benthopelagic and necto-benthic preys. In view of these findings, tub gurnard and
348 grey gurnard in the Adriatic area considered may be better classified as opportunistic-
349 generalist predators, regardless of size class, while red gurnard may be classified as a
350 specialist feeder, in line with the literature on this species (Colloca *et al.*, 1994; Morte *et al.*,
351 1997; Colloca *et al.*, 2010). Niche breadth is also affected by local resource availability
352 (Colloca *et al.*, 1994). Four crustaceans were found to be present in the stomachs of gurnard
353 individuals of all size classes considered: *Goneplax rhomboides*, *Liocarcinus* sp., *Alpheus*
354 *glaber* and *Solenocera membranacea*. These crustaceans are in fact among the most common
355 prey items for both fish and other decapod crustaceans in the muddy bottom communities of
356 the lower continental shelf and upper slope of the western Mediterranean Sea (Rufino *et al.*,
357 2006).

358 In view of the foregoing, it may therefore be concluded that prey item distribution,
359 predator size range and depth distribution are the main variables affecting trophic niche
360 breadth and hence the relative differences encountered among the Adriatic gurnards
361 considered in the present study. No intra-specific juvenile-adult difference (high values) in the
362 trophic niche overlap index was however observed by us for *A. cuculus* and *E. gurnardus*,

363 leading us to conclude that both size groups feed on similar preys. A low value for the trophic
364 niche overlap index was instead reported in the case of *C. lucerna*, thus pointing to the fact
365 that this species colonizes different trophic niches during its ontogenetic growth. An inter-
366 specific comparison showed partial competition among all size-groups of *A. cuculus* and *E.*
367 *gurnardus* (fairly high values) and adults of *C. lucerna* and *E. gurnardus*. These findings
368 suggest habitat partitioning mainly at the interspecific and trophic segregation/competition at
369 the intra-specific level, in line with studies on Atlantic gurnards (Lopez-Lopez *et al.*, 2011).
370 According to Amorim & Hawkins (2004), smaller grey gurnards compete for food by contest
371 tactics whereas larger specimens predominantly scramble for food, probably because their
372 larger body size affords them an advantage in locating, capturing and handling prey. Food
373 resource availability depends not only on prey abundance, but also on the interaction of other
374 factors, including prey size, micro-distribution, capture success and speed of movement.

375 The present quali-quantitative study of trophic ontogeny of gurnards offers a good basis on
376 which to develop adequate fishery management practices for marine communities in the
377 north-middle Adriatic Sea. Moreover, its findings may be useful for ecological modelling
378 aimed at better representing the trophic flows associated with possible changes in feeding
379 habits during ontogeny.

380

381

382 **Acknowledgements**

383 We thank Professor Corrado Piccinetti for his assistance in the bottom trawl surveys and for
384 his valuable suggestions in the drafting of the paper. We also thank Dr. Alessandra Anibaldi
385 and Dr. Claudia Benassi Franciosi for their precious help regarding MEDITS surveys and for
386 biometric parameter measurements.

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Table I. Summary of some biological and ecological parameters relating to *A. cuculus*, *C. lucerna* and *E. gurnardus* from north-middle Adriatic Sea. sampled between 2005-2009.

For each gurnard, samples were classified into two ontogenetic groups: juvenile and adult

	<i>Aspitrigla cuculus</i>		<i>Chelidonichthys lucerna</i>		<i>Eutrigla gurnardus</i>	
	Juveniles	Adults	Juveniles	Adults	Juveniles	Adults
N° of fish examined	118	272	325	648	138	317
TL range (mm)	72 - 113	111 - 262	63 - 214	175 - 415	62 - 102	101 - 264
W range (g)	2.8 - 12.7	9.6 - 180.5	2.2 - 95.2	49.7 - 650.9	2.0 - 9.0	7.3 - 166.4
Depth range (m)	55.25 - 176.5	82.3 - 246	13.55 - 94.05	12.4 - 232	37.15 - 170	37.15 - 181.5

Table II. Diet analysis of the main taxa preyed by *A. cuculus*, *C. lucerna* and *E. gurnardus* in the north-middle Adriatic Sea

	<i>Aspitrigla cuculus</i>						<i>Chelidonichthys lucerna</i>						<i>Eutrigla gurnardus</i>					
	Np	Wp	%Cn	%Cw	%F	%IRI	Np	Wp	%Cn	%Cw	%F	%IRI	Np	Wp	%Cn	%Cw	%F	%IRI
CRUSTACEA	935	49.42	93.88	91.15	76.98	98.26	5816	842.56	90.0	58.11	85.2	96.7	1505	29.79	93.65	61.25	70.77	98.5
TELEOSTEI	10	1.83	1.0	3.38	1.79	0.03	444	581.79	6.87	40.12	31.35	2.93	43	11.95	2.68	24.57	8.79	0.53
MOLLUSCA	8	0.16	0.8	0.3	1.54	0.02	112	8.77	1.73	0.6	7.3	0.16	39	4.2	2.43	8.63	6.37	0.27
ECHINODERMATA	3	0.15	0.3	0.28	0.77	<0.01	–	–	–	–	–	–	1	<0.01	0.06	<0.01	0.22	<0.01
ANELLIDA	–	–	–	–	–	–	2	0.18	0.03	0.01	0.21	<0.01	–	–	–	–	–	–
NEMATODA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
BRYOZOA	–	–	–	–	–	–	1	0.02	0.02	<0.01	0.1	<0.01	7	0.01	0.44	0.02	1.1	0.01
UNIDENTIFIED REMAINS	–	2.65	4.02	4.89	30.26	1.69	–	16.66	1.35	1.15	12.02	0.2	–	2.69	0.75	5.53	44.62	0.69

Np = number of prey types; Wp = weight of prey types; Cn% = percentage by number; Cw% = percentage by weight; F% = frequency of occurrence; IRI% = percentage of index of relative importance

Table III. Diet analysis by ontogenetic group of *A. cuculus*, *C. lucerna* and *E. gurnardus* from north-middle Adriatic Sea.

	<i>ASPITRIGLA CUCULUS</i>						<i>CHELIDONICHTHYS LUCERNA</i>						<i>EUTRIGLA GURNARDUS</i>					
	Juveniles			Adults			Juveniles			Adults			Juveniles			Adults		
	%Cn	%Cw	%IRI	%Cn	%Cw	%IRI	%Cn	%Cw	%IRI	%Cn	%Cw	%IRI	%Cn	%Cw	%IRI	%Cn	%Cw	%IRI
CRUSTACEA	97.62	96.35	99.12	92.61	90.51	97.92	97.4	83.38	99.52	78.03	56.15	88.35	97.35	81.05	96.82	92.2	64.88	98.06
Lophogastrida	62.96	70.45	79.73	70.21	50.12	77.88	–	–	–	0.22	0.03	<0.01	21.7	47	16.17	27.56	20.2	15.65
<i>Lophogaster typicus</i>	72.34	74.11	97.08	83.64	68.95	97.81	–	–	–	0.29	0.03	0.01	29.2	57.7	64.31	32.22	21.96	47.46
Decapoda	31.48	26.18	20.06	24.56	43.81	21.64	98.21	86.6	99.91	82.38	57.87	95.76	50.94	47.57	76.35	57.19	49.0	81.86
<i>Goneplax rhomboides</i>	6.38	1.74	0.75	7.83	5.65	1.87	3.84	30.15	2.7	38.61	29.17	80.63	8.76	8.76	6.83	26.15	23.65	43.01
<i>Philocheras bispinosus</i>	–	–	–	–	–	–	20.82	9.53	8.7	1.49	0.16	0.08	8.76	3.43	5.08	10.25	1.58	2.98
<i>Liocarcinus depurator</i>	–	–	–	1.15	2.79	0.01	4.4	15.62	1.75	11.59	20.91	7.34	0.73	0.29	0.05	2.51	7.31	0.95
<i>Liocarcinus</i> sp.	8.51	2.4	0.75	1.15	2.00	0.06	8.02	11.75	6.74	4.59	3.72	1.18	11.68	2.7	8.46	5.65	2.82	1.76
<i>Alpheus glaber</i>	2.13	1.81	0.11	1.15	2.07	0.06	0.27	3.33	0.03	2.24	0.86	0.48	1.46	1.06	0.25	2.09	6.46	0.73
<i>Philocheras</i> sp.	–	–	–	–	–	–	61.21	16.51	79.99	4.59	0.16	0.63	2.92	1.24	0.43	5.65	0.68	1.36
<i>Solenocera membranacea</i>	6.38	3.87	0.80	0.92	7.33	0.08	0.27	0.94	0.02	1.32	1.44	0.13	13.87	13.72	8.08	1.05	1.51	0.16
<i>Processa</i> sp.	–	–	–	–	–	–	0.11	0.49	<0.01	18.65	1.78	7.56	2.92	0.58	0.57	2.72	0.2	0.19
<i>Squilla mantis</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Corystes cassivellanus</i>	–	–	–	–	–	–	–	–	–	0.4	1.87	0.02	–	–	–	–	–	–
<i>Munida</i> sp.	–	–	–	–	–	–	–	–	–	0.06	0	0	–	–	–	0.42	3.54	0.03
<i>Pontophilus</i> sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.21	1.43	0.01
<i>Pontophilus spinosus</i>	–	–	–	–	–	–	0.05	0.22	<0.01	0.06	0.07	<0.01	–	–	–	0.21	1.07	0.01
<i>Callianassa</i> sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.21	0.27	0.01
<i>Jaxea nocturna</i>	–	–	–	–	–	–	0.03	0.13	<0.01	0.57	0.5	0.04	0.73	2.66	0.15	–	–	–
<i>Philocheras echinulatus</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.21	0.14	0.01
<i>Philocheras trispinosus</i>	2.13	7.94	0.35	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Ebalia</i> sp.	–	–	–	0.46	0.58	0.01	–	–	–	–	–	–	–	–	–	–	–	–
<i>Ebalia granulosa</i>	–	–	–	–	–	–	0.03	0.04	<0.01	–	–	–	–	–	–	–	–	–
<i>Chlorotocus crassicornis</i>	2.13	8.14	0.36	0.46	1.08	0.01	–	–	–	0.11	0.05	<0.01	–	–	–	–	–	–
<i>Plesionika</i> sp.	–	–	–	0.46	0.31	0.01	–	–	–	–	–	–	–	–	–	–	–	–
<i>Pirimela denticulata</i>	–	–	–	0.23	1.19	<0.01	–	–	–	–	–	–	–	–	–	–	–	–
<i>Macropipus tuberculatus</i>	–	–	–	0.23	0.84	<0.01	–	–	–	–	–	–	–	–	–	–	–	–
<i>Liocarcinus maculatus</i>	–	–	–	–	–	–	0.05	0.67	<0.01	0.57	0.37	0.03	–	–	–	–	–	–
<i>Liocarcinus corrugatus</i>	–	–	–	0.23	0.26	<0.01	–	–	–	–	–	–	–	–	–	–	–	–
<i>Liocarcinus vernalis</i>	–	–	–	–	–	–	–	–	–	0.23	0.09	<0.01	–	–	–	–	–	–
<i>Liocarcinus pusillus</i>	–	–	–	–	–	–	–	–	–	0.06	0.02	<0.01	–	–	–	–	–	–
<i>Galathea intermedia</i>	–	–	–	0.23	0.02	<0.01	–	–	–	–	–	–	2.19	1.24	0.17	0.21	0.04	0.01
<i>Galathea dispersa</i>	–	–	–	–	–	–	–	–	–	0.06	0.01	<0.01	–	–	–	–	–	–

<i>Galathea</i> sp.	–	–	–	1.15	0.49	0.03	–	–	–	–	–	–	2.92	1.86	0.48	–	–	–
<i>Macropodia</i> sp.	–	–	–	–	–	–	–	–	–	0.86	0.16	0.02	–	–	–	–	–	–
<i>Upogebia</i> sp.	–	–	–	–	–	–	–	–	–	0.23	0.29	<0.01	–	–	–	–	–	–
<i>Sicyonia carinata</i>	–	–	–	–	–	–	–	–	–	0.17	0.09	<0.01	–	–	–	–	–	–
<i>Brachynotus</i> sp.	–	–	–	–	–	–	–	–	–	0.11	0.05	<0.01	–	–	–	–	–	–
<i>Pisidia</i> sp.	–	–	–	–	–	–	–	–	–	0.11	0.02	<0.01	–	–	–	–	–	–
<i>Pisidia longimana</i>	–	–	–	–	–	–	–	–	–	0.06	0.02	<0.01	–	–	–	–	–	–
<i>Parapenaeus longirostris</i>	–	–	–	–	–	–	0.03	0.04	<0.01	0.06	0.15	<0.01	–	–	–	–	–	–
<i>Penaeus kerathurus</i>	–	–	–	–	–	–	0.03	0.01	<0.01	–	–	–	–	–	–	–	–	–
<i>Pachygrapsus marmoratus</i>	–	–	–	–	–	–	0.54	3.33	0.06	0.11	0.04	<0.01	–	–	–	–	–	–
<i>Processa modica</i>	–	–	–	–	–	–	–	–	–	0.06	0	<0.01	–	–	–	–	–	–
 Mysida	–	–	–	0.58	0.45	0.02	–	–	–	–	–	–	13.68	3.05	4.26	9.19	0.54	1.62
<i>Acanthomysis longicornis</i>	–	–	–	–	–	–	–	–	–	–	–	–	10.22	2.37	4.71	5.65	0.28	0.67
<i>Gastrosaccus sanctus</i>	–	–	–	–	–	–	–	–	–	–	–	–	0.73	0.15	0.05	0.42	0.02	0.01
<i>Gastrosaccus</i> sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.21	0.02	<0.01
 Stomatopoda	1.85	2.78	0.13	–	–	–	0.05	0.32	1.51	1.26	3.1	0.08	–	–	–	–	–	–
<i>Squilla mantis</i>	–	–	–	–	–	–	0.05	0.35	<0.01	1.26	2.99	0.2	–	–	–	–	–	–
 Isopoda	1.85	0.19	0.04	3.48	0.86	0.43	–	–	–	0.18	0.12	<0.01	0.47	0.14	0.01	–	–	–
 Tanaidacea	–	–	–	–	–	–	0.49	0.13	0.02	–	–	–	–	–	–	–	–	–
 Euphausiacea	–	–	–	–	–	–	–	–	–	–	–	–	0.47	1.11	0.02	–	–	–
<i>Meganyctiphanes norvegica</i>	–	–	–	–	–	–	–	–	–	–	–	–	0.73	1.42	0.1	–	–	–
 Amphipoda	–	–	–	0.58	0.07	0.01	0.49	0.04	0.02	0.81	0	0.02	11.79	0.54	3.13	1.39	0.04	0.1
<i>Ampelisca</i> sp.	–	–	–	–	–	–	–	–	–	–	–	–	0.73	0.07	0.04	–	–	–
<i>Leucothoe</i> sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.21	<0.01	<0.01
 Cumacea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.17	<0.01	<0.01
 TELEOSTEI	0.40	0.22	<0.01	1.21	3.77	0.04	0.7	13.18	0.08	16.86	42.21	11.0	0.88	1.35	0.04	3.38	30.06	0.93
 Clupeiformes	–	–	–	–	–	–	–	–	–	1.66	11.46	0.14	–	–	–	–	–	–
<i>Engraulis encrasicolus</i>	–	–	–	–	–	–	–	–	–	2.12	12.98	0.59	–	–	–	–	–	–
 Perciformes	1.85	0.41	0.05	0.39	4.35	0.02	0.54	10.71	0.05	10.47	22.42	3.92	–	–	–	2.25	18.29	0.6
<i>Gobius niger</i>	–	–	–	–	–	–	0.03	1.76	<0.01	2.07	10.29	0.49	–	–	–	1.05	11.34	0.4
<i>Lesuerigobius friesii</i>	–	–	–	–	–	–	0.03	0.19	<0.01	1.61	1.72	0.22	–	–	–	–	–	–
<i>Cepola rubescens</i>	–	–	–	–	–	–	–	–	–	0.46	2.28	0.03	–	–	–	–	–	–
<i>Lesuerigobius suerii</i>	–	–	–	–	–	–	0.05	0.01	<0.01	0.69	0.87	0.04	–	–	–	–	–	–
<i>Callionymus</i> sp.	–	–	–	0.46	5.99	0.03	–	–	–	0.11	0.13	<0.01	–	–	–	0.84	2.95	0.14
<i>Gobius</i> sp.	–	–	–	–	–	–	–	–	–	0.23	0.52	0.01	–	–	–	–	–	–
<i>Callionymus maculatus</i>	–	–	–	–	–	–	–	–	–	0.23	0.35	0.01	–	–	–	0.21	2.54	0.02
<i>Deltentosteus quadrimaculatu.</i>	–	–	–	–	–	–	–	–	–	0.11	0.15	<0.01	–	–	–	–	–	–
<i>Pomatoschistus minutus</i>	–	–	–	–	–	–	–	–	–	0.06	0.01	<0.01	–	–	–	–	–	–

<i>Serranus hepatus</i>	-	-	-	-	-	-	0.03	3.41	<0.01	0.06	0.51	<0.01	-	-	-	-	-	-
<i>Callionymus risso</i>	-	-	-	-	-	-	0.03	0.17	<0.01	0.17	0.15	<0.01	-	-	-	-	-	-
Gadiformes	-	-	-	-	-	-	0.03	0.91	<0.01	0.45	2.98	0.01	-	-	-	-	-	-
<i>Trisopterus minutus</i>	-	-	-	-	-	-	-	-	-	0.29	2.11	0.01	-	-	-	-	-	-
<i>Merlangius merlangus</i>	-	-	-	-	-	-	-	-	-	0.06	0.93	<0.01	-	-	-	-	-	-
<i>Merluccius merluccius</i>	-	-	-	-	-	-	-	-	-	0.06	0.24	<0.01	-	-	-	-	-	-
<i>Gaidropsarus biscayensis</i>	-	-	-	-	-	-	0.03	0.99	<0.01	0.17	0.09	<0.01	-	-	-	-	-	-
Pleuronectiformes	-	-	-	-	-	-	0.05	0.74	<0.01	0.27	1.41	<0.01	-	-	-	1.04	11.8	0.14
<i>Arnoglossus laterna</i>	-	-	-	-	-	-	-	-	-	0.23	1	0.01	-	-	-	0.21	10.04	0.05
<i>Microchirus variegatus</i>	-	-	-	-	-	-	0.03	0.33	<0.01	-	-	-	-	-	-	-	-	-
MOLLUSCA	-	-	-	1.08	0.34	0.03	0.23	0.63	0.01	4.18	0.6	0.57	1.1	0.49	0.06	2.95	0.45	0.31
Bivalvia	-	-	-	-	-	-	0.05	0.02	<0.01	1.16	0.31	0.03	-	-	-	1.04	0.12	0.02
<i>Corbula gibba</i>	-	-	-	-	-	-	0.05	0.02	<0.01	1.09	0.29	0.13	-	-	-	-	-	-
<i>Tellina</i> sp.	-	-	-	-	-	-	-	-	-	0.11	0.01	<0.01	-	-	-	0.42	0.09	0.02
<i>Mactra corallina</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.21	0.03	0.01
<i>Tapes decussatus</i>	-	-	-	-	-	-	-	-	-	0.06	0.01	<0.01	-	-	-	-	-	-
<i>Arca tetragona</i>	-	-	-	-	-	-	-	-	-	0.11	0.02	<0.01	-	-	-	-	-	-
<i>Anadara demiri</i>	-	-	-	-	-	-	-	-	-	0.11	0.01	<0.01	-	-	-	-	-	-
<i>Saccella commutata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.21	0.01	<0.01
<i>Hyalopecten similis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.21	0.01	<0.01
<i>Flexopecten glaber proteus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.21	<0.01	<0.01
Gastropoda	-	-	-	-	-	-	0.03	0.02	<0.01	0.89	0.23	0.02	0.94	0.57	0.05	0.17	0.01	<0.01
<i>Turritella communis</i>	-	-	-	-	-	-	-	-	-	0.8	0.23	0.08	1.46	0.73	0.22	-	-	-
<i>Epitonium</i> sp.	-	-	-	-	-	-	-	-	-	0.06	0.03	<0.01	-	-	-	-	-	-
<i>Nassarius</i> sp.	-	-	-	-	-	-	-	-	-	0.17	0.01	<0.01	-	-	-	-	-	-
<i>Nassarius pygmaeus</i>	-	-	-	-	-	-	-	-	-	0.06	0.01	<0.01	-	-	-	-	-	-
<i>Odostomia acuta</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.21	0.01	<0.01
<i>Acteon tornatilis</i>	-	-	-	-	-	-	-	-	-	0.06	<0.01	<0.01	-	-	-	-	-	-
Cephalopoda	-	-	-	-	-	-	0.08	0.52	<0.01	0.013	0.06	<0.01	-	-	-	-	-	-
ECHINODERMATA	0.40	0.02	<0.01	0.27	0.32	<0.01	-	-	-	-	-	-	-	-	-	0.09	<0.01	<0.01
Regularia	-	-	-	0.19	0.33	<0.01	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cidaris cidaris</i>	-	-	-	0.23	0.46	<0.01	-	-	-	-	-	-	-	-	-	-	-	-
ANELLIDA	-	-	-	-	-	-	-	-	-	0.08	0.01	<0.01	-	-	-	-	-	-
Aciculata - Phyllococida	-	-	-	-	-	-	-	-	-	0.04	0.01	<0.01	-	-	-	-	-	-
<i>Aphrodita aculeata</i>	-	-	-	-	-	-	-	-	-	0.06	0.01	<0.01	-	-	-	-	-	-
Sternaspida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Sternaspis scutata</i>	-	-	-	-	-	-	-	-	-	0.06	0.01	<0.01	-	-	-	-	-	-
NEMATODA	-	-	-	-	-	-	-	-	-	-	-	-	0.22	0.02	<0.01	0.52	0.02	0.01

BRYOZOA	-	-	-	-	-	-	-	-	-	0.04	<0.01	<0.01	-	-	-	-	-	-
UNIDENTIFIED REMAINS	1.59	3.42	0.87	4.84	5.07	2.00	1.68	2.8	0.39	0.81	1.02	0.09	0.44	17.09	3.07	0.87	4.59	0.68

Cn% = percentage by number; Cw% = percentage by weight; IRI% = percentage of index of relative importance

Table IV. Indexes of Levins (Bi), Shannon-Wiener (H'), evenness measure (J') for the diet of each ontogenetic group (juvenile and adult) of *A. cuculus*, *C. lucerna* and *E. gurnardus*

	<i>A. cuculus</i>			<i>C. lucerna</i>			<i>E. gurnardus</i>		
	Bi	H'	J'	Bi	H'	J'	Bi	H'	J'
Juveniles	0.14	1.04	0.53	0.06	1.20	0.38	0.36	2.24	0.79
Adults	0.03	0.77	0.28	0.07	2.26	0.56	0.15	2.09	0.62

Table V. Morisita's index of dietary overlap for each ontogenetic group (juvenile and adult) of *A. cuculus*, *C. lucerna* and *E. gurnardus*

	AspJuv	AspAdu	CheJuv	CheAdu	EutJuv	EutAdu
AspJuv	1					
AspAdu	0.982**	1				
CheJuv	0.020	0.008	1			
CheAdu	0.086	0.077	0.174	1		
EutJuv	0.686*	0.596	0.172	0.291	1	
EutAdu	0.696*	0.647*	0.230	0.589	0.835**	1

AspJuv = juveniles of *A. cuculus* \leq 110 mm; AspAdu = adults of *A. cuculus* $>$ 110 mm

CheJuv = juveniles of *C. lucerna* \leq 180 mm; CheAdu = adults of *C. lucerna* $>$ 180 mm

EutJuv = juveniles of *E. gurnardus* \leq 100 mm; EutAdu = adults of *E. gurnardus* $>$ 100 mm

**=intraspecific overlap; *=interspecific overlap

FIGURE CAPTIONS

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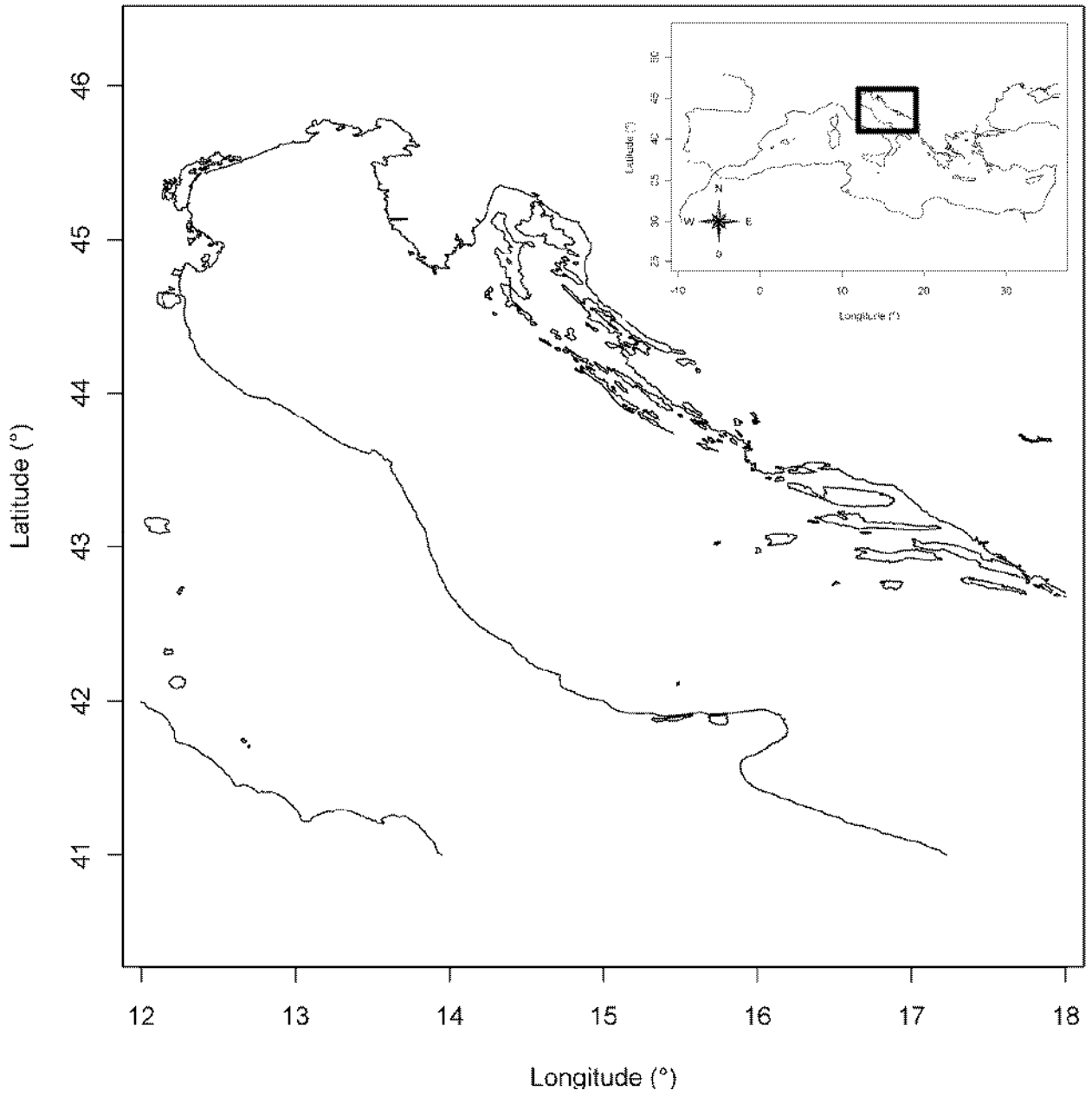
FIG. 1 Map of sampling area in north-middle Adriatic Sea (north-eastern Mediterranean).

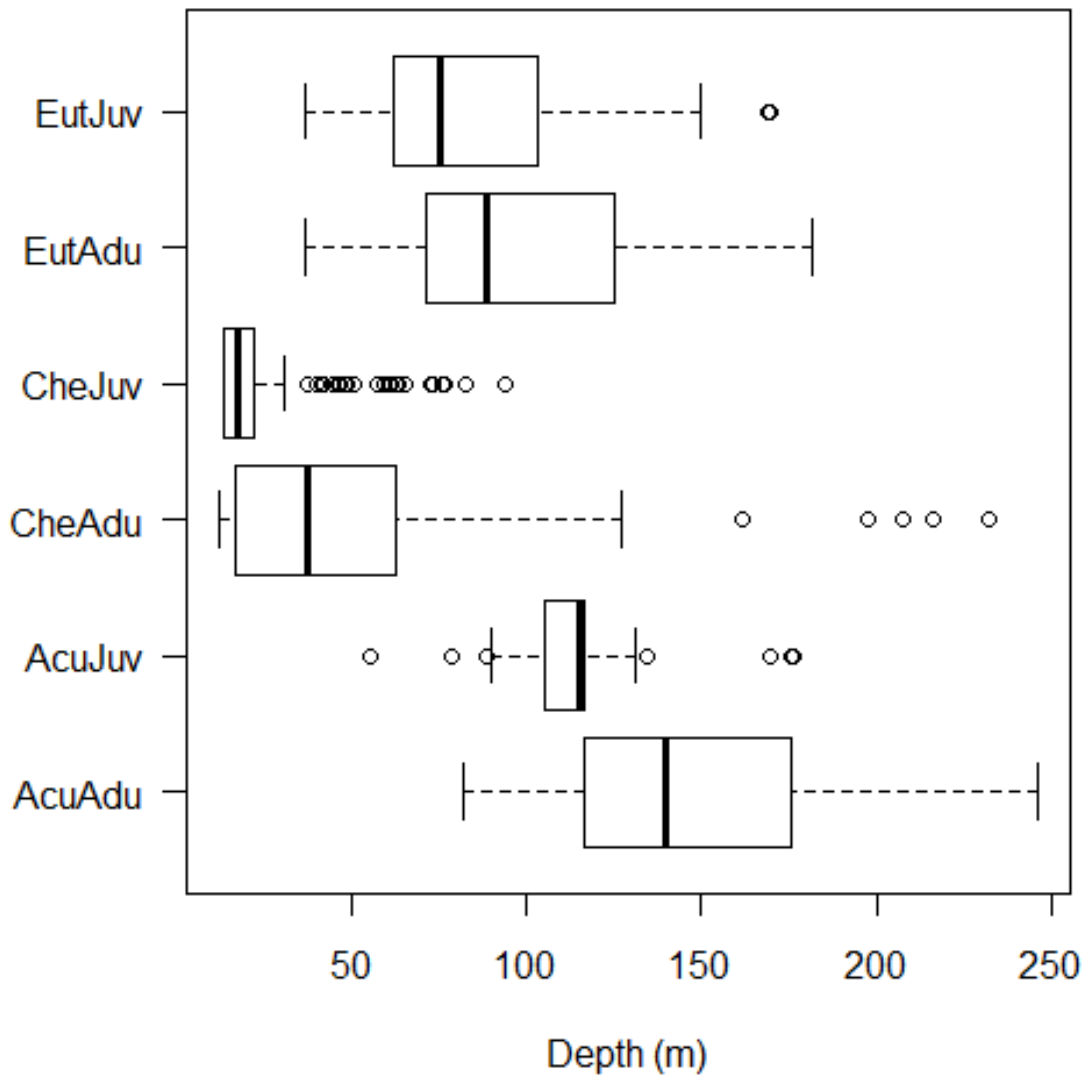
FIG. 2 Boxplot of depth distribution of the three gurnard species divided into two principal size classes: juvenile and adult.

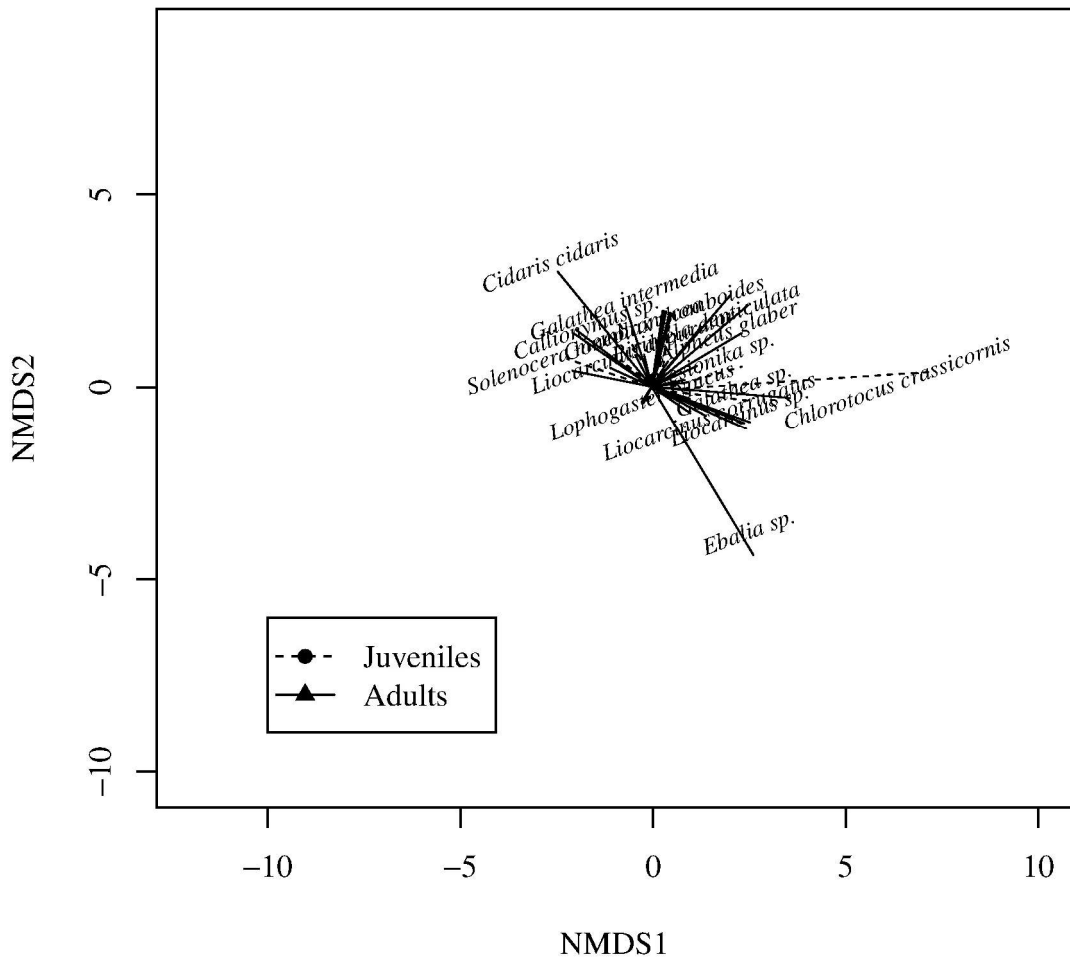
FIG. 3 nMDS plot between two principal size classes (juvenile and adult) based on numerical abundance of prey species. (3a *A. cuculus*; 3b *C. lucerna*; 3c *E. gurnardus*).

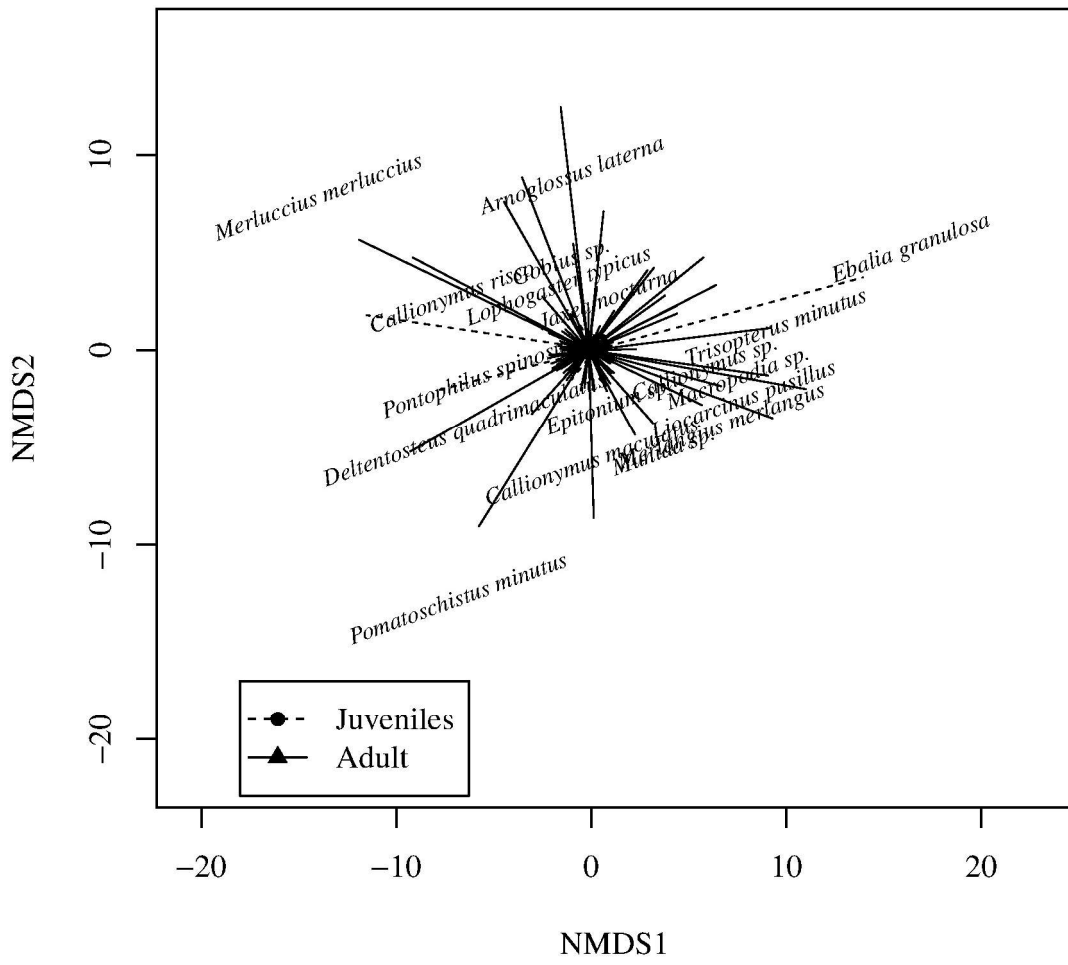
FIG. 4 nMDS plot of dietary overlap based on numerical abundance of prey species. Two main size classes (juvenile and adult) were considered for each gurnards (AspJuv = juveniles of *A. cuculus* \leq 110 mm; AspAdu = adults of *A. cuculus* $>$ 110 mm; CheJuv = juveniles of *C. lucerna* \leq 180 mm; CheAdu = adults of *C. lucerna* $>$ 180 mm; EutJuv = juveniles of *E. gurnardus* \leq 100 mm; EutAdu = adults of *E. gurnardus* $>$ 100 mm).

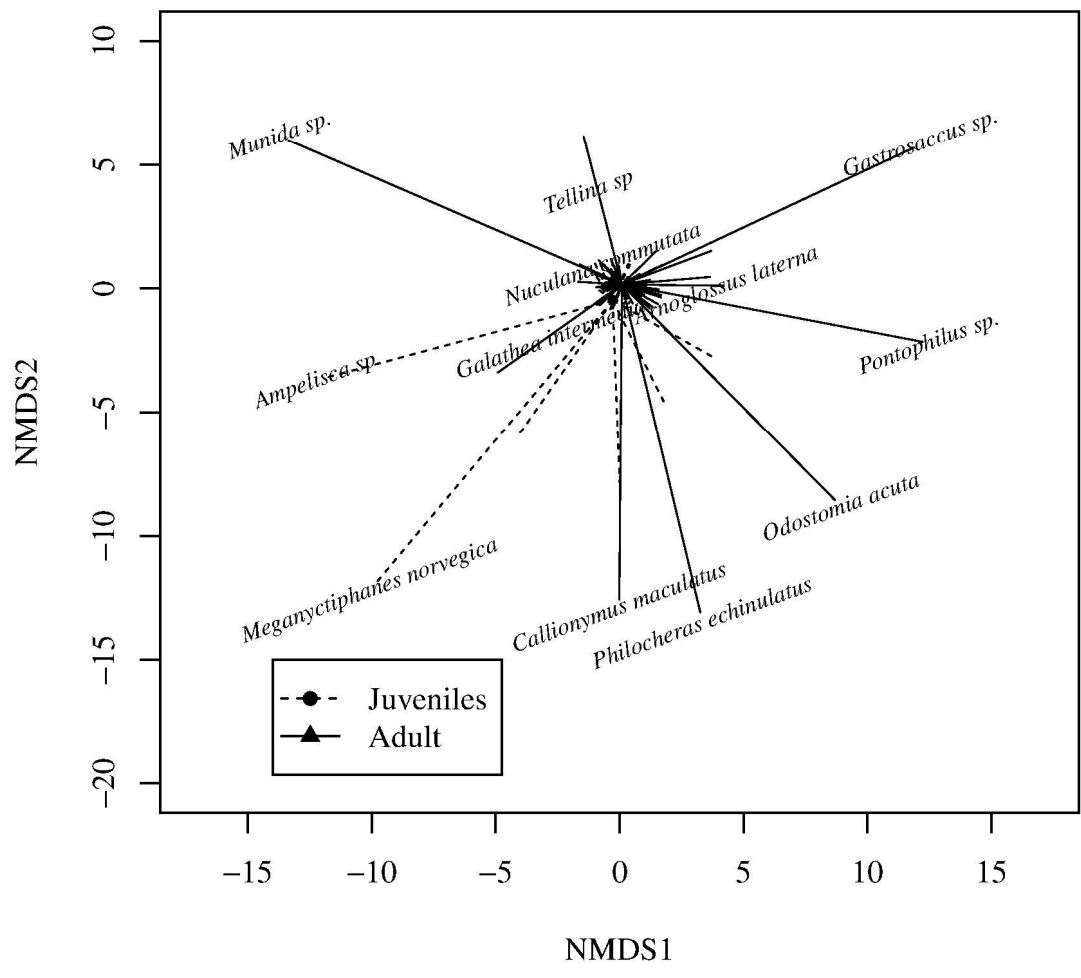
FIG. 5 nMDS plot of dietary overlap based on numerical abundance of prey species. For each species, data was grouped according to size-classes (juvenile and adult) and three strata of depth distribution (0-50 m, 50-100 m, 100-250 m). (AJ = juveniles of *A. cuculus* \leq 110 mm; AA = adults of *A. cuculus* $>$ 110 mm; CJ = juveniles of *C. lucerna* \leq 180 mm; CA = adults of *C. lucerna* $>$ 180 mm; EJ = juveniles of *E. gurnardus* \leq 100 mm; EA = adults of *E. gurnardus* $>$ 100 mm).

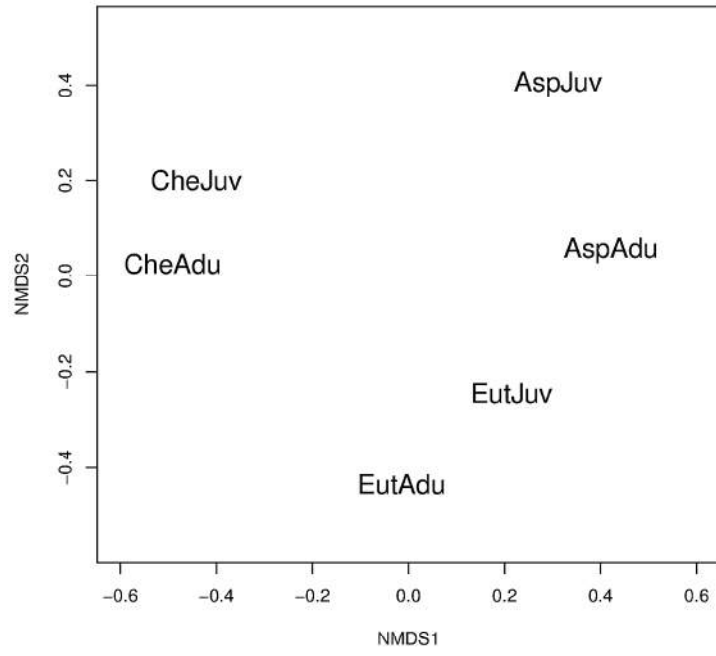


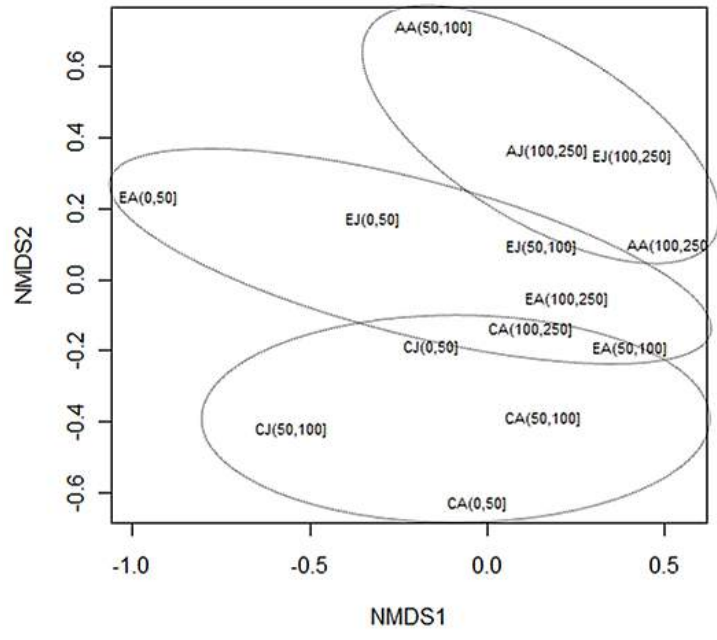












Diet of the grey gurnard, *Eutrigla gurnardus* in the Adriatic Sea, north-eastern Mediterranean

by

Stefano MONTANINI* (1, 2), Marco STAGIONI (1, 2) & Maria VALLISNERI (1)

ABSTRACT. - A total of 449 individuals of grey gurnard *Eutrigla gurnardus* (Linnaeus, 1758) were collected between December 2007 and July 2008 by bottom trawl surveys in the Adriatic Sea, north-eastern Mediterranean. Stomach contents were investigated in order to analyse diet composition according to fish size and depth. The most important prey were Crustacea, mostly Mysidacea such as *Lophogaster typicus* and Decapoda such as *Goneplax rhomboides* and *Philocheiras bispinosus*, followed by Teleostei, mostly Perciformes as *Gobius niger* and *Callionymus* spp. Dietary differences were found in relation to a critical predator size of 120 mm (TL). Decapoda were more frequently found in the diet of juveniles and Teleostei in that of adults living at a greater depth. Feeding strategy analyses revealed trophic specialization towards reference prey, thus narrowing the trophic niche.

RÉSUMÉ. - Régime alimentaire d'*Eutrigla gurnardus* (Triglidae) en mer Adriatique (nord-est Méditerranée).

Au total, 449 spécimens de grondin gris, *Eutrigla gurnardus* (Linnaeus, 1758), ont été capturés entre décembre 2007 et juillet 2008 dans le cadre de campagnes de pêche à la traîne en mer Adriatique. Les contenus stomacaux ont été étudiés afin d'analyser leur régime alimentaire, en fonction de la taille et de la profondeur. Les proies les plus importantes sont des Crustacea, surtout des Mysidacea tels que *Lophogaster typicus* et des Decapoda tels que *Goneplax rhomboides* et *Philocheiras bispinosus*, suivis par des Teleostei, surtout les Perciformes tels que *Gobius niger* et *Callionymus* spp. On a constaté une modification du régime alimentaire à partir de la taille critique de 120 mm (LT) : les Decapoda sont plus fréquents dans le régime alimentaire des jeunes et les Teleostei dans celui des adultes vivant à de plus grandes profondeurs. Les résultats de l'analyse du régime alimentaire ont mis en évidence une spécialisation trophique pour des proies de référence et donc une niche trophique étroite.

Key words. - Triglidae - *Eutrigla gurnardus* - Adriatic Sea - Diet - Feeding habits.

Eutrigla gurnardus (L., 1758) is a widely distributed demersal species that occurs in the Mediterranean and eastern Atlantic. This fish inhabits depths of 20-300 m in muddy and sandy substrata (Relini *et al.*, 1999). Its trophic biology in the Mediterranean Sea is poorly known. Current literature only refers to the North Sea (Ursin, 1975; Agger and Ursin, 1976; De Gee and Kikkert, 1993; Floeter *et al.*, 2005; Floeter and Temming, 2005; Weinert *et al.*, 2010) and the Spanish (Catalan) Mediterranean coast (Moreno-Amich, 1994). As to Italian coastal areas, available data are very limited, dating back several decades (Valiani, 1934).

The present study sets out to analyse stomach contents and dietary changes in relation to abiotic (depth) and biotic (length) variables with a view to better understanding the ecological role of this species in Adriatic demersal marine communities similar to those in the North Sea where the species is considered to be an emerging key predator that feeds on invertebrates as well as on fish (Floeter *et al.*, 2005; Weinert *et al.*, 2010). Knowledge of the feeding ecology of both commercial and non-commercial fish species is essential for

implementing a multispecies approach to fishery management (Gulland, 1997).

MATERIAL AND METHODS

Sampling

A total of 449 samples of *E. gurnardus* were collected seasonally by international bottom trawl surveys in the Mediterranean Sea in winter and summer at depths between 31 and 185 m in the northern-middle Adriatic Sea from the Gulf of Trieste (45°40'N; 13°37'E) to the line joining the Gargano to Dubrovnik (42°08'N; 15°16'E). The bottom trawl surveys were carried out from December 2007 to March 2008 and from June to July 2008 along the Italian coasts (Fig. 1).

Total length (TL, mm) and weight (W, g) of each individual were measured in the laboratory to an accuracy of 0.1 g. Sex was determined on the basis of macroscopic gonads, and specimens were classified as females (F), males (M), juveniles (J, macroscopically unidentifiable sex) or not deter-

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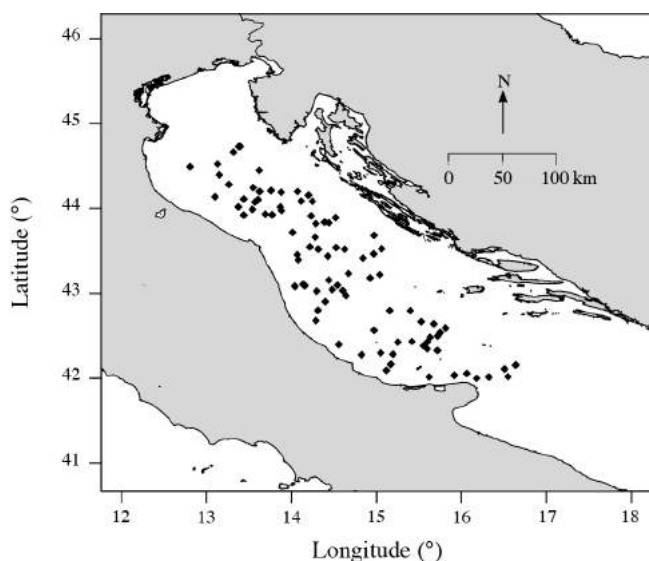


Figure 1. - Sampled sites in the north Adriatic Sea, north-eastern Mediterranean.

mined (ND, individual not examined) (Relini *et al.*, 2008). 156 females, 118 males, 109 juveniles and 66 undetermined were recorded. The stomachs of each of these specimens were removed and preserved in 70% ethanol for subsequent examination in the laboratory.

Diet analyses

Food items were identified to the lowest taxonomic level possible, taking into account each prey type and digestion state. Food items were counted, weighed (nearest 0.001 g) and photographed by means of a NIKON P5100 digital camera connected to a Leica MZ6 stereomicroscope. Inorganic particles and detritus present in the stomachs were also recorded. Data were collected in a database and processed with R software, version 2.10.1.

The relative importance of each item was calculated on the basis of item quantity (number) divided by the total number of overall prey (%N = number of prey *i* / total number of prey x 100), weight proportion (%W = weight of prey *i* / total weight of overall prey x 100) and frequency of occurrence (%O = number of stomachs containing prey *i* / total number of stomachs containing prey *i* x 100). These values were used to calculate the relative importance index (IRI): $IRI = \%O (\%N + \%W)$ and IRI proportion (%IRI). Stomach content coefficients were determined according to an empirical scale as empty (A) = stomach barely bloated with no and/or with only a few small prey; semi-empty (B) = stomach slightly bloated with only a few and/or with considerably small prey; semi-full (C) = stomach sufficiently bloated with considerable amounts of small prey or with only a few large prey; full (D) = stomach massively bloated with large amounts of small prey or with only a few large

prey (Chrisafi *et al.*, 2007). These indexes were used to calculate a fullness index ($FI = \text{number of stomachs with the same degree of fullness} / \text{total number of stomachs examined} \times 100$) and to determine changes in trophic level according to two main size classes correlated to depth.

Group average hierarchical cluster analysis was carried out using the Bray-Curtis similarity index (Clarke and Warwick, 1994) to assess differences between predator length and number (%N) of ingested food categories, data being transformed by Wisconsin double standardization to mitigate prey item range. This analysis was made to determine ontogenetic changes in fish diet. Specimens were subdivided into 6 size-classes.

General feeding strategy was analysed according to the Costello method (1990) as revised by Amundsen *et al.* (1996). The diagram generated by this method provides a two-dimensional representation in which each prey point is obtained by plotting the frequency of occurrence (%O) against prey-specific abundance (PSA) calculated according to the following formula: $P_i = (\sum S_i / \sum S_{t_i}) \times 100$ where S_i is the stomach content (weight) of prey item *i* and S_{t_i} is the total stomach content (weight) of only those predators with prey item *i* in their stomach. Prey importance and predator feeding strategy were determined on the basis of dot distribution along diagram diagonals and axes.

RESULTS

General diet composition

Five taxa (Tab. I) and namely Arthropoda (represented by Crustacea), Chordata (represented by Teleostei), Mollusca, Nematoda and Echinodermata, and 33 prey items (Tab. II), were identified in this study. The diet of *E. gurnardus* was mostly made up of crustaceans (%N = 94.2; %W = 71.9), and to a lower extent of teleost fishes (%N = 2.6; %W = 27.5) and molluscs (%N = 2.3; %W = 0.3). Other prey were nematodes (%N = 0.5; %W < 0.1) and echinoderms (%N = 0.1; %W < 0.1). Teleost fishes were more important in terms of

Table I. - Prey taxa recorded in the stomach content of *Eutrigla gurnardus* from the Adriatic Sea, all individuals merged. %N = numerical composition; %W = weight proportion; %O = frequency of occurrence; IRI = index of relative importance; %IRI = IRI proportion.

Prey Taxa	%N	%W	%O	IRI	%IRI
Arthropoda	94.15	71.93	94.04	15618.6	97.61
Chordata	2.57	27.49	11.91	358.05	2.24
Mollusca	2.25	0.34	8.46	21.94	0.14
nd	0.51	0.24	2.51	1.9	0.01
Nematoda	0.45	0	1.57	0.71	0
Echinodermata	0.06	0	0.31	0.02	0

Table II. - Diet composition of *Eutrigla gurnardus* from the Adriatic Sea, all individuals merged. %N = numerical composition; %W = weight proportion; %O = frequency of occurrence; IRI = index of relative importance; %IRI = IRI proportion.

Prey species	%N	%W	%O	IRI	%IRI
Mysidacea					
<i>Lophogaster typicus</i>	31.73	24.72	21.94	1238.58	50.49
<i>Acanthomysis longicornis</i>	6.81	0.48	3.76	27.42	1.12
<i>Gastrosaccus sanctus</i>	0.5	0.03	0.63	0.33	0.01
<i>Gastrosaccus</i> sp.	0.17	0.03	0.31	0.06	0
Decapoda: Brachyura					
<i>Goneplax rhomboides</i>	21.59	22.06	20.69	903.19	36.82
<i>Liocarcinus</i> sp.	7.14	2.93	7.21	72.59	2.96
<i>Liocarcinus depurator</i>	2.16	6.81	3.45	30.92	1.26
Decapoda: Macrura-Natantia					
<i>Philocheas bispinosus</i>	10.13	1.7	6.27	74.2	3.02
<i>Alpheus glaber</i>	1.99	6.06	3.45	27.77	1.13
<i>Philocheas</i> sp.	5.15	0.78	3.76	22.29	0.91
<i>Solenocera membranacea</i>	3.99	2.51	3.45	22.39	0.91
<i>Processa</i> spp.	2.66	0.21	1.57	4.49	0.18
<i>Pontophilus</i> sp.	0.17	1.34	0.31	0.47	0.02
<i>Pontophilus spinosus</i>	0.17	0.99	0.31	0.36	0.02
<i>Philocheas echinulatus</i>	0.17	0.12	0.31	0.09	0
Decapoda: Anomura					
<i>Galathea</i> sp.	0.66	0.18	0.63	0.53	0.02
<i>Galathea intermedia</i>	0.66	0.12	0.63	0.49	0.02
<i>Munida</i> spp.	0.33	3.28	0.31	1.13	0.05
Decapoda: Macrura-Reptantia					
<i>Callianassa</i> spp.	0.17	0.24	0.31	0.13	0.01
<i>Jaxea nocturna</i>	0.17	0.21	0.31	0.12	0
Teleostei					
<i>Gobius niger</i>	0.83	10.54	1.57	17.82	0.73
<i>Callionymus</i> sp.	0.5	2.72	0.94	3.02	0.12
<i>Arnoglossus laterna</i>	0.17	9.31	0.31	2.97	0.12
<i>Callionymus maculatus</i>	0.17	2.36	0.31	0.79	0.03
Bivalvia					
<i>Tellina</i> spp.	0.33	0.09	0.63	0.26	0.01
<i>Mactra corallina</i>	0.17	0.03	0.31	0.06	0
<i>Flexopecten glaber proteus</i>	0.17	0	0.31	0.05	0
<i>Hyalopecten similis</i>	0.17	0	0.31	0.05	0
Opisthobranchia					
<i>Turritella communis</i>	0.33	0.06	0.63	0.25	0.01
<i>Odostomia acuta</i>	0.17	0	0.31	0.05	0
Euphausiacea					
<i>Meganyctiphanes norvegica</i>	0.17	0.12	0.31	0.09	0
Amphipoda					
<i>Ampelisca</i> spp.	0.17	0	0.31	0.05	0
<i>Leucothoe</i> spp.	0.17	0	0.31	0.05	0

weight proportion than of occurrence frequency and proportion (Fig. 2).

Decapod crustaceans were the most important prey items of this population of *E. gurnardus*. Amongst Decapoda, the dominant species in order of importance were: *Goneplax rhomboides*, *Philocheas bispinosus*, *Liocarcinus* spp., *Solenocera membranacea*, *Alpheus glaber*. *Lophogaster typicus* (Mysidacea) appeared to be the most abundant food in the stomachs examined. Amongst teleost fishes, the most preyed species were *Gobius niger* and *Callionymus* spp.

Fullness index

E. gurnardus fullness index varied significantly for the two size classes calculated as the smaller and the larger than the median, the latter being 120 mm ($\chi^2 = 12.7752$, $df = 3$, $p < 0.05$) (Fig. 3). Stomach fullness was greater in adults living at depths greater than the juveniles ($\chi^2 = 26.0871$, $df = 6$, $p < 0.001$). Adults were found to feed on larger prey (teleost fish and decapods such as crabs) with a greater leathery content, related to increased energy requirements (Fig. 4). Sex-wise, greater fullness was recorded for females than for males ($\chi^2 = 12.9224$, $df = 3$, $p < 0.05$).

Diet variation with fish size

The diet similarity dendrogram (Fig. 5) reveals a first dichotomy marking out two distinct main groups depending on total length (TL) being greater or less 120 mm. Other dichotomies were found, marking out a further six groups according to total fish length, and namely class I containing fish of up to 80 mm; class II from 81 to 100 mm; class III from 101 to 120 mm; class IV from 121 to 140 mm; class V from 141 to 160 mm, and class VI longer than 160 mm. Juveniles (class I; class II) were active predators since their first life stages. Their predation was directed at smaller crustaceans such as *Philocheas bispinosus* and *Processa* spp. Specimens in class III were found to start to feed on some molluscs but the most significant change in feeding behaviour was observed for speci-

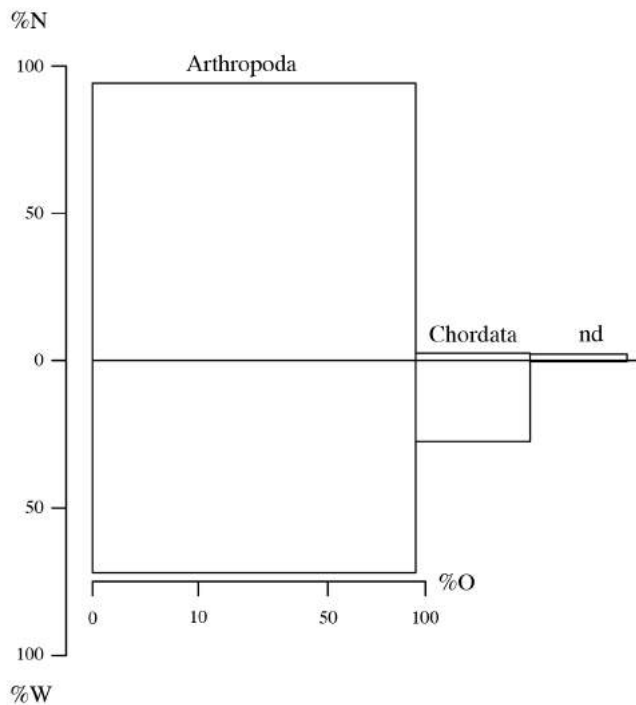


Figure 2. - Proportion of main prey taxa in the stomachs of *E. gurnardus*, all individuals merged. %N = numerical composition; %W = weight proportion; %O = frequency of occurrence; nd = not determined.

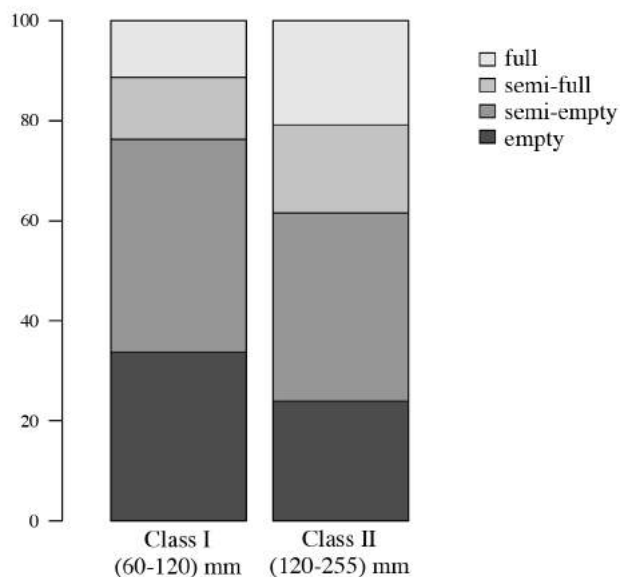


Figure 3. - Variations in stomach fullness of *E. gurnardus* in relation to two size classes. Class I (60-120 mm); Class II (120-255 mm).

mens in classes IV and V. The variety of food items eaten by grey gurnards was seen to increase during life stages corresponding to classes IV and V, when the highest value of niche breadth was attained. Preys include benthic food items

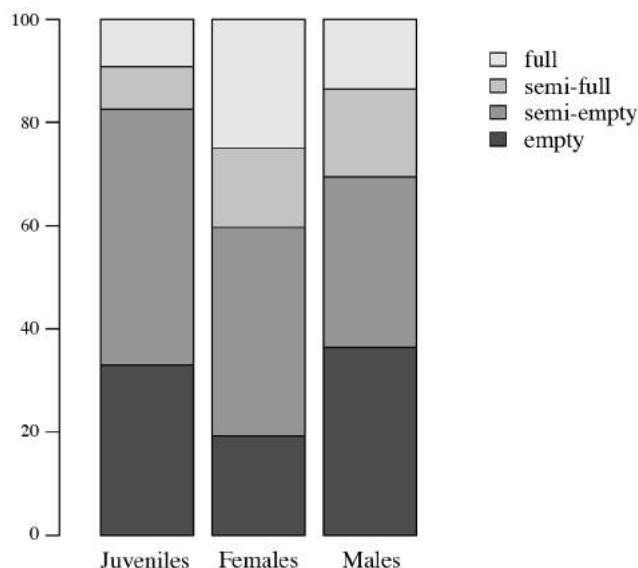


Figure 4. - Variations in stomach fullness of *E. gurnardus* in relation to sex. Juveniles and adults (females and males).

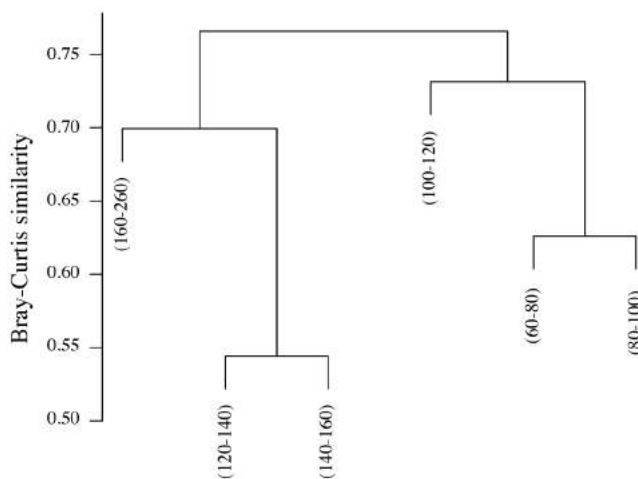


Figure 5. - Dendrogram showing feeding similarities among six *E. gurnardus* size classes from the Adriatic Sea. Class I (60-80 mm); Class II (80-100 mm); Class III (100-120 mm); Class VI (120-140 mm); Class II (140-160 mm); Class III (160-260 mm). Species diversity Bray-Curtis similarity index (cluster method: average).

such as amphipods, bigger decapods such as *Brachyura* and *Alpheus glaber*, mysids, and molluscs. Specimens in these classes were also found to begin to prey necto-benthic items such as *Callyonimus maculatus*. Specimens of more than 160 mm (class VI), although preying crustaceans such as *Pontophilus* spp. and *Liocarcinus depurator*, were found to mainly exhibit piscivorous feeding habits. Their prey were seen to include Callyonimidae, *Gobius niger* and Pleuronectiformes such as *Arnoglossus laterna*.

Feeding strategy

Prey-wise, feeding strategy analysis on the basis of stomach contents revealed crustaceans *Lophogaster typicus* and *Goneplax rhomboides* to be the most dominant prey for the predator populations considered (see top right corner of the graph). Such feeding strategy is indicative of an overall predator population with a narrow feeding niche (Fig. 6). Despite their low occurrence, the teleost *Gobius niger* and the crustaceans *Liocarcinus depurator*, *Alpheus glaber* and *Solenocera membranacea* were observed with great specific abundance. These more palatable preys were consumed by only a few individuals displaying high specialization (see upper left corner of the graph). Smaller crustaceans such as *Acanthomysis longicornis*, *Philocheras* spp., *Processa* spp. and teleost fishes *Callionymus* spp. represented rare and less important prey (see lower left corner of the graph).

Graphical feeding strategy analysis shows inter-phenotype contribution to be quite high (high BPC), with a fair number of the majority of prey points being positioned toward the upper left corner of the graph. This points to the fact that a number of individuals of the predator population of *E. gurnardus* considered were specialized in the predation of different resource types, reflecting dietary changes over the lifecycle of this species.

DISCUSSION

The present study bears out the importance of investigating dietary habits, predator-prey relationships and trophic levels in order to quantify the ecological role of the various components of any marine community. Ideally, priority should be given to studying the trophic biology of scanty known stocks such as, for instance, *E. gurnardus*, especially when it is considered that they may limit or affect the trophic niches of other species of commercial importance.

Very few studies have been carried out on the feeding habits of the Mediterranean grey gurnard particularly in Italian seas (Valiani, 1934). On the basis of stomach content analysis, some authors have reached the conclusion that this predator feeds on crustaceans (mainly Decapoda natantia and Mysidacea) and teleost fishes (Reys, 1960 - Gulf of Lions; Moreno-Amich, 1994 - Catalan coast). Molluscs were also observed to be present in the stomachs, but were deemed to contribute only moderately to the overall diet.

Our findings based on the study of Adriatic Sea populations of the predator are in line with those of these authors. Grey gurnards are epibenthic feeders preying mainly crustaceans. Depending on lifecycle stage, it was observed that *E. gurnardus* increases its predation of fish. Such behaviour probably depends on the predator's changing energetic requirements with growth as well as on its changing predation capacities in relation to fish size (Ursin, 1975;

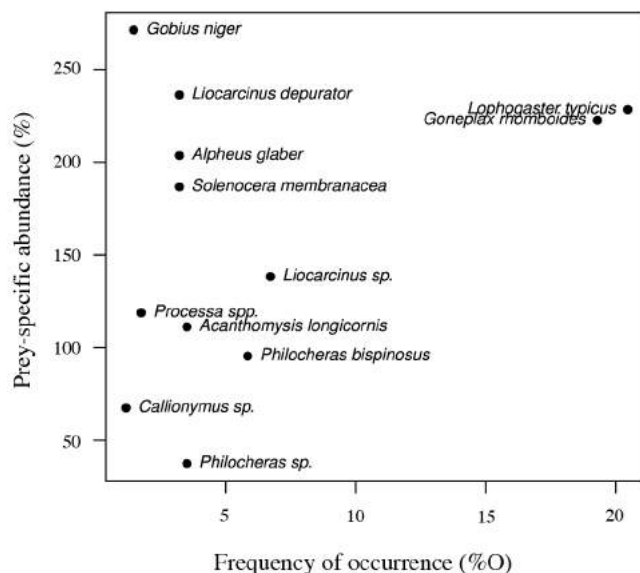


Figure 6. - Prey-specific abundance plotted against frequency of most occurring prey items in the diet of *E. gurnardus* from the Adriatic Sea. %O = frequency of occurrence; PSA (%) = prey-specific abundance.

Agger and Ursin, 1976; De Gee and Kikkert, 1993; Moreno-Amich, 1994; Floeter *et al.*, 2005; Floeter and Temming, 2005). Ontogenetic body increase allows growing predators to successfully ingest more elusive, larger prey (Karpouzi and Stergiou, 2003). In addition, adults move faster and are therefore capable of feeding on prey with higher motility (i.e., fish). They become more opportunistic, apparently engaging in a more generalist-oriented foraging behaviour. According to the plotted pattern, smaller crustaceans were seen to be important prey for the smaller sized classes of the predator, while larger crustaceans and fishes were preferred by larger individuals.

The critical size at which *E. gurnardus* in the area examined was found to switch over from feeding mainly on crustaceans to fishes, to predate at greater depths and to start reaching sexual maturity, was observed to be at around 120 mm TL. The differentiation between females and males starts at lengths close to and less than 100 mm TL. 50% of males have been found to attain maturity at around 120 mm TL, while 50% of females at around 150 mm TL (Vallisneri *et al.*, unpublished data). As to size at maturity, our findings are comparable to those reported in the literature, regardless of location (Papaconstantinou, 1982 - Greece; Dorel, 1986 - Gulf of Biscay, France; Muus and Nielsen, 1999 - Denmark). Minor differences as were observed may be attributed to local conditions, especially temperature that stimulates sexual maturation, and to the study methodology adopted by the different authors.

Stomach fullness is greater in females than in males, a fact that may be accounted for by the higher fitness costs

(i.e., big eggs and large fecundity) incurred by females and by their access to the best spawning sites.

The analysis of prey-specific abundance against frequency of occurrence shows that *E. gurnardus* has a narrow niche width, *Lophogaster typicus* and *Goneplax rhomboides* being the dominant prey. The most abundant food in their stomachs was found to be represented by the species *Lophogaster typicus* (Mysidacea); this finding is in line with that of the literature relating to the stomach contents of other species of the same Triglidae family in the south Mediterranean (Terrats *et al.*, 2000). It is also in line with the results reported by Colloca *et al.* (2010) for other Mediterranean gurnards (*Aspitrigla cuculus*, *Lepidotrigla cavillone* and *L. dieuzeidei*) that were found to be characterized by a specialist trophic behaviour. A significant negative correlation between prey type similarity and depth distribution similarity was also observed for Mediterranean demersal fish, with gurnards overlapping in both food and depth preferences (Colloca *et al.*, 2010).

In line with the literature (Floeter *et al.*, 2005; Montanini *et al.*, 2008), the Adriatic population studied by us shows that *E. gurnardus* potentially competes as a predator with other fish species. As reported by Floeter and Temming (2005), in the North Sea grey gurnard predation is in fact a very critical process with a significant top-down effect on whiting (*Merlangius merlangus*) and potentially also on cod recruitment.

Acknowledgements. - We thank Pr Corrado Piccinetti for his assistance in bottom trawl surveys and his valuable suggestions.

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Reçu le 21 juin 2010.

Accepté pour publication le 16 novembre 2010.

Da tempo si sa che gli organismi marini sono un serbatoio enorme di composti naturali potenzialmente attivi contro virus, batteri o cellule cancerogene. Svariate sostanze ad azione antimicrobica sono state isolate sia da batteri, alghe unicellulari e multicellulari, sia da molte specie di invertebrati. Relativamente a tale problematica gli studi sui policheti risultano molto esigui e mancano dati sulla presenza nelle loro secrezioni mucose di sostanze antimicrobiche. Nel presente lavoro è riportata un'indagine sull'attività antibatterica nel muco dell'anellide polichete, *Sabella spallanzanii* che, vivendo in ambienti eutrofici come le aree portuali ha dovuto mettere a punto dei meccanismi di difesa che possono giocare un ruolo cruciale nella protezione contro potenziali patogeni e parassiti, inclusi batteri e funghi. A tal riguardo campioni di muco sono stati prelevati mensilmente nell'arco di un anno da esemplari prelevati nel Mar Adriatico Meridionale (Brindisi). Dai dati ottenuti è stata dimostrata l'esistenza nel muco di questo polichete di un'elevata attività lisozima-simile. Tale attività è sempre presente tranne durante il periodo riproduttivo (gennaio-marzo). Dall'analisi elettroforetica è stato evidenziato un diverso pattern proteico del muco prelevato nel periodo riproduttivo rispetto a quello prelevato nel resto dell'anno. L'assenza di attività antibatterica durante questo periodo può essere spiegata in relazione alla riproduzione di questa specie. È stato infatti osservato che la fecondazione delle uova si verifica all'interno del tubo del polichete (fecondazione in situ) e non ha successo se non quando le uova si trovano immerse nel muco prodotto dal verme. Dopo la fecondazione le uova sono fatte fuoriuscire dal tubo ancora all'interno del muco e solo a questo punto le trocofore in sviluppo vengono liberate nel mezzo. È quindi ipotizzabile che una forte attività antibatterica possa essere di disturbo per la fecondazione. Dai risultati ottenuti si può quindi dedurre che in momenti diversi del ciclo vitale di questo polichete il muco possa svolgere un ruolo differente ma comunque sempre rivolto alla protezione assicurando la difesa dell'adulto per la maggior parte dell'anno e la salvaguardia dei gameti nel periodo riproduttivo.

Strategia alimentare di *Trigla lucerna* (Teleostei, Triglidae) in Alto-Medio Adriatico: note di 7 campagne di pesca a strascico

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Nell'ambito della gestione delle risorse alieutiche, di particolare risalto sono gli studi relativi alle strategie alimentari, ai rapporti preda-predatore ed ai livelli trofici, essenziali per quantificare gli effetti della pesca sull'ecosistema. Obiettivo di questo studio, svolto nell'ambito di un progetto ministeriale MIPAAF, è la messa a punto di un modello di analisi della strategia alimentare di una "specie target" del Mediterraneo centrale: *Trigla lucerna* (Linnaeus, 1758), detta cappone o gallinella. A tale scopo, nel corso di sette campagne di pesca a strascico in Alto-Medio Adriatico, fra il golfo di Trieste (45° 40' N 13° 37' E) e le isole Tremiti (42° 0,8' N 15° 16' E), sono stati effettuati 204 campionamenti fra 10 e 260 m di profondità. Sul totale di 1.096 esemplari di taglia compresa fra 63 e 415 mm, sono state condotte analisi quali-quantitative dell'attività alimentare e dei contenuti stomacali in relazione ai principali parametri somatici (taglia, sesso) e ambientali (stagione, profondità, comunità bento-nectonica). L'analisi dei "cluster" basata sulla tipologia delle prede, ha evidenziato la separazione fra due gruppi di taglia a ridosso di 180 mm, che segnano l'inizio del differenziamento sessuale. L'attività alimentare è maggiore nei giovanili (<180mm), durante l'estate e a basse profondità, rispecchiando la distribuzione e l'abbondanza delle reclute. L'analisi qualitativa dei contenuti stomacali ha permesso l'identificazione di 55 specie, appartenenti a 6 categorie sistematiche principali. L'analisi quantitativa evidenzia che l'alimento preferito a tutte le taglie è costituito da Crustacea (89,7% del numero e 58% in peso) e Teleostea (6,7 % in numero totale e 39,7% in peso). Prede accessorie sono rappresentate da Mollusca (1,6 % in numero totale e 0,6% in peso). Nell'ambito Crustacea, le specie dominanti sono, tra i Decapoda reptantia i Brachiura *Goneplax rhomboides* (14,2% del numero e 29,5% in peso) e *Liocarcinus* sp. (13% del numero e 24,7% in peso), tra i Decapoda natantia *Philocheira* sp. (59,5% del numero e 2,5% in peso). In relazione al sesso, non si manifestano differenze significative. In relazione alla taglia, si delineano due modelli di "strategie alimentari": il primo, tipico dei giovanili, che si nutrono prevalentemente di crostacei, numerosi e di piccole dimensioni; il secondo, proprio degli adulti, che si nutrono prevalentemente di pesci in numero minore ma di dimensioni maggiori, parallelamente all'accrescimento, al differenziamento sessuale ed al miglioramento della capacità predatoria. In sintesi, *T. lucerna* si presenta come "consumatore opportunista", caratterizzato da una certa biodiversità delle prede; tuttavia, la comunità alimentare di cui si nutre, che rispecchia la comunità dell'area, risulta composta da un insieme di nicchie trofiche presumibilmente correlate a differenze ontogenetiche.

La carabidofauna del Parco Naturale Regionale Lama Balice (BA) (Coleoptera, Carabidae)

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ABITUDINI ALIMENTARI DI *EUTRIGLA GURNARDUS* IN ALTO-MEDIO ADRIATICO

FEEDING HABITS OF EUTRIGLA GURNARDUS IN THE NORTHERN-MIDDLE ADRIATIC SEA

Abstract - A total of 774 individuals of grey gurnard, *Eutrigla gurnardus* were collected in the Northern-Middle Adriatic Sea by bottom trawl surveys (GRUND 2007-MEDITS 2008). The stomach contents have been analyzed to determine diet according to fish size, sex, depth and season. The results show that crustaceans (%N=94.3; %W=68) are the basic food, (particularly Decapoda) and that teleostean fishes are quite important in the adult diet (particularly *Callionymus sp.*, *Gobius niger*).

Key-words: feeding habits, *Eutrigla gurnardus*, Triglidae, North-Middle Adriatic Sea.

Introduzione - La gallinella, *Eutrigla gurnardus* (L., 1758) è una specie comune nel Mediterraneo e nell'Atlantico orientale, tuttavia la sua biologia trofica è poco nota. Le uniche pubblicazioni si riferiscono al mare del Nord (Ursin, 1975; Agger e Ursin, 1976; De Gee e Kikkert, 1993; Floeter *et al.*, 2005; Floeter e Temming, 2005) ed alla costa catalana (Moreno-Amich, 1994-Mediterraneo nord-ovest). Relativamente ai mari italiani, i dati sono particolarmente scarsi e risalenti ad alcuni decenni orsono (Valiani, 1934).

Materiali e metodi - Un totale di 774 esemplari di gallinella sono stati collezionati fra 30,9 e 185 m di profondità nel Nord-Medio Adriatico fra il golfo di Trieste e la congiungente Gargano-Dubrovnik durante le campagne di pesca a strascico GRUND 2007 e MEDITS 2008. Su tutti gli esemplari sono stati registrati i parametri biometrici (LT, mm), peso corporeo (P, g), sesso (valutato sulla base dell'analisi macroscopica delle gonadi). È stato stimato lo stato di riempimento degli stomaci, sono state identificate le prede (fino al livello tassonomico più basso possibile), successivamente contate, pesate e fotografate mediante sistema computerizzato di immagini. I dati sono stati informatizzati mediante database relazionale "Microsoft Access" e analizzati mediante "pacchetto statistico R.2.8.1". Sono stati calcolati i principali indici, al fine di individuare le variazioni del regime trofico in rapporto al sesso, alla taglia (2 classi, minore e maggiore della mediana che è 120 mm), alla stagione e alla profondità. È stato valutato lo stato di riempimento degli stomaci (in base al peso del contenuto stomacale) in relazione alle variabili su indicate.

Risultati - In relazione allo stato di riempimento degli stomaci, gli adulti, che vivono a profondità maggiori rispetto agli indeterminati, presentano un grado di riempimento maggiore (X^2 test $p < 0,001$). Relativamente alla stagione, gli stomaci risultano più pieni durante la stagione primaverile-estiva (X^2 test $p < 0,05$). La dieta di *E. gurnardus* si basa in generale su Crustacea (%N=94,3; %P=68), Teleostei (%N=2,6; %P=26,2) e Mollusca (%N=2,3; %P=0,4). Prede accessorie sono costituite da Nematoda (%N=0,4; %P=0,02) ed Echinodermata (%N=0,1; %P=0,002). I Crustacea sono rappresentati principalmente da Decapoda (%N=27,9; %P=42,6), Mysidacea (%N=17,3; %P=18,9), Amphipoda (%N=2,1; %P=0,1) Euphausiacea (%N=0,1; %P=0,1). Fra i Decapoda le specie dominanti sono nell'ordine: *Goneplax rhomboides* (%N=21,5;

%P=22), *Philocheras bispinosus* (%N=10,2; %P=1,7), *Liocarcinus* sp. (%N=6,8; %P=2,8), *Solenocera membranacea* (%N=4,1; %P=2,5), *Alpheus glaber* (%N=2; %P=6,1). La specie *Lophogaster typicus* (Mysidacea) (%N=32,4%; %P=24,8) risulta essere l'alimento più abbondante negli stomaci. Tra i Teleostei, le specie maggiormente predate sono *Gobius niger* (%N=1%; %P=10,6) e *Callionymus* sp. (%N=0,5%; %P=2,7). Sulla base della tipologia delle prede l'analisi dei cluster ha separato 3 gruppi: quelli con taglia inferiore a 82 mm; quelli con taglia compresa tra 82 e 162 mm; quelli con taglia superiore a 162 mm. Sulla base dell'analisi della strategia alimentare le specie *Lophogaster typicus* e *Goneplax rhomboides* risultano essere dominanti negli stomaci ma non le più appetite. *Galathea intermedia* e *Gobius niger* appaiono le prede più appetite, evidenziandone la specializzazione. I nostri risultati sono sostanzialmente in accordo con la letteratura (Ursin, 1975; Agger e Ursin, 1976; De Gee e Kikkert, 1993; Moreno-Amich, 1994; Floeter *et al.*, 2005; Floeter e Temming, 2005). Infatti la dieta si basa essenzialmente su crostacei e teleostei e, con l'accrescimento, aumenta la predazione dei pesci in accordo con Floeter e Temming (2005). *E. gurnardus* si conferma anche per l'area adriatica un predatore non selettivo potenzialmente in competizione a livello trofico con altre specie ittiche, in accordo con la letteratura (Floeter *et al.*, 2005; Montanini *et al.*, 2008).

Conclusioni - Si conferma l'importanza degli studi relativi alle abitudini alimentari, ai rapporti preda-predatore e ai livelli trofici, essenziali per quantificare i ruoli ecologici dei diversi componenti delle comunità marine. In particolar modo assume priorità la valutazione della biologia trofica di popolazioni ittiche poco note, come quelle di *E. gurnardus*, considerando il fatto che possono limitare o influenzare le nicchie trofiche di altre specie ittiche anche di interesse commerciale.

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Chapter IV. Sagittal otoliths as potential marker of species life cycle: sclerochronological studies, morphometries and shape analysis, ultrastructural analysis, chemical analysis on bio-mineralization process

Scientific papers are reported below the summary of chapter IV as follows:

MONTANINI S., VALLISNERI M., PÉREZ-MAYOL S., PALMER M., MORALES-NIN B. (2014). Validation of first annulus formation in Triglidae family (Teleostei, Scorpaeniformes): a tool for increase the biological knowledge of a demersal resource. Abstract p. 272. 5th International Otolith Symposium, Paguera (Mallorca, Islas Baleares) 20-25 ottobre 2014.

MONTANINI S., VALLISNERI M., BENNI E., PÉREZ-MAYOL S., MORALES-NIN B. (2015). Pelagic larval duration and settlement marks on the otoliths of tub gurnard, *Chelidonichthys lucerna* from Adriatic Sea. *Biol. Mar. Mediterr.*, ACCEPTED

STAGIONI M., **MONTANINI S., VALLISNERI M.** Otolith shape differences of genus *Lepidotrigla* (Osteichthyes, Scorpaeniformes): an application of geometric analysis. UNPUBLISHED (*in preparation*)

STAGIONI M., **MONTANINI S., VALLISNERI M.** Characterization of triglids (Teleostei, Scorpaeniformes) size through otolith morphometrics and shape analysis. UNPUBLISHED (*in preparation*)

MONTANINI S., STAGIONI M., TOMMASINI S., VALLISNERI M. Fish-otolith size relationships for Triglidae in the Adriatic Sea (north-eastern Mediterranean). UNPUBLISHED (*in preparation*)

MONTANINI S., STAGIONI M., VALDRE' G., TOMMASINI S., VALLISNERI M. (2015). Intra-specific and inter-specific variability of the sulcus acusticus of sagittal otoliths in two gurnard species (Scorpaeniformes, Triglidae). *Fish. Res.* 161: 93-101.

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Scientific papers before 2011-2014 years:

MONTANINI S., STAGIONI M., VALLISNERI M. (2010). Elliptic fourier analysis of otoliths of Triglidae in the north-middle Adriatic sea. *Biol. Mar. Mediterr.*, 17 (1): 346-347

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SUMMARY OF CHAPTER IV.

About otolith, several studies were conducted for increase the knowledge on gurnard life cycles.

About **sclerochronological study** the first growth stages of *C. lucerna* were analyzed, counting and measuring presumed daily growth increments. After embadding otolith in epoxy resin using rectangular silicon moulds, transverse cross-sections of approximately 300 μm to 500 μm were prepared using a low-speed wafering saw (Buehler IsoMet). All mounts were ground and polished with frequent microscopic control, until the core plane was reached. Thin sections of approximately 100 μm were finally obtained for reading and storage. Otolith sections (N=131) were unclear due to difficulties in the sectioning plane (core not located, slanting sections) resulting only in 23% of sections with an increment pattern well defined. Corroboration of interpretation criteria was taken by counting twice (40X magnification under transmitted light) presumed daily increments on two defined radii: 1. along the proximal-distal axis from the primordium to the last increment before the apparition of an accessory growth centre (AGC); 2. from the more dorsal AGC to the proximal edge of the otolith close to the sulcus acusticus and perpendicular to the increment deposition; 3. AGC area, only for counts (widths were not taken). Settlement marks are generally similar to literature descriptions for other demersal fish with a mean of DGI number about 24 ± 2 (mean core width 11.2 ± 0.2 ; mean first increment width 1.49 ± 0.08). Between hatching check and active feeding check 6 DGI were counted. In all sections growth rates seem to be fast than previous results for *C. lucerna*. It's probably due to great growth plasticity at individual and seasons level. The results showed for the first time the growth at invalidated daily level of gurnards and the importance of developing a standard protocol adapted for each specie. The main difficulties encountered were related to the location of the otolith core due to a high opacity probably linked to the big amount of protein matter in the early development.

Shape analysis studied – EFA: fish otolith shape analysis is an important way for describing and characterizing mathematic otolith outlines. It is used with multiple goals, for example, species phylogeny and stock discrimination (Lombarte et al. 2006). In particular the Elliptic Fourier

Analysis (EFA) represents one among the most valuable and time-efficient method since data are automatically normalised in relation to the first harmonic and consequently they become invariant to size, rotation, and starting point (Iwata and Ukai 2002). In this study the EFA method was applied on otoliths collected from 7 species of triglids (*Aspitrigla cuculus*, *Chelidonichthys lastoviza*, *C. lucerna*, *Eutrigla gurnardus*, *Lepidotrigla cavillone*, *L. dieuzeidei* and *Trigla lyra*) (Teleostei, Scorpaeniformes) distributed in the north-middle Adriatic Sea. The aim of this study is to verify the existence of intra and interspecific differences associated with endogenous and exogenous factors. The SHAPE program was used to extract the contour shape of the sagitta and to assess the variability of shapes by means of the study of principal component analysis (PCA). The statistical analysis of the collected data was carried out by means of the R software. The intraspecific variability of adult specimens was higher for *C. lucerna* and *E. gurnardus* while it was lower for the Genera *Aspitrigla* and *Lepidotrigla*. Intraspecific comparison between juveniles and adults showed that in all species there was an increase in otolith shape variability linked to growth and to environmental conditions. According to these results, the EFA method was proved to be a suitable tool for supporting phylogenetic and eco-morphological investigations and assessing affinities among the investigated triglids species. It allowed to find similarity between *Lepidotrigla* sp. and *C. lastoviza*; distance between *C. lucerna* and *T. lyra* and an increase in the variability of otolith shape from juveniles to adults. However, in order to facilitate correct biological interpretation of data, the EFA method should be correlated with appropriate sampling plans (Farias et al. 2009; Stagoni et al. 2009). EFA was used also in another study focused on the species of genus *Lepidotrigla*: *L. cavillone* and *L. dieuzeidei*. They have long been confused because of a very similar morphology. In order to discriminate interspecific variation, otolith shape were investigated in this genus for the first time using external outlines (Fourier shape analysis). Samples were randomly collected at two areas in Adriatic sea (North and South of Ancona-Zara boundaries) during Grund 2007 scientific trawl survey. Fishes were measured for length, weight. Otoliths shape

indices and outline were calculated. Otolith shape analysis showed no variability in *L. cavillone* of the two areas, but discriminated between *L. cavillone* and *L. dieuzeidei*.

Morphometrics studies: the sagittal otoliths of Triglidae family from the Adriatic Sea (north-eastern Mediterranean) were described using morphological and morphometric characters. The morphological descriptions were based on the otolith shape, outline and sulcus acusticus features. The morphometric parameters determined were expressed in terms of 5 shape indices (aspect ratio, roundness, rectangularity, ellipticity, circularity). Shape indices for which “species-length” interactions were significant ($P < 0.001$) were normalised. The data were processed with R (R Development Core Team 2010). Otolith shape intra-inter specific changes have been described providing informations about species identification, fish size, sex and phylogenesis. 1) Univariate analysis (ANOVA): Interspecific comparison, analyzed by univariate analysis, were tested by Kruskal-Wallis and Tukey tests. About shape indices, major shape variation were respectively: for “aspect ratio” LEPTDIE-TRIGLUC; LEPTCAV-TRIGLUC; LEPTCAV-TRIGLYR; for “roundness” TRIGLUC-LEPTCAV.; for “rectangularity” ASPICUC-LEPTCAV.; for “ellipticity” LEPTDIE-TRIGLUC; LEPTCAV-TRIGLUC; TRIPLAS-TRIGLUC; LEPTCAV-TRIGLYR; LEPTDIE-TRIGLYR.; for “circularity” LEPTDIE-LEPTCAV; LEPTDIE-TRIPLAS; LEPTCAV-TRIPLAS. Otoliths morphologically more different, defined by three shape indices, were those belonging to *L. cavillone* and *C. lucerna*. The results were in agreement with the analytical key about the family (FAO 1987) that placed these two species at the extremes. 2) Multivariate analysis (MANOVA): Intraspecific comparison were analyzed by multivariate analysis. The otolith outline shape indices changed with size (between juveniles and adult) and sex for some species. In particular, intraspecific comparison showed significant differences between juveniles and adults for *A. cuculus*, *C. lucerna*, *L. dieuzeidei* and between females and males for *L. dieuzeidei*. Therefore, about the intraspecific relationship, *L. dieuzeidei* was the species more diversified. Another study were carried out focused on using sagittal otoliths for ecomorphological characterization of demersal fish from Adriatic Sea. The aim of this study was to analyze whether the morphological features

could be associated with diet and ecological niche in six demersal species for MEDITS trawl survey project (including a gurnard specie): *Chelidonichthys lucerna*, *Merlangius merlangus*, *Merluccius merluccius*, *Mullus barbatus*, *Pagellus erythrinus*, *Trisopterus minutus capelanus*. By ImageJ 1.47j software the following measurements were recorded from both right and left sagittae: LO (maximum length of sagitta) and WO (maximum width of sagitta) in mm; OA (otolith area) in mm². To detect inter-specific comparisons, total length of fish (TL) and allometry effects were avoided by normalizing all measurements to a given TL according to Leonart et al. (2000). Three percentage indexes were calculated: A%: otolith width/TL of fish%; L%: otolith length/TL of fish%; EL%: otolith width/otolith length%. PCA, PERMANOVA and post-hoc pair-wise analysis on species groups measurements and indexes were performed to test inter-specific differences. The information about feeding habits (IRI%) using to compare the results, were taken from Piccinetti et al. (2007). Two morphologic groups in sagitta shape were found: group 1 with lanceolated otolith including necto-benthic and bento-pelagic gadiforms (*M. merlangus*, *M. merluccius* and *T. minutus* c.) and group 2 with oval otolith including benthic species (*C. lucerna*, *M. barbatus* and *P. erythrinus*). PCA analysis discriminated within group: within group 1 *M. merluccius* differed from *M. merlangus* and *T. minutus* c. both for the diet as active predator of bony fishes that for more slender body; within group 2 *P. erythrinus* differed from *C. lucerna* and *M. barbatus* particularly for the diet that was based mainly on polychaetes. PERMANOVA found significant differences among species and pair-wise test comparison were all significant ($p < 0.001$) except for *C. lucerna* - *M. barbatus* and *M. merlangus* - *T. minutus* c. ($p > 0.05$), as evidence by the PCA plot. The present results support the hypothesis that sagitta morphologic features could be used to characterized the sagittae of fish and provides to be an efficient tool for distinguishing the fish ecotype, according to Volpedo and Echeverría (2003).

Combined studies on *sulcus acusticus*: Morphology, morphometries and compounds of *sulcus acusticus* of sagittal otoliths of two gurnard species (*Aspitrigla cuculus* and *Eutrigla gurnardus*) from north-central Adriatic Sea (north-eastern Mediterranean) were investigated. The study

objectives were to find morphometric variables and shape variability of sulcus acusticus of these two close related sound producers species linked to developmental changes from juvenile to adult stage and species-specific pattern which influenced ecomorphological adaptations of the auditory system. The method used was a combination of multivariate techniques, X-ray diffractions pattern and ultrastructural investigation by SEM. Multivariate analysis (PCA, LDA and PERMANOVA) performed on standardised morphometrics of a total of 202 individuals, significantly separated four groups: *A. cuculus* juveniles, *A. cuculus* adults, *E. gurnardus* juveniles and *E. gurnardus* adults due to different life history characteristics. At intra-specific levels higher mean values of sulcus area/otolith area (S:O) ratio of adults than the juveniles were observed within *A. cuculus* and not for *E. gurnardus*. At inter-specific levels significantly differences were showed between adults and not between juvenile groups. Considering all the groups, the main variability on sulcus area were observed for caudal part of the sensorial macula. Although no difference about compounds were showed by X-ray diffraction at intra-specific level, the crystals on sulcus acusticus were different in shape, size and arrangement between juveniles and adults of each gurnard. The crystals of juveniles were quite enmeshed into otolith matrix and showed a rod/smooth-shape. The crystalline arrangement of adults became more complex with larger, rod/rectangular-shape and well-formed faces. Ontogenetic and specie-specific differences might be related to specie-specific physiological factor (i.e sexual maturity), depth distribution and feeding patterns that changing during gurnards growth. These changes, relating to both endogenous and exogenous factors, were marked by the sulcus acusticus which provided a useful tool for fish ontogenetic, physiological and ecological studies. In conclusion, our study confirmed that the sulcus acusticus morphology can be used as a tool within population structure (intra-specific comparisons) and among species (inter-specific comparisons). Changes in sulcus acusticus shape could occurred in relation to fish growth and greater complexity in the crystalline structure of the sulcus acusticus. These differences might be related to: 1) phylogenetic influence that can be reflected in the sulcus acusticus morphology (Nolf 1985; Torres et al. 2000); 2) physiological factors i.e. sexual maturation. The most significant

changes take place near the specie-specific size at first maturity. At this size the metabolism of the fish undergoes important changes that provide variations in the growth, affecting the otolith morphology (Härkönen 1986); 3) environmental factors i.e. depth range which might effect the pattern of crystallization and shape of otoliths as observed in the study of Jitpukdee and Wannitikul (2004); 4) eco-physiological factors i.e. feeding pattern: a) feeding competition within species; b) ontogenetic diet shifts as a reflection of the changing abilities of fish; c) specie-specific feeding strategy linked to different ecological niches. These changes, relating to both endogenous and exogenous factors, are designed to improve the hearing which it changes and evolves during the life of the fish.

Chemical analyses: The aim of these studies were to analyze whether concentration of the sagitta organic matrices could be related with intraspecific (ontogenetic growth) and interspecific differences in three ecological fish type of the Adriatic Sea (including a gurnard specie): *Chelidonichthys lucerna* (benthic type), *Boops boops* (necto-benthic type) and *Engraulis encrasicolus* (pelagic type). X-ray powder diffraction (XRD) patterns were collected using a PanAnalytic X'Pert Pro equipped with X'Celerator detector powder diffractometer using Cu Ka radiation generated at 40 kV and 40 mA. The content of the organic matter in the otoliths was determined by thermo gravimetric analysis (TGA) using a SDT Q600 simultaneous thermal analysis instrument (TA instrument). The analysis was performed under nitrogen flow from 30 to 120 °C with a heating rate of 10 °C min⁻¹, an isothermal at 120°C for 5 min, and another cycle from 120 to 600 °C with the same heating rate. Results were reported as means of several replicas (generally three for each group of individuals) using a total of 40 otoliths. We have taken into account random and instrumental errors by propagation of uncertainty (Taylor 1997). For all samples, the XRD patterns showed only the diffraction peaks of aragonite. No transition between CaCO₃ crystalline forms were found at ontogenetic level between juveniles and adults of *C. lucerna* and *B. boops*, respectively. The diverse contents of the organic matrix in the sagittal otoliths were related to the fish species class and age, they showed a specie-specific features and variation during ontogenesis.

The otoliths' thermograms showed a first weight loss in a range around 130°-400°C followed by one between about 410 and 500°C. Around 530 °C aragonite converted into calcite in all samples, as showed by XRD diffrattograms. The total weight loss (water + OM) ranged between 2.1 and 3.4% (w/w). The juvenile individuals had greater loss than adults both for *C. lucerna* and *B. boops* suggesting that the organic matrix could have different roles depending on the stage of otolith growth. At inter-specific level, comparing TGA mean results carried out from otoliths of adult individuals, *E. encrasicolus* (pelagic type) showed greater weight loss than *C. lucerna* (benthic type) and than also *B. boops* (necto-benthic type). In conclusion, the preliminary results confirmed data reported for other species (Morales-Nin 1986; Cermeño et al. 2006) and suggest that the biomineral formation is not only genetically but also biologically and ecologically controlled, reflecting environmental and behavioural differences (Parmentier et al. 2002). Another study was the first attempt to analyze at quanti-qualitative level the organic matter of otoliths in sound producers bony fishes of Triglidae family. The role of organic matrix is not fully understood, although different studies have demonstrated that it plays a crucial role in determining the precipitation of the CaCO₃ polymorph (Mann 2001) and controlling the shape of the otolith (Miller *et al.*, 2010). We collected sagittal otoliths from target gurnard species (*Chelidonichthys cuculus*, *Chelidonichthys lucerna*, *Eutrigla gurnardus*, *Lepidotrigla cavillone*) sampled in north-middle Adriatic by MEDITS trawl fisheries. The otoliths were removed from otic capsule, cleaned from adhering tissues with 3% (v/v) H₂O₂ for 15 minutes and then sonicated in MilliQwater in the ultrasonic bath for 5 minutes. The quantitative content of the organic matter in the otoliths was determined by thermo gravimetric analysis (TGA) using a SDT Q600 simultaneous thermal analysis instrument (TA instrument). The qualitative content of the organic matter was determined, after sonication in a denaturing buffer and determination of the protein content by Lowry DC method (Biorad); the sample has been analyzed by SDS-PAGE and bands have been detected by Silver Staining (Sigma). At quantitative level, the preliminary analysis of organic matter for all species showed a very small fraction of the whole otolith ranging from 2.52% to 2.54% in terms of weight

percentage in adults and from 2.81% to 3.50% in juveniles, confirming also ontogenetic differences in organic matrix implication (Cermeño et al. 2006). At qualitative level, the preliminary analysis of the obtained gels for adult *L. cavillone*, showed the presence of three major bands under the thresholds corresponding around at 45, 30 and 20 kDa; another less obvious band is visible around 75 kDa. The band observed around 45 kDa may correspond to otolith matrix protein (OMP), one of the major components of fish sagittae according to Motta et al. (2009).

from the Mediterranean Sea using otoliths. Our main goal was to evaluate the precision of the method and compare the age estimates with results from previous independent studies that were based on either otoliths or dorsal fin spine sections. Sagittal otoliths were obtained from 278 bluefin tuna caught in the Mediterranean Sea during the period 1993-2008. In particular, a part of otoliths were obtained from specimens collected from the catches of professional fishing vessels, at landing or after tuna were transported to the wholesale market, while an additional sample was obtained from reared in sea cages specimens at Bluefin Tuna Farms. Specimens' size ranged from 8.5 to 278 cm in total length and from 7.5 g to 386 kg in total weight. Otoliths from juvenile specimens, smaller than 60 cm in fork length, were aged by enumeration of daily growth increments while otoliths from specimens larger than 60 cm were aged by enumeration of annual growth bands in otolith sections. The age estimates ranged from 20 days to 21 years and the von Bertalanffy growth parameters were determined ($L_{\infty}=330,8$ cm, $k=0,084$ and $t_0=-1,452$ years). The potential longevity of the species was found to be 35.7 years.

Abstract reference: WSAgeP_Montanini_08

Validation of first annulus formation in Triglidae family (Teleostei, Scorpaeniformes): a tool for increase the biological knowledge of a demersal resource

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Gurnards are demersal fishes exploited by commercial trawl fisheries that spend a pelagic phase during their early life history stages. There are eight different species in the Mediterranean showing intra- and inter-specific differences related to feeding habits, depth distribution and biological cycle. Although these changes have been widely reported little is known about their first life stages due to a lack of information on age and size at settlement to the bottom, length at first year of age and otolith formation. The present study is the first attempt to determine the first growth stages of six gurnard species: *Chelidonichthys cuculus*, *C. lucerna*, *Eutrigla gurnardus*, *Lepidotrigla cavillone*, *L. dieuzeidei* and *Trigloporus lastoviza* and to determine the first annuli formation. Indirect validation is made by counting presumed daily increments on two defined radii: the first radius is measured along the proximal-distal axis from the primordium to the last increment before the apparition of an accessory growth centre (AGC); the second radius is measured from the more dorsal AGC to the proximal edge of the otolith close to the sulcus acusticus and perpendicular to the increment deposition. The duration of the pelagic life phase was determined and the first annulus was defined by the position of one year-increment count in terms of distance from primordium and annulus density (*i.e.*, translucent vs. opaque). The location of discontinuous and incremental zones linked to developmental changes, the description of the species-specific patterns, as well as the measurement of increment widths are discussed in the light of the species ecology and biology.

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PELAGIC LARVAL DURATION AND SETTLEMENT MARKS
ON THE OTOLITHS OF TUB GURNARD, *CHELIDONICHTHYS LUCERNA*,
FROM ADRIATIC SEA

*DURATA DEL PERIODO PELAGICO LARVALE E MARCATORI
MICROSTRUTTURALI IN OTOLITI DI GALLINELLA,
CHELIDONICHTHYS LUCERNA, DELL'ADRIATICO*

Abstract - Larval duration of the tub gurnard, *Chelidonichthys lucerna* was estimated for the first time from samples collected in north-middle Adriatic Sea using sagittal otoliths. Settlement marks, characterized by discontinuity of daily growth increments and a re-orientation of the growth axes and otolith shape with the appearance of accessory growth centres, were determined. Pelagic larval duration was a mean of 24 days and it was marked by the development of accessory growth centres.

Key-words: larval duration, settlement mark, daily increments, *Chelidonichthys lucerna*, Adriatic Sea.

Introduction - Tub gurnard (*Chelidonichthys lucerna*, L. 1758) is the most landed and commercially important gurnard species for the Italian fisheries, particularly in Adriatic Sea (Profeta *et al.*, unpubli. data). Despite its importance and the increasingly fishing pressure (FAO, 2007), information on biological traits (especially growth and maturity) are scarce and sparse. Although studies on age and growth counting presumed *annuli* and studies on changes of habitat during the demersal life have been widely reported, little is known about their early pelagic life stages. To date only Padoa (1956) described larvae and post-larvae stages for few gurnard species and Dulčić *et al.* (2001) studied embryonic and larval development on laboratory reared tub gurnard. The development of fish ageing using daily growth increments (DGI) counting in otoliths is an important tool for population dynamic studies of the juvenile phase (Morales-Nin, 2000). The aim of this study is to develop settlement mark identification criteria and estimate settlement age from Adriatic tub gurnard specimens.

Materials and methods - Samples were collected in the north-middle Adriatic Sea from two trawl surveys carried out in autumn-winter (2002) and summer (2011). Sagittal otolith were removed from tub gurnards ranging from 61 to 277 mm TL. After embedding the otolith in epoxy resin using silicon moulds, transverse cross-sections of 300 to 500 µm were prepared using a low-speed wafering saw (Buehler IsoMet). All mounts were ground and polished with frequent microscopic control, until the core plane was reached. Thin sections of about 100 µm were finally obtained for reading using a microscope (brightfield) and storage. Each increment showed a common bipartite structure consisting in a discontinuous zone (D-zone) and an incremental zone (L-zone), which appeared respectively dark and light under transmitted light. It was assumed that the increments were laid down daily. Corroboration of interpretation criteria was made by two operators counting twice (40× magnification) presumed daily

increments along the proximal-distal axis from the *primordium* to the last increment before the apparition of an accessory growth centre (AGC). Counts per otolith were generally within 5-10% between readers, hence none were excluded. The DGI mean was considered the age of the specimen in days.

Results - Otolith sections (N=131) were unclear due to difficulties in the sectioning plane (core not located, slanted sections) resulting only in 23% of sections with an increment pattern well defined. Tub gurnard otoliths showed a quite circular *primordium* masked by organic matter. A hatch check (H) and a strong growth check identified as the presumed active feeding check (F) were visible in all the sections. The distance between the *primordium* and the first increment (H check) was $11.24 \pm 0.15 \mu\text{m}$ (mean \pm sd, standard deviation), with the first increment having a variable thickness (mean $1.49 \pm 0.08 \mu\text{m}$). Six increments were laid down between H and F checks according to Dulčić *et al.* (2001) and coinciding with the complete reabsorption of the yolk-sac. Sub-daily increments were also laid down in core area showing a fast growth. Growth increments located in the core presented a width increasing with the time to the settlement discontinuities, and a thick organic matrix layer. Wider increments were associated with a reshaping of otolith and a change in growth axis. The transition from a rounded shape to additional planes of growth was produced by the development of two to four AGCs to the left and right of dorso-ventral core region. Relative *primordium* was not easily visible because it was masked by protein deposition on the dorso-ventral axis. The deposition of the AGCs is linked to metamorphosis and habitat change (from pelagic to demersal life stage) as observed for other bony fishes (Arneri and Morales-Nin, 2000). From *primordium* to settlement, the mean otolith age was estimated at 24 ± 2.43 days.

Conclusions - DGI microstructural pattern and settlement mark were generally similar to what reported in literature for other Scorpaeniformes in different areas (McBride 2002; Ahrenholz and Morris, 2010). The results showed for the first time the growth at invalidated daily level of tub gurnard and the importance of developing a standard protocol adapted for this species. The main difficulties encountered were related to the location of the otolith core, due to a high opacity probably, linked to the big amount of protein matter in the early development, as reported by Vallisneri *et al.* (2014).

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1 **SHORT COMMUNICATION**

2

3 **Otolith shape differences of genus *Lepidotrigla* (Osteichthyes,**
4 **Scorpaeniformes): an application of geometric analysis**

5

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11

12 **Abstract**

13 The species of genus *Lepidotrigla*: *L. cavillone* and *L. dieuzeidei* have long been confused because of a
14 very similar morphology. In order to discriminate interspecific variation, otolith shape were investigated
15 in this genus for the first time using external outlines (Fourier shape analysis). Samples were randomly
16 collected at two areas in Adriatic sea (North and South of Ancona-Zara boundaries) during Grund 2007
17 scientific trawl survey. Fishes were measured for length, weight. Otoliths shape indices and outline were
18 calculated. Otolith shape analysis showed no variability in *L. cavillone* of the two areas, but
19 discriminated between *L. cavillone* and *L. dieuzeidei*.

20

21 **Keywords:** *otolith, shape indices, elliptical Fourier analysis, taxonomy, Lepidotrigla*

22

23 **Introduction**

24

25 *Lepidotrigla dieuzeidei* BLANC & HUREAU, 1973 was recorded as a new species in 1973,
26 whereas previously it had been confused with *Lepidotrigla cavillone* LACÈPEDE, 1801 and
27 its biology is little known (Olim & Borges, 2006; Vallisneri et al., 2010). The two
28 species live in the Mediterranean Sea and in Eastern Atlantic, however *L. dieuzeidei*
29 shows a more restricted depth range, generally between 100 and 200 m depth, while *L.*
30 *cavillone* occurs between 30 and 330 m depth (Tortonese, 1975; Serena et al., 1990).
31 Almost identical to *L. cavillone*, *L. dieuzeidei* stands for some hardly detectable details:
32 slightly bigger eyes, shallow depression behind the eye and lack of two small spines in
33 front of the eye (Figure 1) (Fisher et al., 1987). However, these somatic characters are

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34 not always obvious. In contrast, otoliths may represent a useful diagnostic feature
35 especially in case of breakage of the fish, or finding of otoliths in stomach contents and
36 in archaeological sites.

37 Geometric morphometrics is an excellent tool applied to biology, in particular
38 Elliptical Fourier analysis (EFA) (Mardia et al., 1979). EFA represents a precise method
39 for describing and characterizing outlines, efficiently capturing outline information in a
40 quantifiable manner. Elliptic Fourier Descriptors (EFDs) can be normalized to be
41 invariant with respect to the size, rotation, and starting point (Kuhl and Giardina 1982).
42 The aim of this study is to investigate potential differences in otolith shape in order to
43 discriminate between the two species of genus *Lepidotrigla* that are so similar for
44 external morphology to be confused.

45 46 **Materials and methods**

47
48 During the scientific bottom trawl survey Grund 2007 in the Adriatic Sea (north-central
49 Mediterranean), a total of 75 samples were collected: 50 *L. cavillone*, including 25
50 North (LC1), 25 South (LC2) of the Ancona-Zara boundary and 25 *L. dieuzeidei* (LD)
51 South. The sampling was random. The Adriatic Sea for different geographical, physical
52 and chemical properties can be divided into three parts: northern, central and south. The
53 seabed in the northern Adriatic degrades slowly from both coasts and reached a
54 maximum depth of 70 m off the coast of Pesaro. The inputs of the Po River influence
55 the nature of the seabed, from coastal sands to mud in the bottom. The central Adriatic
56 lies between Ancona-Zadar and Gargano–Kotor boundary. Its waters degrade slowly,
57 but reaches depths up to 270 m in the Pomo pit, off of St. Benedict.

58 For each specimen the total length (TL, mm) and weight (W, g) were recorded (tab.
59 1). The left sagitta was removed, cleaned using ultrasounds bath and kept dry for later
60 analysis.

61 Otolith digital images were captured using a NIKON P5100 digital camera connected
62 to a Leica MZ6 stereo microscope. The images were processed with ImageJ to calculate
63 morphometrics indices (MI) - Area, Perimeter, Circularity, Aspect ratio, Roundness,
64 Solidity - from binarized pictures (Rasband, 2010). SHAPE packages of programs
65 (Iwata & Ukai, 2002) was used for image processing, contour recording, derivation of
66 EFDs, principal component analysis of EFDs, and visualization of shape variations
67 estimated by the principal components (fig. 1). Were used 10 harmonics (40-EDFs,

68 >90% variance) and normalization of EFDs was performed by procedure based on the
69 ellipse of the first harmonic (Kuhl and Giardina 1982) which caused the degeneration of
70 the first three EFDs to fixed values: $a_1=1, b_1=c_1=0$. Therefore, each individual was
71 represented by 37 EFDs for the shape analysis. The statistical analysis of the collected
72 data was carried out by means of the R software (R Development Core Team, 2010). MI
73 and EFDs could be linked to the fish size. So, we first investigated the potential link
74 between fish size and shape indices. We found a significant relationship (Pearson
75 correlation, $P < 0.01$) between fish size and all morfometrics indices, but not EFDs. We
76 then transform the indices values in order to make them invariant with fish size, using
77 the residuals of the common-within group slope derived from the relationship between
78 fish size and indices values (Lleonart, 2000). Statistical differences in the
79 morphometrics indices measures between the different species were tested by a one-way
80 analysis of variance (ANOVA) and by linear discriminant analysis (LDA). EFDs were
81 tested by LDA to detect species otoliths shape differences. Fitness of LDA analysis
82 were tested by mean group LDA functions ANOVA and Tukey-test. Error rate of
83 classification were tested by leave-one-out cross-validation method.

84

85 **Results and discussion**

86

87 The range of size was uniform for all samples (80-120 mm); the medians were: 104 mm
88 (LC1), 88 mm (LC2), 97 mm (LD); the averages were: 101.8 mm (LC1) 90.6 mm
89 (LC2), 95.2 mm (LD). ANOVA on the distribution of lengths of the three samples are
90 normally distributed (Levene test $F_{2,72}=0.929$ $p=0.3996$) and revealed a significant
91 difference ($F_{2,72}=8.8439$, $p<0.001$); Tukey test for multiple comparisons indicated a
92 difference between LC1 and LC2 ($p < 0.001$), but not between LD and LC1 or LD and
93 LC2. So the two groups of *L. cavillone* were treated separately in subsequent analysis.
94 ANOVA analysis on MI not showed significant difference between species. LDA
95 analysis showed the same overall classification error rate of 32% for MI and EFDs
96 when calculated on three groups, but EFDs discriminated better *L. cavillone* and *L.*
97 *dieuzeidei* (4% error rate) than MI (32% error rate). EFDs LDA1 function explains more
98 variance than MI (94% against 87%, figs. 2 and 3). MI LDA1 function ANOVA
99 showed a minor significant difference between LD and two LC groups ($p<0.05$) than
100 EFDs ($p<0.001$). LC1 were not significant different from LC2 ($p>0.05$).

101 EFA has proved particularly suitable to study the shape of the otoliths, succeeding in

102 our case, to effectively discriminate the two species (*L. cavillone* and *L. dieuzeidei*).
103 Especially significant was the fact that EFA analysis, excluding the first harmonic, can
104 eliminate the “effect size”, facilitating the interpretation of results (Rohlf & Archie,
105 1984; Crampton, 1995). Reconstructing the otoliths outline average using a variable
106 number of harmonics can lead instead of focused analysis: this allows to detect also
107 very small changes in the otolith outline.

108 This study provides support of the potential benefits of otolith shape analysis as
109 cheaper and more time efficient method than either genetic or micro-chemical
110 discrimination tool. Our results in the identification of otoliths confirm that combining
111 image analysis techniques with Elliptical Fourier Analysis provided an efficient method
112 of application in fishery biology, feeding ecology and systematic (Tuset et al., 2006).

114 **Acknowledgements**

115 Financial support for this study was provided by Canziani Program of Department of
116 Evolutionary Experimental Biology, University of Bologna and RFO Program of the
117 Italian MIUR (M.Vallisneri).

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150

151 **Figure captions**

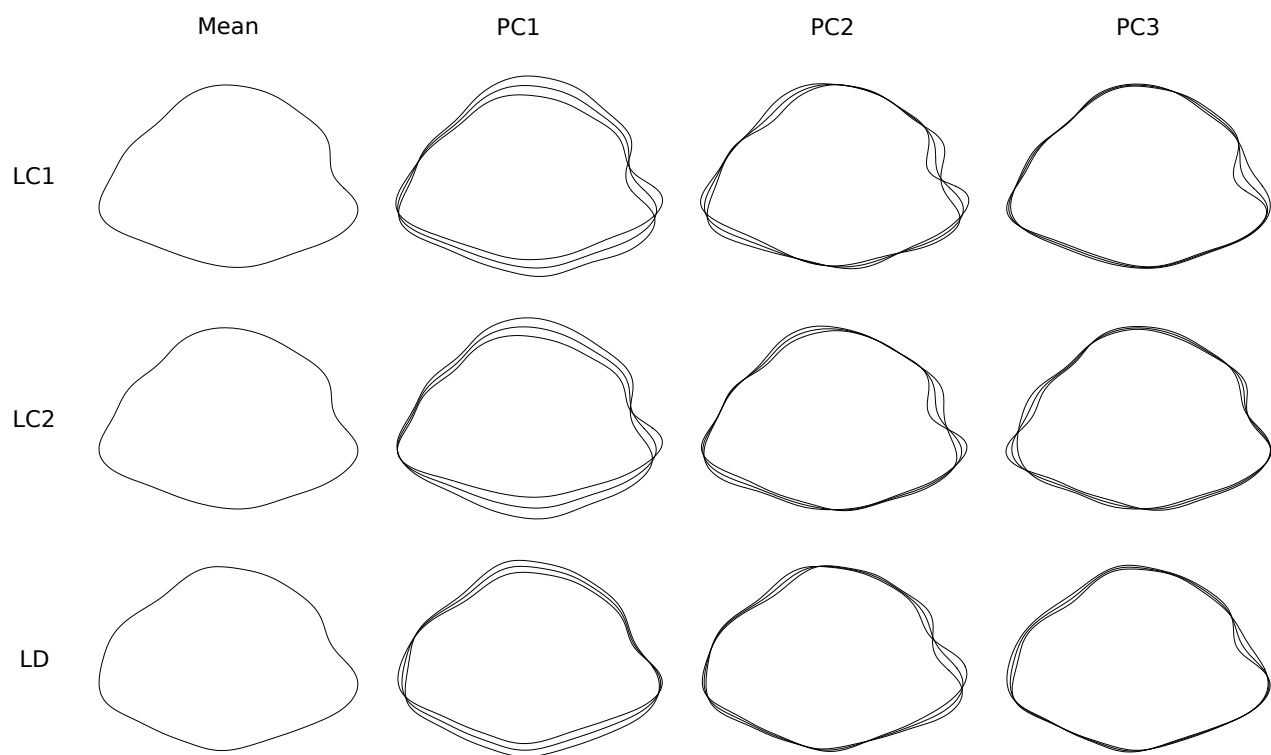
152 Figure 1. Mean outline reconstruction by EFDs principal component analysis (showed first three
153 component with overlayed -2sd and +2sd).

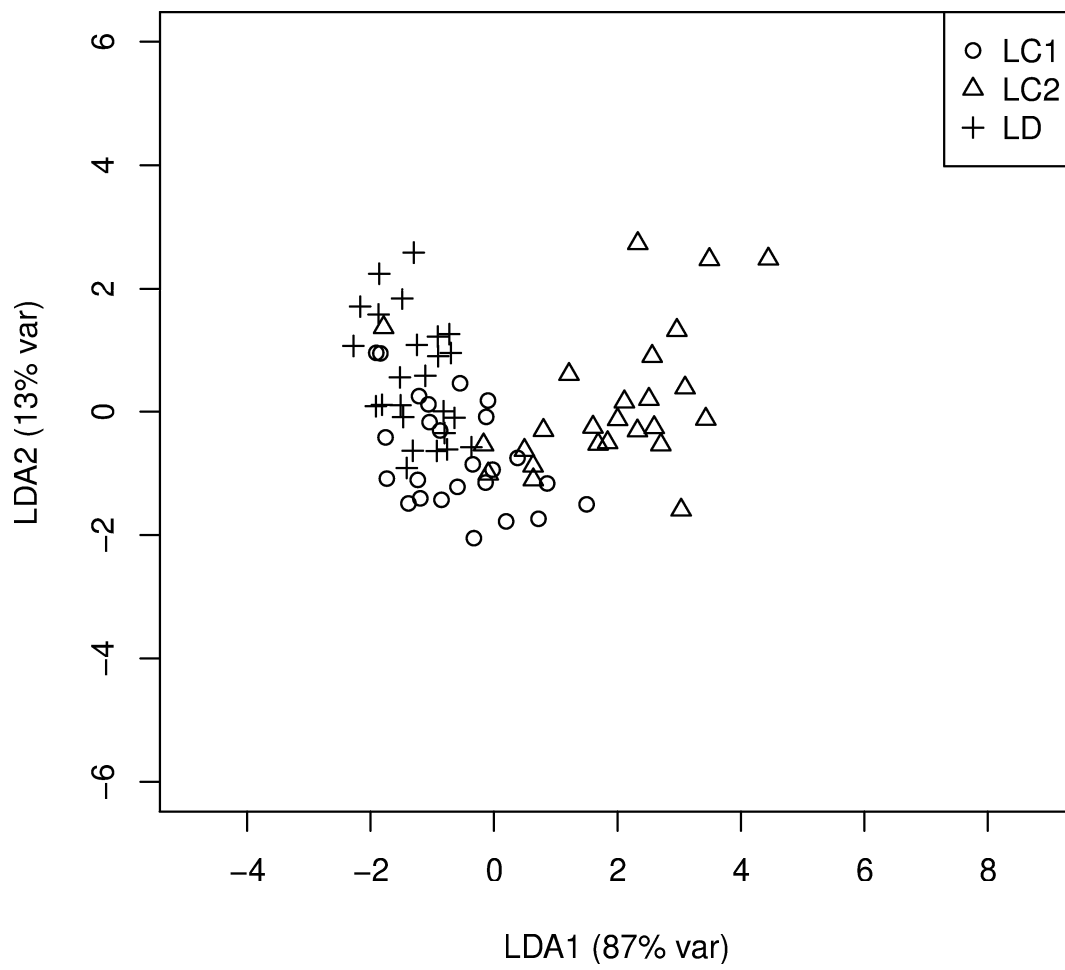
154 Figure 2. LDA plot of MI.

155 Figure 3. LDA plot of EFDs.

156 **Table caption**

157 Table 1. Biometrics data.





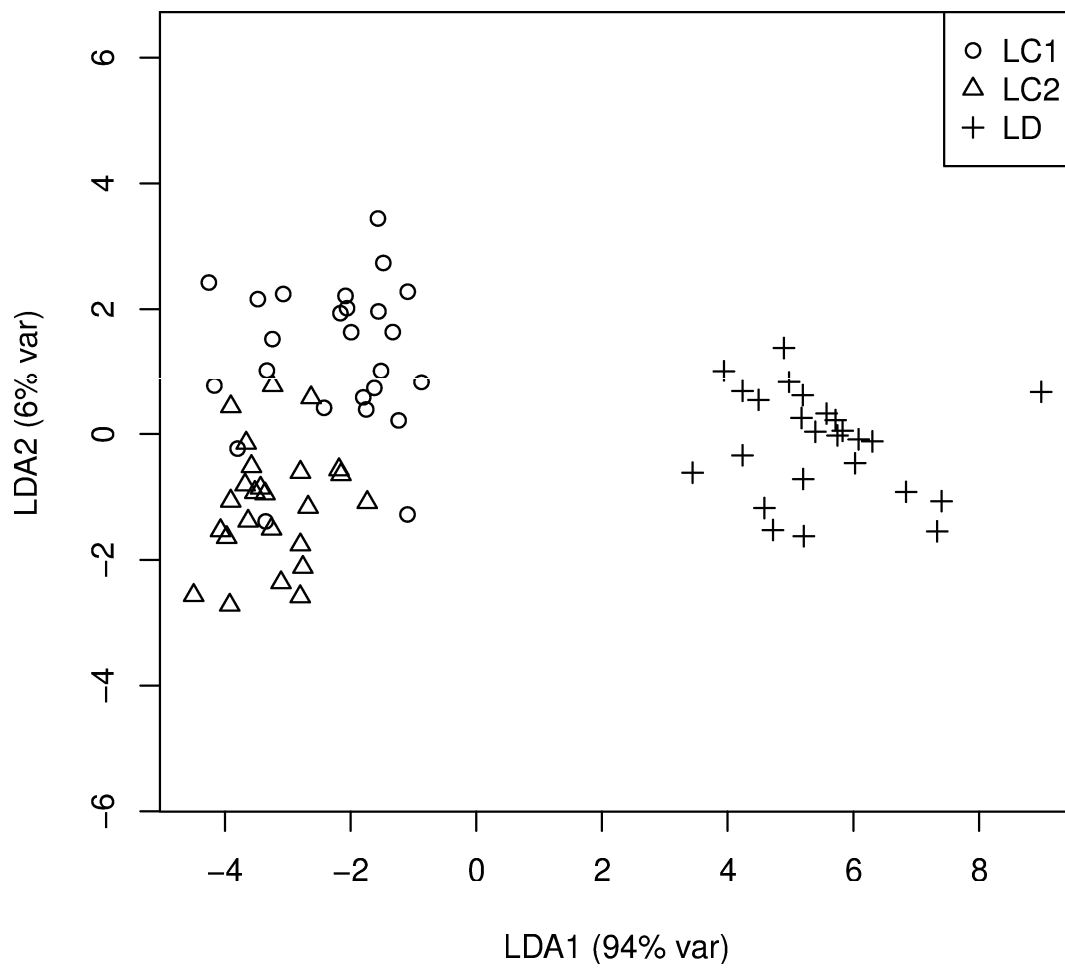


Table I. Biometrics data.

LC1 = *L. cavillone* North of the boundaries Ancona-Zara;

LC2 = *L. cavillone* South; LD = *L. dieuzeidei* South.

	Medians (mm)	Average Length (mm)	Average Weight (g)
LC1	104	101.8	12.5
LC2	88	90.6	8.2
LD	97	95.2	9.5

Short communication

Characterization of triglids (Teleostei, Scorpaeniformes) size through otolith morphometrics and shape analysis

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Abstract

The variability in otolith contour shape of triglids (*Aspitrigla cuculus* and *Chelidonichthys lucerna*) from the Adriatic Sea (north-eastern Mediterranean) was analysed for size discrimination purposes. Shape indices (aspect ratio, roundness, rectangularity, ellipticity, circularity) and Elliptic Fourier Analysis (EFA) could be used to evaluate differences in otolith growth steps. Significant differences were found at critical size, between juveniles and adults. The combined use of the two external outlines methods should be highly informative for intraspecific discrimination. The differences in shape could be related to endogenous (changes in metabolic and physiological conditions as sexual maturity) and exogenous causal factors (change of environmental conditions linked to depth, substrate, diet).

Keywords: Sagitta; Shape indices; Elliptic Fourier Analysis; Triglidae; Adriatic Sea

Introduction

Otoliths are sound transducers and play an important role in fish hearing. Otoliths are considered as very useful tools to study the various events that characterize fish life cycles (Campana 1999; Lecomte-Finiger 1999; Newman and Dunk 2002; Pothin et al., 2004, 2006).

Otolith shape is species-specific (Campana and Casselman, 1993, Aguirre and Lombarte 1999; Parmentier et al., 2001) and it may vary within each species according to fish size, geographical sites, environmental factors as depth (Lombarte and Leonart 1993; De Vries et al., 2002; Cardinale et al. 2004). The ecomorphological link hypothesis of otolith shape was carried out in relation to life history, biological and behavioural characteristics of fish species (Volpedo and Echeverria 2003) or the type of swimming activity (Lychakov and Rebane 2000).

Triglidae (Teleostei, Scorpaeniformes) are known for their sound producing ability in agonistic contexts related to territorial defense, reproduction, and competitive feeding (Amorim *et al.*, 2004).

Aspitrigla cuculus is to be found throughout the Mediterranean and in Eastern Atlantic, from Scandinavia to Mauretania. Along the Italian coasts it lives down to 400 m with the highest density between 100 and 200 m (Relini et al., 1999). Juveniles occur in shallower waters than adults (Serena et al., 1990; Colloca et al., 1994). The biggest individuals live below 200 m. Females reach maturity around 14 cm SL (Colloca et al., 1990).

Chelidonichthys lucerna lives in the Mediterranean Sea, Black Sea and Eastern Atlantic from Norway to Senegal. Along the Italian coasts generally it occurs up to 200 m depth. The juveniles appear in spring-summer on sand-muddy bottoms between 10 and 20 m (Frogliola, 1976). A significant body size-depth relationship was pointed out; tub gurnards, as they grow, leave the nursery and are found mainly on detrital bottoms up to 150 m depth (Colloca et al., 1994; Biagi et al., 1996).

Aspitrigla cuculus and *Chelidonichthys lucerna* change diet during growth with the capture of bigger-sized prey and the replacement of food categories and bathymetric migration (Colloca *et al.*, 1994). Juveniles feed upon benthic crustaceans, mainly Decapoda Natantia, Mysida and Amphipoda. Adults prey increasingly on decapods and teleost fishes as body size increases (Froglia, 1976; Colloca *et al.*, 1994). Juveniles of two species occur in shallower waters than adults (Serena *et al.*, 1990; Colloca *et al.*, 1994) and they showed a significant body size-depth relationship (Colloca *et al.*, 1994).

The aim of this study was to analyze intraspecific shape variations in otolith (through shape indices and elliptic Fourier descriptors) of gurnards belonging to several size-classes and conditions.

Materials and methods

A total of 217 specimens were collected during bottom trawl surveys in the Adriatic Sea, from the Gulf of Trieste (45°40'N 13°37'E) to the Tremiti Islands (42°08'N 15°16'E) between 2007 and 2008. For each specimen the total length (TL, mm), weight (W, g) and sex were recorded. For each specimen the left sagitta was removed, cleaned in ultrasounds bath and kept dry for later analysis. Otolith digital images were captured using a NIKON P5100 digital camera linked to a Leica MZ6 stereomicroscope. The images were processed with ImageJ (Rasband, 2010) to calculate five geometric shape indices (aspect ratio, roundness, rectangularity, ellipticity and circularity). Geometric indices for which “species-length” interactions were significant ($P < 0.001$), were normalised. The SHAPE program (Iwata and Ukai, 2002) was used to extract the outline of the otoliths and to assess the variability of shapes, using the EFA, and estimated it through the study of principal component analysis (PCA). The statistical analysis of the collected data was carried out by means of the R software (R Development Core Team, 2010).

Results

The score plots for the discriminant functions showed a distinct separation between juveniles and adults for the two analyzed species. For shape indices the study of linear discriminant analysis (LDA) showed separation between class-size linked to transition from immature to mature stage. These shots were determined for *A. cuculus* to 120 mm (Fig.1) and for *C. lucerna* to 180 mm (Fig. 2). For the EFA method the study of principal component analysis (PCA) showed an increase in the variability of otolith shapes from juveniles to adults for all the components analyzed. About 99% of variation in otolith shape was explained by a maximum of 20 harmonics. The first 4 discriminated over 80% of the variance. The general allometric trends observed for the otolith morphology corresponded to a relative elongation of the *sulcus acusticus* (linked to PC1) and an increase of *excisura ostii* area (linked to PC3).

Discussion

This finding was probably related to individual genetic factors (Gauldie and Crampton, 2002) and to the different depths of the trophic niches occupied. In fact, *A. cuculus* and *C. lucerna* showed an increase in the variability of otolith shape linked to growth and to environmental conditions. In particular, they showed a critical size between juveniles and adults that coincides with the slope to great depths, the migration by the Italian toward Croatian coast, the start of sexual maturity and the change of their main food, from crustaceans to fishes (Montanini *et al.*, 2008).

In conclusion, it is important evaluate the differences in the shape of sagittal otolith during growth because these age variations could be confused with stocks variations.

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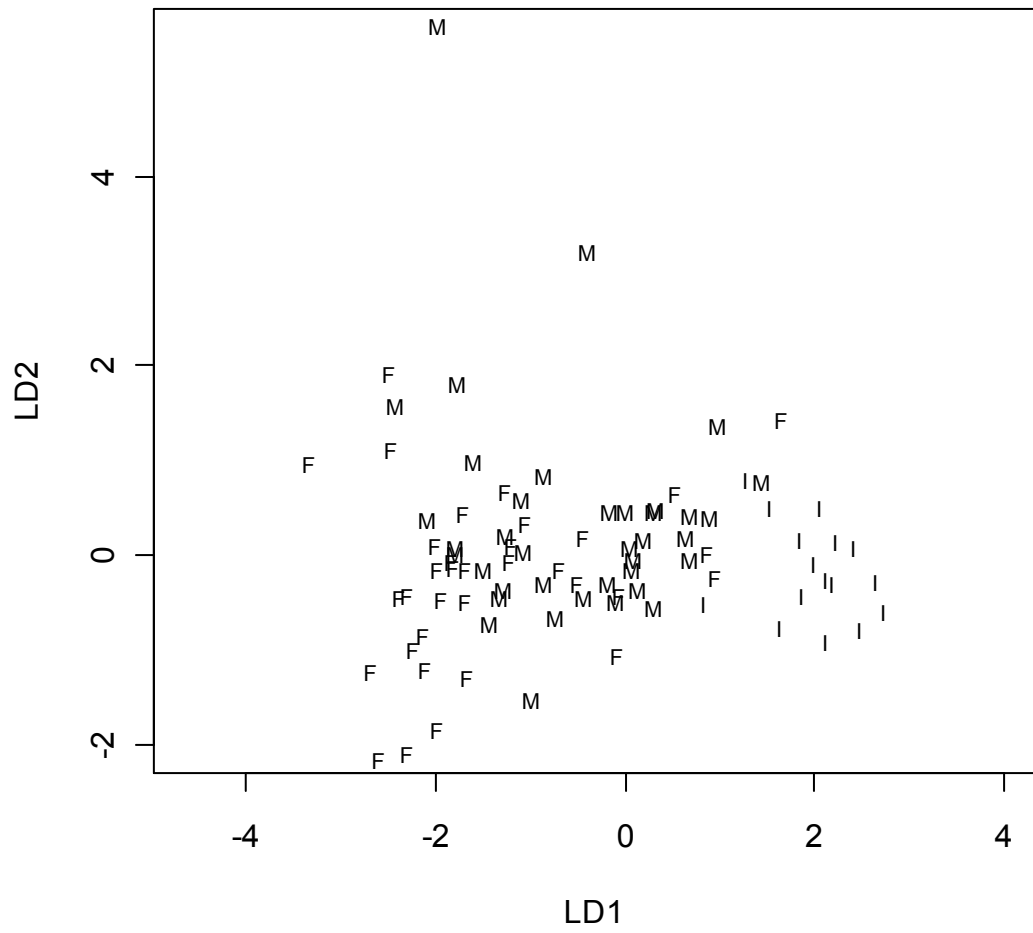
Figure captions

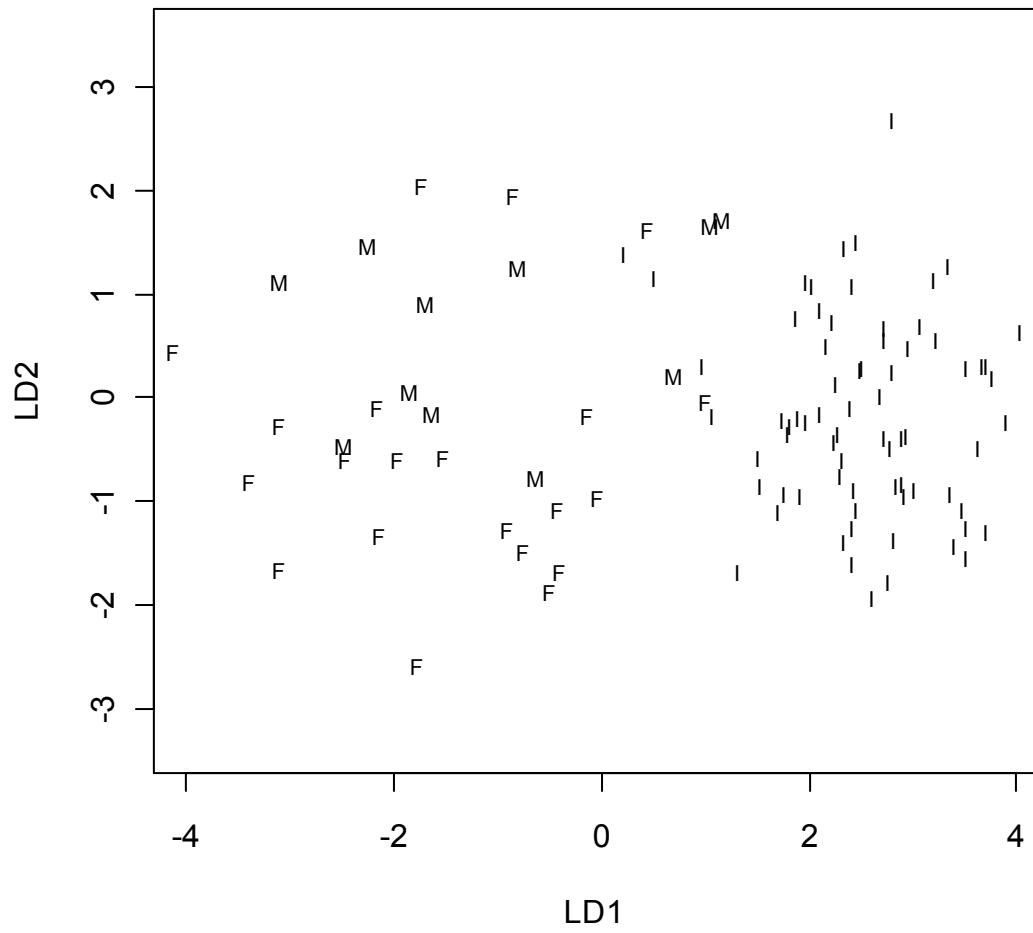
Fig.1. Discriminant analysis function scores for juveniles and adults of *Aspitrigla cuculus* based on geometric indices. Female (F), Male (M), Juveniles (I).

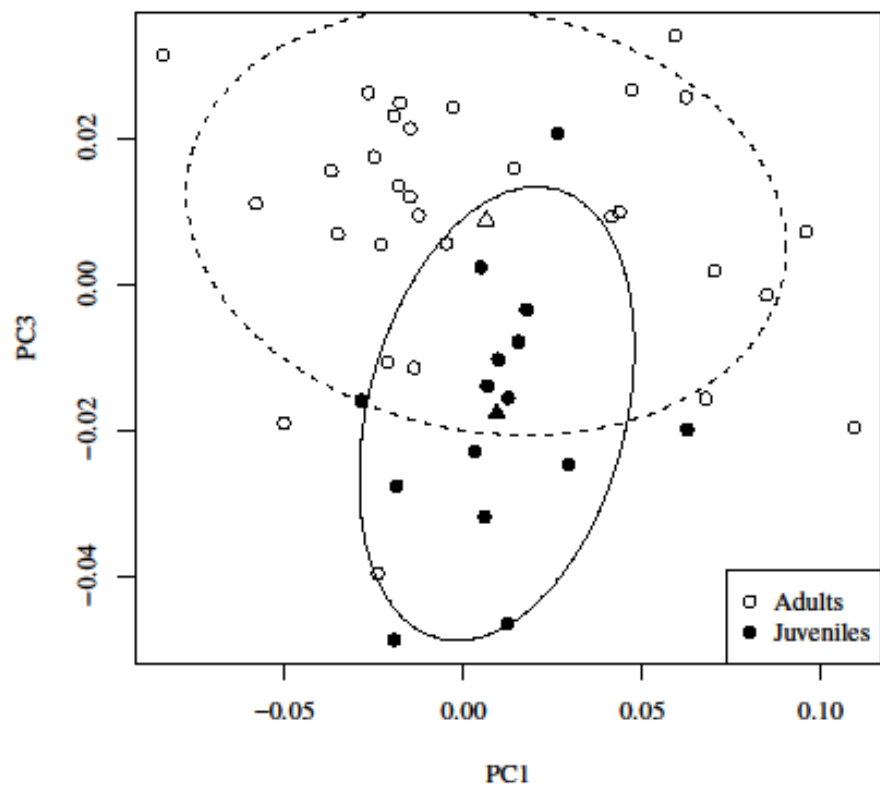
Fig.2. Discriminant analysis function scores for juveniles and adults of *Chelidonichthys lucerna* based on geometric indices. Female (F), Male (M), Juveniles (I).

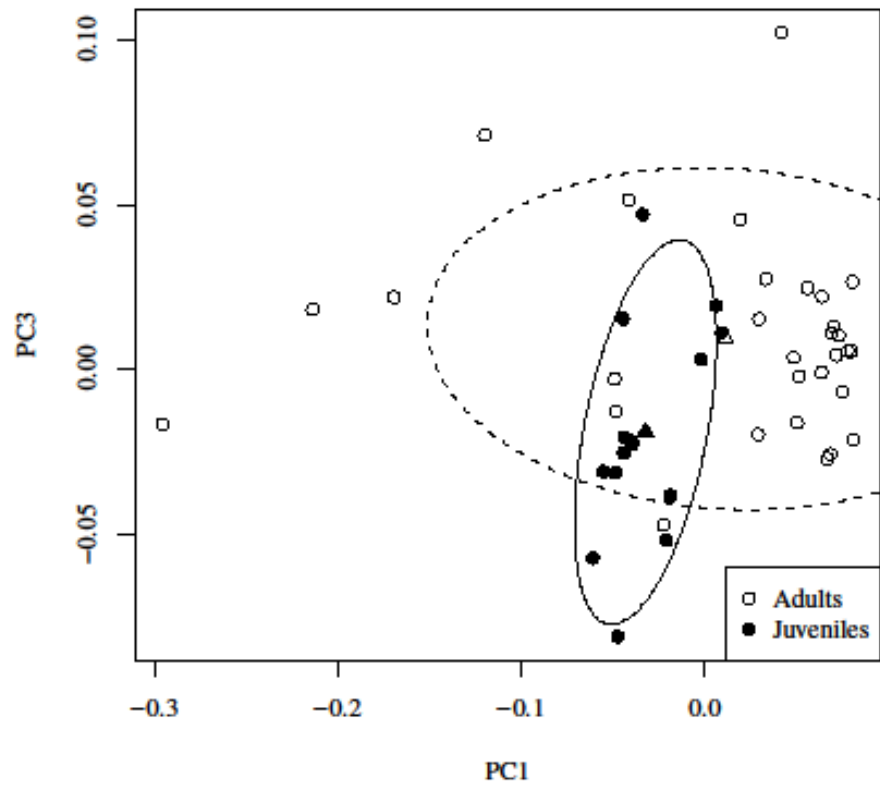
Fig.3. Otoliths PCA plot between adults and juveniles of *Aspitrigla cuculus*. Ellipses include 75% confidence interval.

Fig.4. Otoliths PCA plot between adults and juveniles of *Chelidonichthys lucerna*. Ellipses include 75% confidence interval.









1 **Fish-otolith size relationships for Triglidae in the Adriatic Sea (north-**
2 **eastern Mediterranean)**

3
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12

13 SUMMARY: The aim of the study is to improve the information about age and growth of
14 these species and to value qualitatively and quantitatively the process of somatic growth
15 parameters (fish total length) in relation to bones structure parameters (length, width and total
16 mass of otolith). Positive allometric growth was observed for both females and males and also
17 for all fish, weight increased allometrically with length. The biometric parameter otolith width
18 (OW) expressed greater variance than the other parameters (OL - Otolith Length; OM -
19 Otolith Mass). Comparison between sexes was possible for *C. lucerna*, but the regressions
20 were not statistically different.
21

22 *Keywords:* gurnards, otolith morphology, length-weight relationship, fish-otolith sizes,
23 Adriatic Sea.
24

25 RUNNING TITLE: Fish-otolith size relationships for Triglidae in the Adriatic Sea
26

27 INTRODUCTION

28 Gurnards are demersal fish which inhabit the continental and insular shelves of tropical
29 and temperate seas to depths of 500 m, found on sandy, muddy or rubble substrates (Fischer
30 *et al.*, 1987). There are seven different species in the north-middle Adriatic Sea (13°-15°E,
31 42°-45°N) showing differences related to biometric features and ecological ones such as diet,
32 spawning period and depth distribution (Tsimenides *et al.*, 1992; Colloca *et al.*, 1994, 2003).
33 They spending a pelagic phase during their early life history stage. During demersal life cycle
34 the change of their ecologic behaviours are linked to the start of sexual maturity, the tendency
35 to migrate to greater depths and change of diet from crustaceans to fish with a wider trophic
36 spectrum (Montanini *et al.*, 2008, 2010; Vallisneri *et al.*, unpublished data). Red gurnard
37 (*Aspitrigla cuculus* Linnaeus, 1758), tub gurnard (*Chelidonichthys lucerna* L., 1758) and grey
38 gurnard (*Eutrigla gurnardus* L., 1758) are the three major species landed and most
39 commercially important Triglidae species in Mediterranean and therefore have been included
40 in the list of target species of the Medit project (Relini *et al.*, 2008).

41 Otolith structural analyses have contributed to our understanding of ecological and
42 oceanographic processes relevant to ecosystem-based management (Sponaugle 2010). The
43 greatest application of otoliths to date has been in providing information on the age and
44 growth of fish in years, which has ultimately been used in the management of associated
45 fisheries (Campana and Thorrold 2001, Campana 2005). The knowledge of age and growth
46 provide to give information about the early life stages of fishes, clarifying the effects of
47 changes in the environment on growth and survivals and about factors affecting recruitment
48 success; in adults is used to determine the effect of fishing on the stock, the efficacy of
49 management policies, to understand life history events and to maximize yield while still
50 ensuring the future of the resource (Jones 1992). Otoliths are also useful in trophic biology
51 studies. In most cases hard structure such as sagittae otoliths are the only diagnostic features

52 of preys remain in digestive tracts, faeces and scats of predators and have been extensively
53 used for identification of prey. In order to estimate body size and prey biomass it useful
54 calculate relationships between otolith measurements and fish ones by specific regressions
55 such as has been widely shown in many studies (Correa and Vianna 1993, Granadeiro and
56 Silva 2000, Harvey *et al.* 2000, Waessle *et al.* 2003, Battaglia *et al.* 2010). This type of
57 studies provide to give indirect information of fish distribution and also for quantifying the
58 potential effects of natural predation (Correa and Vianna 1993, Mauco *et al.* 2001, Waessle *et*
59 *al.* 2003). It is important evaluate prey-predator relationships in all the steps of the food chain
60 mostly for species that have commercially relevant or could feed other commercial fish and
61 crustaceans as in the case of gurnards in Adriatic Sea. They prey anchovy, shrimps and mantis
62 such as reported in many studies (Frogliola *et al.*, 1974, Colloca *et al.*, 1994, Floeter and
63 Temming 2005, Montanini *et al.*, 2008).

64 The aim of the study is to improve the information about age and growth of these species
65 and to value qualitatively and quantitatively the process of somatic growth parameters (fish
66 total length) in relation to bones structure parameters (length, width and total mass of otolith).
67 There are not previous studies of the above species in the area where the present study took
68 place. These data could be use to feeding studies, to value which parameters influenced
69 growth pattern of otoliths and to provide a helpful tool for giving information about structure
70 and biology of gurnards in the Adriatic area.

71

72 **MATERIALS AND METHODS**

73 A total of 322 gurnards (101 *A. cuculus*, 114 *C. lucerna*, 107 *E. gurnardus*) were collected
74 from December 2007 to December 2008 in a sampling area of 59400 km² during seasonally
75 oceanographic bottom trawl surveys (winter survey: GRUND project, GRUpo Nazionale
76 Demersali; summer survey: MEDITS project, MEDiterranean International Trawl Survey)

77 using as sampling gear a bottom trawl made of four panels and a mesh codend size of 20 mm
78 (stretched mesh) (Relini *et al.*, 2008). The surveys were carried out at depths ranging from 14
79 to 245 m in the north-east Mediterranean (north-middle Adriatic Sea from the Gulf of Trieste
80 to the line joining the Gargano-Dubrovnik) (Fig. 1).

81 The total body length (TL, mm) and total weight (W, g to the nearest 0.1 g) were
82 measured. According to the codes of sexual maturity for fish (Relini *et al.* 2008) four
83 categories of sexes, Females, Males, Undetermined (impossible to determine it by eye), Not
84 Determined (the individual has not been examined) and maturity stages were recorded.
85 Length-weight relationship for every species was performed using the potential function:

$$86 \quad \log W = \log(a) + b \log(L)$$

87 where W is the total weight of the fish (g), L is the total length (mm), *a* is the intercept on the
88 Y-axis of the regression curve and *b* is the regression coefficient. To test for possible
89 significant differences between the estimated *b*-values and *b* = 3 *t*-test was used and 95%
90 confidence limits for parameters *a* and *b* were calculated.

91 The left sagitta of all specimens were extracting from the skulls by the sagittal cut method,
92 cutting the heads from mouth to the back. Otoliths were removed from otic capsule by a pair
93 of forceps, cleaned using ultrasound bath and kept dry. Digital images of all the otolith
94 samples were collected using a NIKON P5100 digital camera linked to a Leica MZ6
95 stereomicroscope. Each sagitta was photographed with the *sulcus acusticus* facing up and the
96 *rostrum* to the right. The left sagittal otolith of representative adult of each species was
97 photographed by scanning electron microscopy (SEM HITACHI S-2400) in order to describe
98 otolith morphology according to the terminology used by Tuset *et al.* (2008). Maximum
99 otolith length (OL) and width (OW) were measured by ImageJ program. Otolith total mass
100 (OM) was weighed to the nearest 0.1 mg. For each species the relationships between left
101 otolith measurements and fish total length were described by both linear and non-linear

102 regression models. Statistical procedures followed Zuur *et al.* 2009. (The Shapiro-Wilk test
103 was performed to check if residuals look normal.) For $p\text{-value} < 0.001$ data were divided into
104 subgroups (small-sized individual, medium-sized individual and large-sized individual) linked
105 to size range and maturity stage according to the null hypothesis that residuals were normal.
106 The following morphometric relationships were analysed: fish length (TL) – otolith length
107 (OL), fish length (TL) – otolith width (OW) and fish length (TL) – otolith total mass (OM)
108 and the parameters a , b and the coefficient of determination (r^2) were estimated. In each case
109 we adopted the relationship which explained the highest proportion of variance (highest value
110 of r^2). The significance of all regressions was verified using the F -test. Also we proceeded to
111 investigate differences between sexes (females and males). To check no significance
112 differences of size range t -test was employed ($p > 0.05$). ANOVA was using to test differences
113 between regression coefficients of females and males. All analyses were performed with R
114 Development Core Team ver. 2.10 (2010).

115

116 **RESULTS**

117 **Otoliths morphology**

118 Representative otoliths for each species are shown in Figure 2. Within the family
119 Triglidae showed a tendency towards a similar structural morphology. The family tended to
120 show a oval shape with irregular margin. The anterior region has one to two spikes. The
121 posterior region has tended to round up to irregular or oblique bifida. Basically *sulcus*
122 *acusticus* appears ostial, that opens to the *ostium* in the anterior region. In addition, the *sulcus*
123 is generally located on the longitudinal midline of otolith so ventral and dorsal areas have
124 more or less the same size. *Ostium* and *cauda* are different: the *ostium* is generally funnel-
125 shaped and shorter than the *cauda*. The *cauda* is generally tubular and curved, more or less
126 bent towards the posterior region.

127 **Length – weight relationships**

128 The regression of total wet body weight (W) as a function of total length (TL) were
129 statistically significance in all species ($p < 0.001$). The coefficient of determination being
130 higher than 0.97 in all species (Table 1). b values ranged from 3.07 to 3.22. Statistical
131 analyses (t -test) and calculation of confidence limits of a and b revealed significant
132 differences from the theoretical value of 3 ($p < 0.001$) and positive allometric growth for all the
133 gurnards considered. Considering sexes (females and males) b values ranged from 3.02 to
134 3.35. For both sexes of each gurnard isometric growth was showed, in fact no significance
135 difference were found to value $b = 3$ (t -test $p > 0.001$) (Table 1).

136 **Fish – otolith size relationships**

137 Three morphometric measurements of sagitta were analysed. The range values of
138 individual maximum otoliths lengths (OL), otolith width (OW) and otolith total mass (OM)
139 for each species were reported in Table 2.

140 The Shapiro-Wilk normality test was high significant in all the regressions of each species
141 (nonnormality distribution) so sample were divided into 3 subgroups (smaller, medium and
142 large) linked to size range and maturity stage associated. Only for *C. lucerna* subgroup
143 medium was further divided into 2 groups (medium1 and medium2) due to high range
144 parameters variability.

145 There was a linear relationships between otolith morphometric parameters vs fish total
146 length (TL) (F -test $p < 0.001$ in all cases) (Table 3; Fig. 3). Comparing the values of r^2 , data
147 fitted well to the regression model for OW vs TL for each species which explained from 91%
148 to 94% of the variance (*A. cuculus* $r^2 = 0.94$, *C. lucerna* $r^2 = 0.94$, *E. gurnardus* $r^2 = 0.90$,
149 respectively). OL vs TL and OM vs TL relationships showed significance values of r^2 for two
150 species (OL vs TL: *A. cuculus* $r^2 = 0.90$ and *C. lucerna* $r^2 = 0.95$, OM vs TL: *A. cuculus* $r^2 = 0.94$

151 and *C. lucerna* $r^2=0.93$). Only for *E. gurnardus* potential regression model explained lower
152 than 90% of the variance (OL vs TL $r^2=0.86$, OM vs TL $r^2=0.86$).

153 Only for *C. lucerna* it was possible performed ANOVA to test if the slope of the otolith
154 size to body size differ between sexes (size range t -test $p>0.001$). Eliminating the contribution
155 of interaction between regression and sex (no significance interaction F -test $p>0.001$), no
156 significance difference were found between females and males (b values weren't statistically
157 different).

158

159 **DISCUSSION**

160 Data from this study provides to widen the knowledge about the Mediterranean gurnards,
161 in particular using otoliths as a powerful taxonomic feature for fish species identification and
162 for better understanding structure and trophic relationships of demersal communities.

163 Positive allometric growth was observed for both females and males and also for all fish,
164 weight increased allometrically with length (Uckun and Togulca 2007). Male of red gurnard
165 and tub gurnard grow faster than the females (which had greater longevity) (Papaconstantinou
166 1983, Papaconstantinou 1984, Uckun and Togulca 2007) and reach smaller lengths than males,
167 according to literature about other areas (Serena *et al.* 1998-Tuscany coast, Ismen *et al.*, 2004-
168 Iskenderun Bay, Eryilmaz and Meric 2005-Sea of Marmara, Boudaya *et al.* 2008-Gulf of
169 Gabès, Vallisneri *et al.* in press-Adriatic coast).

170 Papaconstantinou (1984), Tsimennides *et al.* (1992) and Montanini *et al.*, 2008 stated that
171 the larger fish migrate to greater depths and that this more evident among females, mainly
172 because of their greater length and life-span. A significant size-depth relationship was
173 observed for juveniles (T.L. < 7 cm) and adult of large scaled-gurnard (Colloca *et al.* 1994):
174 at the end of the first year life juvenile migrate deeper form the coastal nursery; the latter
175 moved shallower toward the spawning grounds. Reduction of growth coincided with

176 attainment of sexual maturity and a change in the feeding strategy of the species (Colloca *et*
177 *al.* 1994). Studies of distribution of grey gurnard in North Sea (ICES 2006) showed seasonal
178 northwest-southeast migration pattern from cold to warmer coasts linked to spawn. For the
179 North sea case, this withdrawal may reflect the southerly origin of the species.

180 Analysis of otolith morphometric parameters related to the total length of the fish, shows
181 that the regression model expresses well the data for the two species (*Aspitrigla cuculus* and
182 *Chelidonichthys lucerna*) since the coefficients of the report are generally good (coefficient
183 determination $r^2 > 0.90$). Only in the case of *Eutrigla gurnardus* coefficients are lower than
184 90% ($r^2 > 0.86$) The biometric parameter otolith width (OW) expressed greater variance than
185 the other parameters (OL - Otolith Length; OM - Otolith Mass). This seems to be due to the
186 fact that the measure of the otolith width, obtained from the digital images, lends itself to be
187 more accurate than both the length measurement otolith, for the presence dell'excisura ostii
188 more or less defined and / or from the rostrum more or less elongated, and the measure of the
189 weight otolith. For species with $r^2 < 0.90$ would be desirable to continue the search with
190 appropriate sampling that take into account also the homogeneity of the environmental
191 parameters that may affect the regression, such as the depth, as well as the influence of
192 genetic factors.

193 About sexes (male and females) comparison was possible for *C. lucerna*, but the
194 regressions were not statistically different.

195 In conclusion, the present work has helped to provide new information on otolith
196 morphometry in relation to body size of fish species belonging to Triglidae, a family of
197 bottom fish still quite unknown. In fact the results are used to assess in terms of the process
198 and quantitative growth of somatic parameters (length, body weight) in relation to the
199 parameters of bone structures as otoliths (length, width, weight otolith). This makes it
200 possible to determine the growth trend related to age, to the population, habitat, geographic

201 area. Furthermore, these data could be used in studies of trophic biology or archaeozoology to
202 determine the length and weight of fish preyed using morphometric parameters of the otoliths
203 recovered in the food or in the fossil remains. Ultimately, this information may be used at the
204 management level, as indirect method for assessing the distribution of fish preyed species and
205 to quantify the potential effects of predation.

206

207 **ACKNOWLEDGEMENTS**

208 We thank Pr. Corrado Piccinetti for his assistance in bottom trawl surveys and for his
209 valuable suggestions in the drafting of the paper.

210

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276 TABLE 1. – Relationship between fish length and fish weight of gurnards from the Adriatic
 277 Sea referring to total samples (females, males, undetermined and not determined) and
 278 sexes (females and males). Number of individuals (n), ranges of fish lengths (TL), fish
 279 weights (W), confidence limits of a (95% CL (a)), confidence limits of b (95% CL (b))
 280 and coefficient of determination (r^2) were indicated. All regressions were statistically
 281 significant at $p < 0.001$.

282

Species	n	TL range (mm)	W range (g)	Equation	95% CL (a)	95% CL (b)	r^2
<i>A. cuculus</i>	101	82-209	4.5-96.6	$W=2.927e-06*TL^{3.223}$	2.046e-06 - 4.188e-06	3.150 - 3.295	0.987
Females	34	102-209	7.7-96.6	$W=1.564e-06*TL^{3.347}$	5.521e-07 - 4.431e-06	3.143 - 3.551	0.972
Males	38	112-190	9.6-64	$W=2.403e-06*TL^{3.260}$	9.246e-07 - 6.246e-06	3.067 - 3.452	0.971
<i>C. lucerna</i>	114	87-340	5.7-446.1	$W=6.762e-06*TL^{3.071}$	4.175e-06 - 7.597e-06	3.045 - 3.164	0.990
Females	20	153-340	33.4-446.1	$W=5.564e-06*TL^{3.111}$	1.748e-06 - 1.771e-06	2.898 - 3.323	0.907
Males	11	136-306	23.3-252.9	$W=8.017e-06*TL^{3.030}$	2.146e-06 - 2.995e-05	2.784 - 3.275	0.987
<i>E. gurnardus</i>	107	74-234	2.9-113.5	$W=3.824e-06*TL^{3.156}$	2.481e-06 - 5.892e-06	3.066 - 3.245	0.972
Females	51	100-234	8.1-113.5	$W=2.468e-06*TL^{3.244}$	9.417e-07 - 6.466e-06	3.050 - 3.438	0.958
Males	37	93-161	6.2-33.8	$W=7.368e-06*TL^{3.019}$	2.979e-06 - 1.822e-05	2.830 - 3.209	0.968

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284

285 TABLE 2. – Range values of observed otolith dimensions (otolith lengths OL, otolith width
 286 OW and otolith total mass OM) for each gurnard.

287

Species	n	OL range (0.1 mm)	OW range (0.1 mm)	OM range (0.1 mg)
<i>A. cuculus</i>	101	20.8-40.7	15.2-29.4	15-110
<i>C. lucerna</i>	114	16.4-54.1	12.5-36.9	8-135
<i>E. gurnardus</i>	107	17.8-39.6	12.9-26.6	9-60

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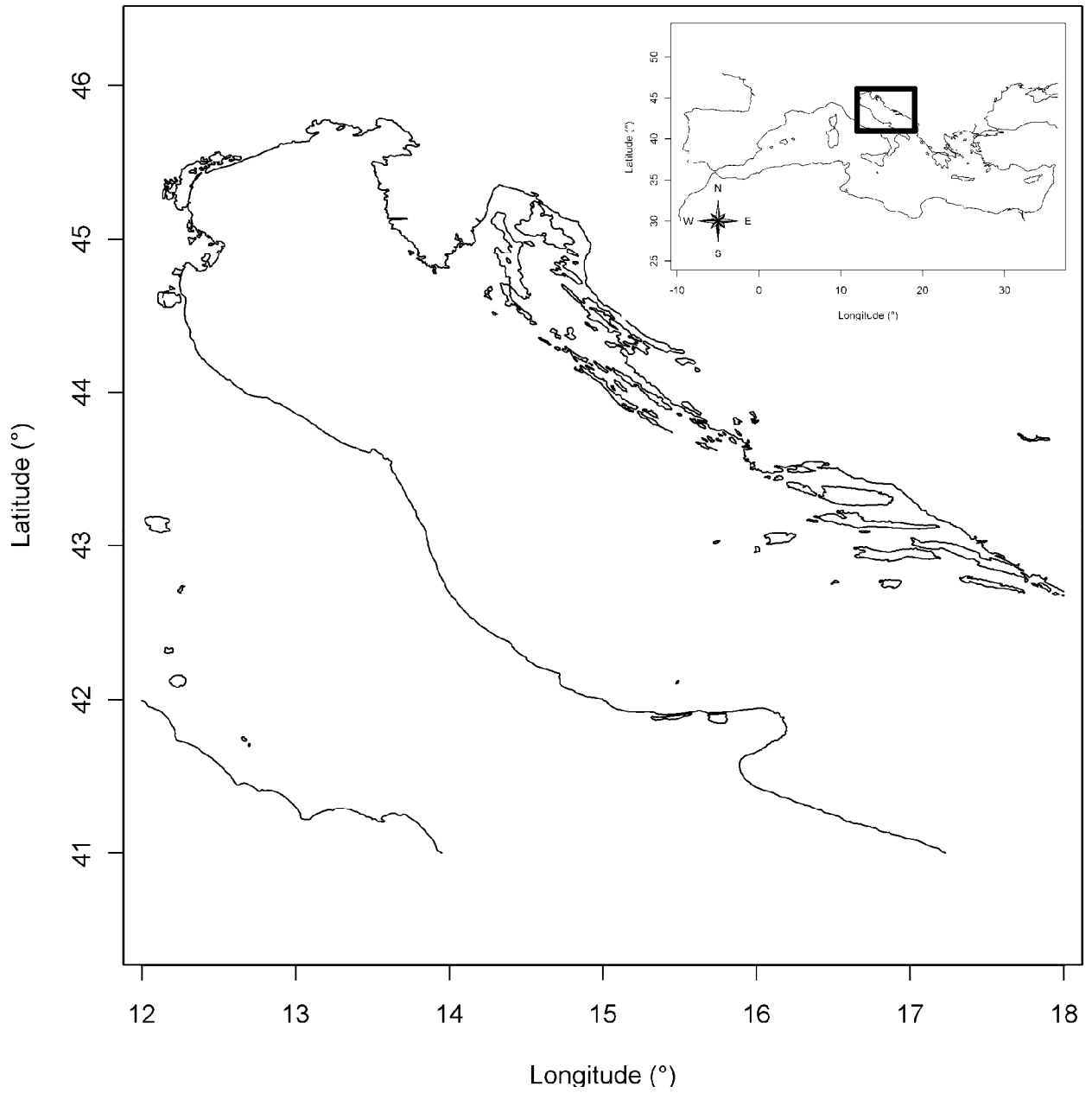
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291 TABLE 3. – Relationship between otolith morphometric parameters and total length (TL) of
 292 gurnards sampled in the Adriatic Sea. Number of individuals (n) and coefficient of
 293 determination (r^2) were indicated. OL: otolith length, OW: otolith width, OM: otolith
 294 mass. All regressions were statistically significant at $p < 0.001$.

Species	n	OL vs TL	r^2	OW vs TL	r^2	OM vs TL	r^2
<i>A. cuculus</i>	101	$TL=1.1228*OL^{1.435}$	0.903	$TL=1.8085*OW^{1.411}$	0.940	$TL=2205.8*OM^{0.506}$	0.938
<i>C. lucerna</i>	114	$TL=3.1174*OL^{1.170}$	0.948	$TL=2.6332*OW^{1.319}$	0.942	$TL=2292.1*OM^{0.468}$	0.934
<i>E. gurnardus</i>	107	$TL=2.6115*OL^{1.225}$	0.861	$TL=2.2563*OW^{1.389}$	0.901	$TL=2583.7*OM^{0.485}$	0.862

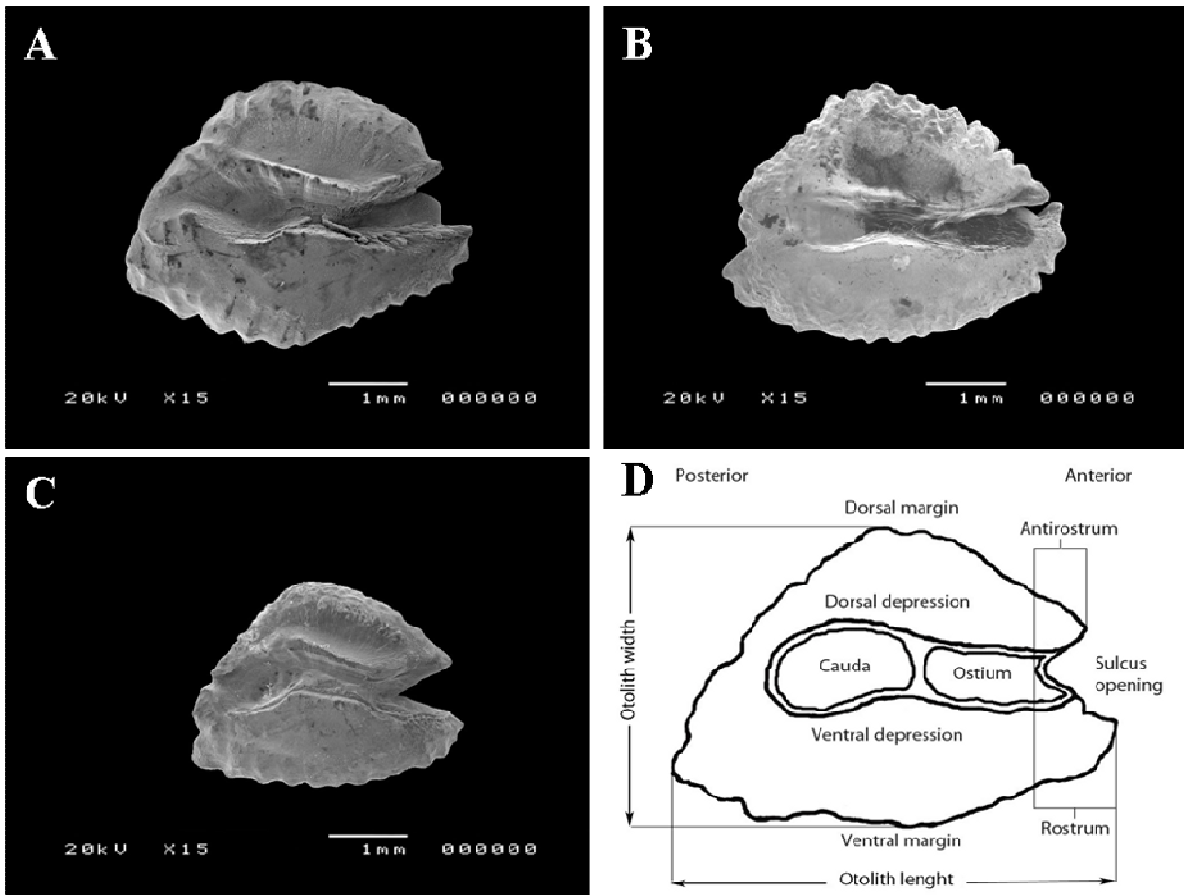
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312 FIG. 1. – Map showing the sampling area of garnards in the north-middle Adriatic Sea (north-
313 eastern Mediterranean).

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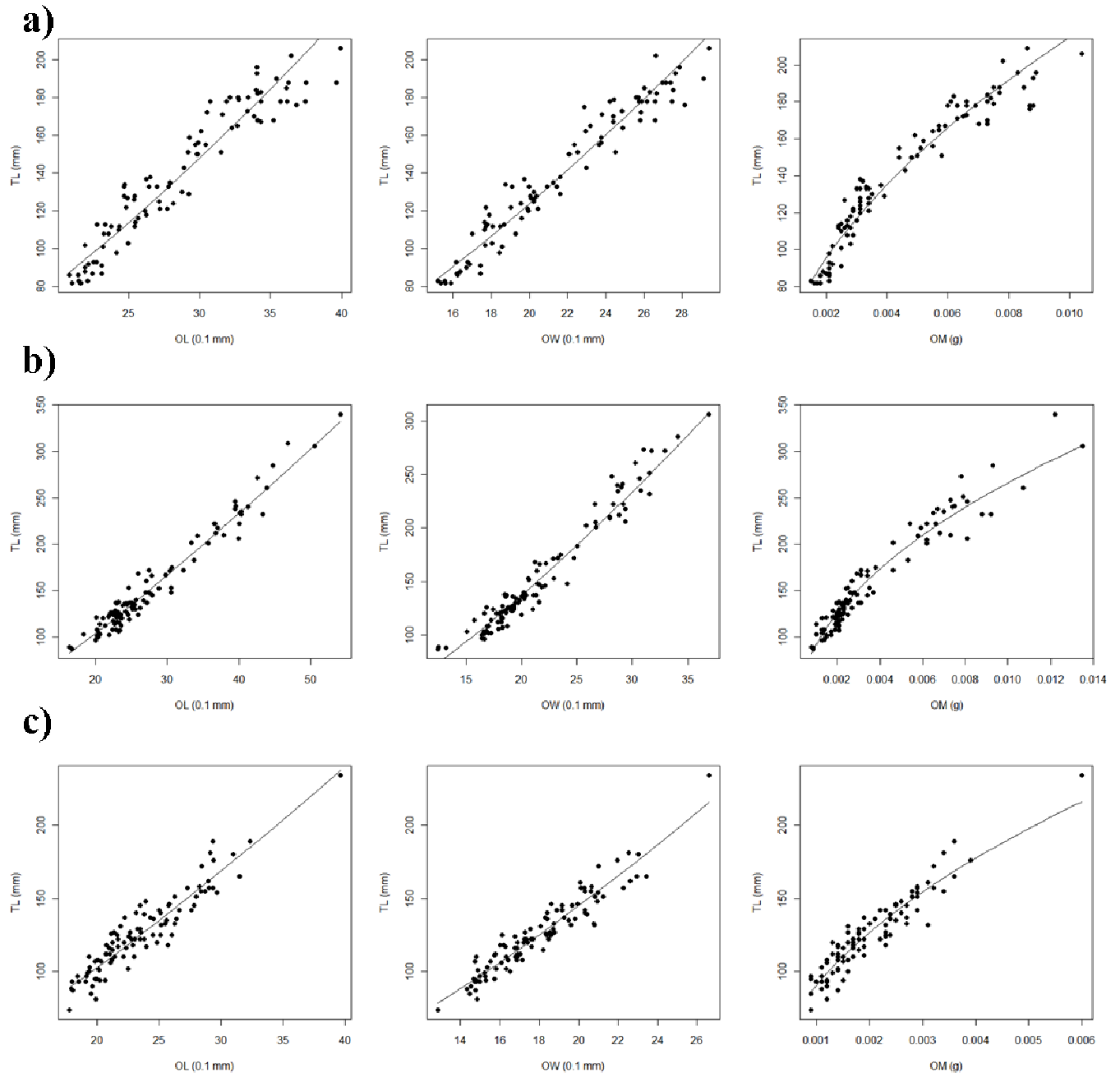
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FIG. 2. – Left sagitta morphology of a representative adult individual of *A. cuculus* (A), *C. lucerna* (B) and *E. gurnardus* (C) photograph by SEM. Idealized otolith of Triglidae family that show principal structure and key to measurements (D).



327

328 FIG. 3. – Relationships of otolith length (OL), width (OW) and mass (OM) vs total length

329 (TL) observe in *A. cuculus* (a), *C. lucerna* (b) and *E. gurnardus* (c) from Adriatic Sea.

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Intra-specific and inter-specific variability of the sulcus acusticus of sagittal otoliths in two gurnard species (Scorpaeniformes, Triglidæ)



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ARTICLE INFO

Article history:

Received 11 March 2014
Received in revised form 19 June 2014
Accepted 3 July 2014
Handling Editor B. Morales-Nin
Available online 31 July 2014

Keywords:

Sulcus acusticus
Ecomorphology
Morphometry
Crystalline structures
Triglidæ

ABSTRACT

Morphology, morphometrics and compounds of sulcus acusticus of sagittal otoliths of two gurnard species (*Aspitrigla cuculus* and *Eutrigla gurnardus*) from north-central Adriatic Sea (north-eastern Mediterranean) were investigated. The study objectives were to find morphometric variables and shape variability of sulcus acusticus of these two closely related sound producers species linked to developmental changes from juvenile to adult stage and species-specific pattern which influenced ecomorphological adaptations of the auditory system. The method used was a combination of multivariate techniques, X-ray diffractions pattern and ultrastructural investigation by SEM. Multivariate analysis (PCA, LDA and PERMANOVA) performed on standardized morphometrics of a total of 202 individuals, significantly separated four groups: *A. cuculus* juveniles, *A. cuculus* adults, *E. gurnardus* juveniles and *E. gurnardus* adults due to different life history characteristics. At intra-specific levels higher mean values of sulcus area/otolith area (S:O) ratio of adults than the juveniles were observed within *A. cuculus* and not for *E. gurnardus*. At inter-specific levels significant differences were seen between adults and not between juvenile groups. Considering all the groups, the main variability on sulcus area were observed for caudal part of the sensorial macula. Although no differences in compounds were shown by X-ray diffraction at intra-specific level, the crystals on sulcus acusticus were different in shape, size and arrangement between juveniles and adults of each gurnard. The crystals of juveniles were quite enmeshed into otolith matrix and showed a rod/smooth-shape. The crystalline arrangement of adults became more complex with larger, rod/rectangular-shape and well-formed faces. Ontogenetic and specie-specific differences might be related to specie-specific physiological factor (i.e. sexual maturity), depth distribution and feeding patterns that change during gurnards growth. These changes, relating to both endogenous and exogenous factors, were marked by the sulcus acusticus which provided a useful tool for fish ontogenetic, physiological and ecological studies.

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1. Introduction

The shape of otolith would appear to be an ideal natural phenotypic marker for fish populations. The otolith shape is markedly specie-specific and once deposition, otolith material is unlikely to be resorbed or altered (Campana and Neilson, 1985; Campana and Casselman, 1993). The morphology, the morphometry and the microstructure of otoliths could be affected by environmental factors, such as depth, water temperature and type of substrate (Gauldie, 1993; Lombarte and Leonart, 1993; Volpedo and

Echeverría, 2003), feeding habits (Amorim et al., 2004; Cardinale et al., 2004), ontogeny (Amorim and Hawkins, 2005; Monteiro et al., 2005; Capoccioni et al., 2011), physiology, such as the hearing capabilities associated with specialization in acoustic communication (Arellano et al., 1995; Lombarte and Cruz, 2007) and phylogeny (Torres et al., 2000). An important aspect would be the study of shape variability in relation to ontogenetic changes in sulcus acusticus area and shape which is related to the frequency response and auditory threshold of the inner ear. The sulcus acusticus is a depression situated in the proximal surface of the sagitta and it allows the connection between the sensorial macula and the otolith (Dunkelberger et al., 1980; Platt and Popper, 1981). The sagitta acts as transmitter of mechanical stimuli to the cilia of the macula inserted in the sulcus acusticus (Gauldie, 1988). The sulcus is

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commonly divided into two sections, the ostium and the cauda. The ostium is anterior and generally reaches the rostral position of the otolith where it opens; the cauda is posterior and most frequently closed, often with a ventrally curved tip (Tuset et al., 2008). Ostium and cauda filled with the colliculi constituting the area where the macula acustica touches the otolith. Several questions arise concerning the adaptative function of the relationship between the otolith and macula. Gauldie (1988) considers that the existence of a close morphological relationship between the sensorial macula and the otolith is fundamental for the mechanical efficiency in the process of transduction of the sound in which the inner ear is implicated. Torres et al. (2000) confirmed that the development of the sulcus acusticus could be related to the ecomorphological adaptations of the auditory system for the genus *Merluccius*.

About microstructure, otoliths are composed of CaCO_3 that normally precipitates as aragonite. The calcite and vaterite morphs of calcium carbonate are other crystalline forms that can occur in otoliths of teleost fishes. Each otolith has a variable external crystalline morphology which may be related to different growth rates of the crystals. The kinetics of deposition of the carbonate may change in response to external factors such as changes in salinity and temperature, physiological factors such as the sexual maturation and changes in diet (Gauldie and Nelson, 1990). Three classes of information are stored in the crystalline structure of the otolith: shape conservation, coexisting crystal morphs, and consecutive changes in crystal morph (Gauldie, 1993). Several studies had shown ontogenetic comparison (juvenile and adult size classes) of crystalline otoliths finding differences linked to different proportion of calcium carbonate polymorph (i.e. Sweeting et al., 2004) or trace elements concentrations. Other studies take into account the shape of the crystals in relation to different habitats and depth (i.e. Jitpukdee, 2009; Schulz-Mirbach et al., 2010). No studies considered any ontogenetic changes of morphology of crystals according to metabolic differences.

Gurnards are demersal fish which inhabit the continental and insular shelves of tropical and temperate seas to depths of 500 m, found on sandy, muddy or rubble substrates (Fischer et al., 1987). They spend a pelagic phase during their early life history stage. During demersal life cycle the change of their ecologic behaviors are linked to the start of sexual maturity, the tendency to migrate to greater depths and change of diet expanding the trophic spectrum (Vallisneri et al., 2012). Red gurnard (*Aspitrigla cuculus* Linnaeus, 1758) and grey gurnard (*Eutrigla gurnardus* L., 1758) have been included in the list of target species of the Medits project (trawl survey) (Relini et al., 2008). In the Italian Seas (Colloca et al., 1994—Tyrrhenian Sea; Manfredi et al., 2014—Adriatic Sea) these gurnards showed a wide bathymetric overlap although they differed in seasonal bathymetric distribution linked to recruitment pattern and individual size growth. Along the Italian coasts red gurnard lives down to 400 m with the highest density between 100 and 200 m, while it lacks to under 50 m. In north-central Adriatic Sea the distribution area of red gurnard covers the whole central Adriatic, with the exception of the deep water of the Pomo/Jabuka Pit and the coastal areas of both western side and eastern channel area (Manfredi, 2011; Piccinetti et al., 2012). In the central Adriatic area the species shows a depth distribution between 50 and 250 m (Vallisneri et al., 2013). Recruits were found at shallower depth (mostly between 70 and 150 m) while adults were observed at greater depth (mostly deeper than 150 m) (Manfredi et al., 2014). Spatial separation between juveniles and adults was found and the depth migration of the juveniles has been documented for other Mediterranean gurnard such as *Chelidonichthys lucerna* as for searobin species (Lewis and Yergey, 1976; Richards et al., 1979; Colloca et al., 1994). Grey gurnard is widely distributed in the whole study basin, with the exception of the western shallow coastal water and of the area deeper than 300 m close to the

southern boundary of the north-central Adriatic Sea (Manfredi, 2011; Piccinetti et al., 2012). For this species no differences in trends of abundance along depth were found between recruits and spawners, their depth abundances varying concordantly (Manfredi et al., 2014). About diet, red gurnard feeds quite exclusively on crustaceans and it does not vary significantly around the size of sexual maturity and depth, appearing as a specialist predator that feeds on necto-benthic Lophogastrida (Vallisneri et al., 2013) in agreement with data from other Mediterranean areas (Colloca et al., 1994, 2010—Tyrrhenian Sea; Terrats et al., 2000—Aegean Sea). Instead grey gurnards are epibenthic feeders preying mainly crustaceans (mainly Decapoda natantia, Lophogastrida and Mysida) and teleost fishes; molluscs are also observed to be present in the stomachs, but are deemed to contribute only moderately to the overall diet (Montanini et al., 2010) according to many authors (Reys, 1960—Gulf of Lions; Moreno-Amich, 1994—Catalan coast). Grey gurnard shows a generalistic feeding behavior and diet changes with ontogenesis: decapoda are more frequently found in the diet of juveniles and teleostei in that of adults (Montanini et al., 2010).

Gurnards present a small relative size of sagitta otoliths (Lombarte and Cruz, 2007), approximately oval or triangular to oval (Tuset et al., 2008), and are known for their ontogenetic and competitive feeding differences in sound production (Amorim et al., 2004; Amorim and Hawkins, 2005).

The aim of the present paper is to determine if sulcus acusticus of gurnards sagittal otoliths should be used as a useful discriminate marker between life cycle stages. For this purpose intra- and also inter-specific differences in the morphometric features and mineral compounds of the sulcus acusticus were investigated in two Mediterranean gurnards and ecomorphological pattern were discussed.

2. Materials and methods

2.1. Sampling

A total of 202 sagittae were extracted randomly from *A. cuculus* ($n = 101$) and *E. gurnardus* ($n = 101$) collected between 2007 and 2012 from several oceanographic trawl surveys in the north central Adriatic Sea (north-eastern Mediterranean) at depth ranging from 37.15 to 237.5 m. For each samples, the total body length (TL, mm) and total weight (W , g to the nearest 0.1 g) were measured. According to the codes of sexual maturity for fish (MEDITS, freely available at <http://archimer.ifremer.fr/doc/00002/11321/>) macroscopic maturity stages were recorded and specimens of each species were classified in two groups according to the codes: juvenile and adult individuals. To support macroscopic evaluation of gonads, juvenile individuals were also identified by means of the Bhattacharya's method while for adult ones we referred to Vallisneri et al. (2012). Samples size and biometric measurements were summarized in Table 1. Sagittal otolith images by SEM were showed for all size classes considered (Fig. 1a and b).

2.2. Morphological measures

The sagittal otoliths of all specimens were extracting from the skulls by the sagittal cut method, cutting the heads from the mouth to the back. Otoliths were removed from otic capsule by a pair of forceps, cleaned from adhering tissues with 3% H_2O_2 for 15 min and then with Milli-Q water in the ultrasonic bath for 5 min (two successive cycles). They were finally kept dry. Digital images of all the otolith samples were collected using a NIKON P5100 digital camera linked to a Leica MZ6 stereomicroscope. Each sagitta was photographed with the sulcus acusticus facing up and a 2592×1944

Table 1
Eco-biological parameters of gurnards.

Gurnard size classes	N. otolith	Cut off size (mm)	TL range (mm)	TL mean \pm SD (mm)	Depth range (m)
<i>A. cuculus</i> juvenile–A1	50	<100	57–99	79.98 \pm 9.92	63.6–178
<i>A. cuculus</i> adult–A2	51	\geq 150	150–196	167.96 \pm 12.30	88.9–237.5
<i>E. gurnardus</i> juvenile–E1	51	<100	56–99	82.01 \pm 10.14	31.3–170
<i>E. gurnardus</i> adult–E2	50	\geq 122	127–197	152.34 \pm 18.79	41.3–181.5

TL (total length), SD (standard deviation).

pixel digital picture was taken. The images were first orientated horizontally along their longest axis and binarized for contour extraction by ImageJ 1.48p software (Rasband, freely available at <http://rsb.info.nih.gov/ij/>). Spatial calibration against known values of 2 mm was applied to all images.

By ImageJ nine morphological features were recorded from sagittae and their relative sulcus acusticus: otolith area (mm²), sulcus area (mm²), sulcus perimeter (mm), sulcus length (mm), sulcus height (mm), vertical height of the collum (mm), sulcus ostial area (mm²), sulcus caudal area (mm²), sulcus ostial length (mm), sulcus caudal length (mm) (Fig. 2).

2.3. Data analyses

Values of coefficients a , b , r^2 and 95% confidence limits (CL) for parameters of the potential function fitted by each sulcus acusticus measurement in relation with fish total length (TL) were calculated on raw data. Correlation among parameters measured and TL was tested by Pearson correlation coefficient and before morphometrical analyses, allometry effects were removed by normalizing all measurements to a given median TL for each groups according to Leonart et al. (2000). Data were tested for normal distribution using the Shapiro–Wilk test and for homogeneity of variance using Bartlett's test (Sokal and Rohlf, 1995). Due to the results of the tests, parametric or non-parametric univariate statistics were used to compare mean values of each measurements.

The S:O ratio (sulcus acusticus area/otolith area \times 100) which expressed the percentage of the sulcus acusticus surface against otolith were calculated. ANOVA ($p < 0.001$) and pair-wise

comparison by t -test ($p < 0.05$) were used to test for differences among size groups and species. With the objective to investigate also ostium and cauda region of sulcus acusticus, we calculated Ostium:Sulcus ratio (ostium area/sulcus acusticus area \times 100) which expressed the percentage of the ostium surface against sulcus acusticus and Cauda:Sulcus ratio (cauda area/sulcus acusticus area \times 100), which expressed the percentage of the cauda surface against sulcus acusticus. Kruskal–Wallis rank sum tests ($p < 0.001$) and paired Wilcoxon rank test ($p < 0.05$) were used to test for differences among size groups and species.

Multivariate analyses by PCA (principal component analysis) were performed to examine all morphological variables simultaneously and in order to evaluate eco-morphological patterns. The purpose of principal component analysis is to find the best low-dimensional representation of the variation in a multivariate data set. Screen plot showed the fraction of total variance in the data as represented by each principal component. Cumulative variance was explained by the strong meaning of eigenvalues ranked from the greatest to the least. Loadings for the Principal Components were showed. LDA (linear discriminant analysis) was carried out on the PCA samples scores. This method expects a grouping variable (specie size classes groups) and sulcus acusticus morphological variables to be used in calculating the discriminant function. The difference on morphometrics between species and life stage was tested with nonparametric permutational multivariate analysis of variance (PERMANOVA) using distance matrices and pair-wise comparison. The PERMANOVA was based on the Bray–Curtis dissimilarity measure (999 random permutations). Significance tests were done using F -tests ($p < 0.001$) based on sequential sums of

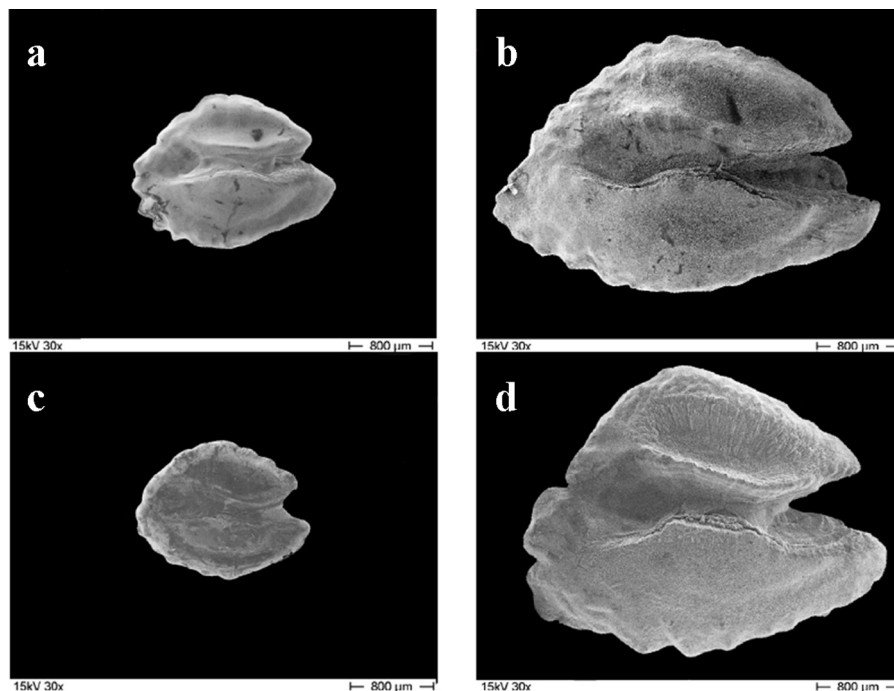


Fig. 1. Proximal surface of sagittal otolith of juvenile and adults of *Aspitrigla cuculus* (a and b respectively) and *Eutrigla gurnardus* (c and d respectively) scanning by SEM.

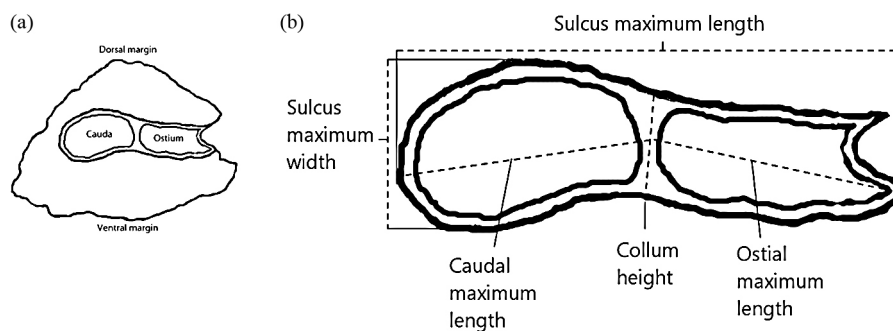


Fig. 2. Morphological measurements of the sulcus acusticus of a sagittal otolith: (a) schematic drawing of the proximal surface of the sagittal otoliths of gurnard species showing ostium and cauda region of sulcus acusticus; (b) sulcus acusticus indicating interactive measurements.

squares from permutations of the raw data. The statistical analysis of the collected data was carried out by means of the R software (R-project, freely available at <http://www.r-project.org/>).

2.4. X-ray diffraction

The mineralogical composition of unfixed otoliths was determined by means of X-ray powder diffraction patterns using an automated diffractometer (Philips PW1710) equipped with a graphite monochromator and using $\text{CuK}\alpha$ radiation 1.5418 \AA , 40 kV and 30 mA; divergence and detector slits both of 1° , 2θ range $5\text{--}65^\circ$; $0.5^\circ 2\theta \text{ min}^{-1}$ speed. The program (X Powder PRO) was used to evaluate the profiles and the results were compared with International Centre for Diffraction Data (ICDD) database.

2.5. SEM investigations

A representative otolith sub-sample ($n=55$) of each species and size was investigated by scanning electron microscopy (SEM HITACHI S-2400) in order to describe morphology of sulcus acusticus. Sagittal otoliths were mounted on a brass stub and sputter-coated with a thin layer of gold. External crystalline structures of the inner sulcus acusticus were investigated for both ostium and cauda region of each sagitta at 3000 and 4000 magnification.

3. Results

Only sulcus area, perimeter and sulcus maximum length r^2 values are higher than 0.50 in all the four groups considered. Results on other morphological features suggested a nonlinear

relationship against TL despite statistically significant correlation (cor.test values tend to ± 1 , $p > 0.05$). Table 2 summarized the means and the standard deviations for the standardized morphometries in gurnard groups analyzed. Significant variations of mean values were detected for each measure among specie size groups (parametric ANOVA, $p < 0.05$; non parametric Kruskal–Wallis test, $p < 0.05$) except for some variables in the comparison of juvenile otoliths between *A. cuculus* vs. *E. gurnardus* (collum: Wilcoxon's test, $W=396$, $p=0.19$; sulcus ostial area: Wilcoxon's test, $W=405$, $p=0.13$; sulcus caudal area: Wilcoxon's test, $W=410$, $p=0.11$; sulcus caudal length: Tukey's test, $p=0.37$). Generally *A. cuculus* reached higher values despite *E. gurnardus* considering also size classes comparisons.

3.1. Ratio indexes

At intra-specific level, the S:O ratio was significantly higher in the adults than the juveniles of *A. cuculus* (Student's t -test: $t=4.758$, $p < 0.001$, Table 2) while did not vary between size classes in *E. gurnardus* (Student's t -test: $t=0.389$, $p=0.699$, Table 2). At inter-specific level, S:O values was higher in the adult of *A. cuculus* than the adults of *E. gurnardus* (Student's t -test: $t=4.032$, $p < 0.001$, Table 2) while does not vary between juveniles (Student's t -test: $t=-0.492$, $p=0.625$, Table 2).

Table 2 summarized also ostium:sulcus areas ratio and cauda:sulcus areas ratio. In relation to the differential development of the two areas of the sulcus acusticus (ostium and cauda region), the main variability existing in the caudal part of the sensorial macula: cauda:sulcus areas ratio detached significant difference in means from all paired data (Table 2) except in the inter-specific comparison of adult specimens of the two gurnards (Wilcoxon's

Table 2
Means and standard deviations of the sulcus acusticus standardized morphometries and ratio indexes.

Specie class	<i>Aspitrigla cuculus</i>		<i>Eutrigla gurnardus</i>	
	Juvenile	Adult	Juvenile	Adult
Mean variables \pm standard deviations				
Sulcus area (S , mm^2)	0.34 ± 0.06	1.17 ± 0.16	0.27 ± 0.04	0.65 ± 0.12
Perimeter (mm)	3.36 ± 0.21	6.06 ± 0.42	2.91 ± 0.21	4.70 ± 0.49
Maximum length (mm)	1.42 ± 0.10	2.49 ± 0.19	1.21 ± 0.09	1.90 ± 0.19
Maximum width (mm)	0.39 ± 0.03	0.73 ± 0.06	0.36 ± 0.04	0.58 ± 0.07
Collum vertical height (mm)	0.14 ± 0.02	0.28 ± 0.05	0.13 ± 0.02	0.21 ± 0.04
Ostial area (mm^2)	0.11 ± 0.02	0.43 ± 0.08	0.09 ± 0.03	0.21 ± 0.05
Caudal area (mm^2)	0.16 ± 0.03	0.61 ± 0.09	0.15 ± 0.04	0.32 ± 0.07
Ostial maximum length (mm)	0.60 ± 0.07	1.12 ± 0.14	0.53 ± 0.06	0.89 ± 0.14
Caudal maximum length (mm)	0.64 ± 0.08	1.23 ± 0.12	0.61 ± 0.06	0.88 ± 0.11
Ratio indexes				
Sulcus area:Otolith area	0.17 ± 0.02	0.21 ± 0.02	0.18 ± 0.02	0.18 ± 0.02
Ostial area:Sulcus area	0.32 ± 0.05	0.37 ± 0.06	0.35 ± 0.10	0.33 ± 0.05
Caudal area:Sulcus area	0.47 ± 0.08	0.53 ± 0.07	0.56 ± 0.01	0.49 ± 0.08

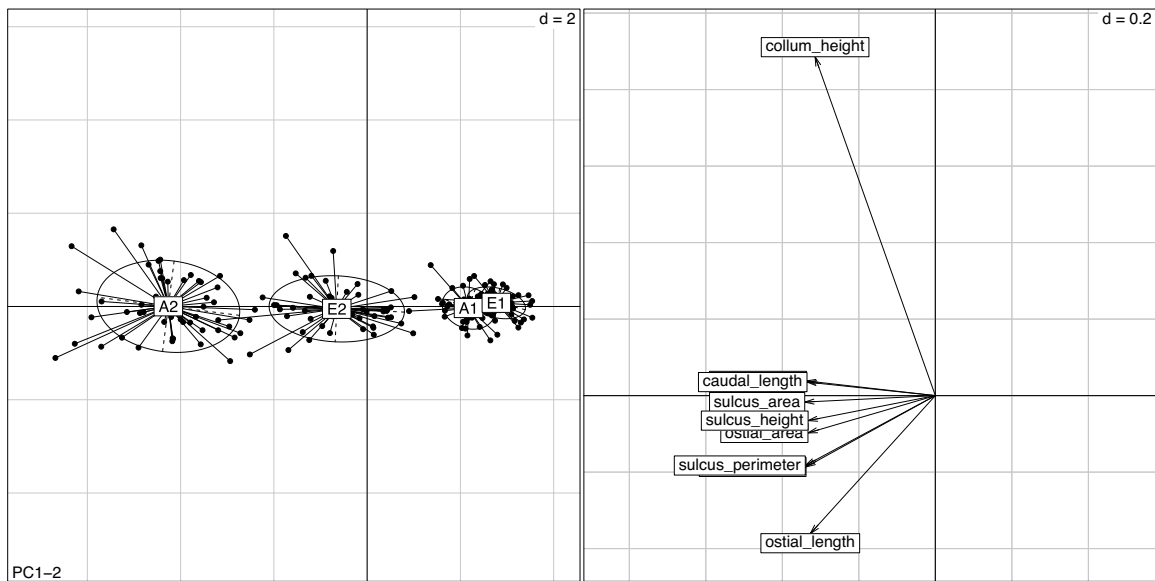


Fig. 3. Principal component analysis (PCA) scatter plot of the first two principal components indicating the separation among four gurnard size classes: A1: juveniles of *A. cuculus*; A2: adults of *A. cuculus*; E1: juveniles of *E. gurnardus*; E2: adults of *E. gurnardus*. Vector directions/lengths represent eigenvectors of morphological variables for each axis.

test, $W = 413$, $p = 0.10$, Table 2). Instead, about ostium:sulcus areas ratio, same values are detached between juvenile of the two species (Wilcoxon's test, $W = 246$, $p = 0.14$, Table 2) and at intra-specific level between juveniles and adults of *E. gurnardus* (Wilcoxon's test, $W = 347$, $p = 0.69$, Table 2).

3.2. Multivariate analyses

PCA on morphological variables of sulcus acusticus of the two gurnards resulted in the first principal components (PC) axes explaining over 93% of the variance (eigenvalue = 8.37) indicating a strong size vector (Fig. 3; Table 3). A separation amongst gurnard groups related with morphological variables was observed. The first PC axis was positively associated with the width and collum height of sulcus acusticus while was negatively associated with all other variables. All the variables displayed similar values (Fig. 3; Table 4). The second PC axis identified in collum height and ostial length higher values as performed by the scatter diagrams of the projection of a vector basis (Fig. 3; Table 4).

About LDA from the scatterplot of the first two discriminant functions, we can see that gurnard groups were quite well separated in the scatterplot (Fig. 4). The first discriminant function (x-axis) separates at intra-specific level very well between juvenile and adult groups within species, but cannot separate juveniles at inter-specific comparison. The second discriminant function (y-axis) achieves a little separation at the inter-specific level between juveniles of *E. gurnardus* from juveniles of *A. cuculus*. To achieve

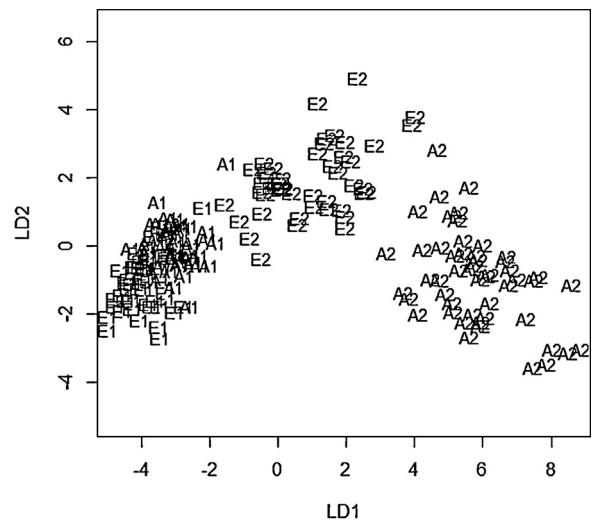


Fig. 4. Linear discriminant analysis (LDA) plot against four gurnard size classes: A1: juveniles of *A. cuculus*; A2: adults of *A. cuculus*; E1: juveniles of *E. gurnardus*; E2: adults of *E. gurnardus*.

a very good separation it would be best to use both the first and second discriminant functions together.

Significant differences were observed in mean otolith shapes among all samples (PERMANOVA; $F_{3,151} = 471$, $p < 0.001$), and the

Table 3
Eigenvalues of morphometrical variables and cumulative variance explained for the principal components of the PCA analysis associated with them.

Component	Eigenvalue	Cumulative variance
1	8.37	93.03
2	0.20	95.30
3	0.13	96.74
4	0.12	98.12
5	0.07	98.93
6	0.06	99.60
7	0.02	99.83
8	0.01	99.97
9	0.003	100.00

Table 4
The loadings for the first and the second principal component.

Morphometrical variables	PC1	PC2
Sulcus area	-0.341	-0.017
Perimeter	-0.341	-0.183
Maximum length	-0.338	0.186
Maximum width	0.333	-0.065
Collum vertical height	0.314	0.886
Ostial area	-0.333	-0.098
Caudal area	-0.337	0.038
Ostial maximum length	-0.327	-0.361
Caudal maximum length	-0.336	0.035

Table 5
P-values for pair-wise comparisons conducted after PERMANOVAs of biometrical data (adonis package in R software).

Pair-wise comparison	P-value
Intra-specific	
<i>A. cuculus</i> juveniles vs adults	<0.001
<i>E. gurnardus</i> juveniles vs adults	<0.001
Inter-specific	
<i>A. cuculus</i> vs <i>E. gurnardus</i> all samples	<0.001
<i>A. cuculus</i> vs <i>E. gurnardus</i> juveniles	<0.001
<i>A. cuculus</i> vs <i>E. gurnardus</i> adults	<0.001

subsequent pair-wise comparisons showed that all samples were significantly differently from each other (Table 5).

3.3. X-ray diffraction

All of the otoliths examined were composed of calcium carbonate. The results suggested that the biomineralization of otolith (both for intra- and inter-specific comparisons) occurred predominantly in the aragonite phase.

3.4. SEM analyses

Using SEM, the external textural organization of the sulcus acusticus of the sagittae traced different crystalline arrangement at the intra-specific level. For each species considered, crystalline units changed in shape, size and direction as a function of fish growth (from juvenile to adult stage).

The inner crystalline units of the sulcus acusticus of juveniles showed poorly formed habit with rounded edges, slightly oriented in the vertical and oblique plane with the long axis of the crystals oriented perpendicularly to the growth increment, from the nucleus to the outer edge of the growth (Fig. 5a and b; Fig. 6a and b). In rare cases more defined and larger crystals have been found singly or grouped and they presented a rod/smooth-shape. In all the samples the crystalline units were enmeshed into an insoluble organic material from the otolith matrix. This matrix, which appears darker than mineral areas, was laid down and made a continuous network that in some cases bridged all the crystalline units.

Concerning adult individuals they showed more defined and developed prismatic structures (Figs. 5c and d and 6c and d). Crystalline units presented a homogeneous distribution having a rod/rectangular-shape. The crystals have sharp edges clearly visible and well-formed faces and were iso-oriented with most stacked in the vertical and oblique plane perpendicularly to the growth increment. The mineral fraction was found to be predominant. Darker areas, defined as residues of the organic matrix were observed only in specimens with very large sizes and they looked like a fusion of many crystals.

Morphological differences of ostium and cauda region of sulcus acusticus had been detached for both juvenile and adult individuals. Crystalline units from ostium region showed well-defined and regular prismatic habit, thicker and were uniformly distributed (Figs. 5a and c and 6a and c). The cauda region presented a non-uniform distribution of crystals which differ in size and habit from the ostium region (Figs. 5b and d and 6b and d). Crystals were smaller, with irregular habit, faces much smoother, tended to have lamellar/columnar-shape and a flat surface.

4. Discussion and conclusions

Our study characterized the use of three distinct approaches for discriminate changes in the auditory system of Mediterranean

gurnards as a function of different life stages and specie-specific ecological patterns. Particularly we have combined the morphometric analysis with the biochemical diffraction and ultrastructural investigation in order to use the sulcus acusticus of sagittal otolith as a potential marker at both intra- and inter-specific level.

The present study provided evidence to assert that morphological features and shape of sulcus acusticus not only discriminated between stocks (Castonguay et al., 1991; Campana and Casselman, 1993; Begg and Brown, 2000; Bolles and Begg, 2000; Murta, 2000; Begg et al., 2001) or species (Crech, 1992; Torres et al., 2000) but could be used to detached size-related differences within population, varying during ontogenesis according to environmental, biological and ecological behavior of the species. The purpose is to use variations in phenotypic characteristics to discover critical size within species that may be correlated to specific exogenous and endogenous factors as describe by Morales-Nin (2000).

Multivariate analysis (PCA, LDA and PERMANOVA) performed on standardized morphometrics of individuals significantly separated into four groups: *A. cuculus* juveniles, *A. cuculus* adults, *E. gurnardus* juveniles and *E. gurnardus* adults showing different life history characteristics among each group. Gurnard species showed changes of habitat during the demersal life, which have been related to the growth, feeding strategy generally coinciding with the start of sexual maturity, the tendency to migrate to greater depths and an increase in the variety of food items eaten (Papaconstantinou, 1984; Colloca et al., 1994, 2003; Vallisneri et al., 2012). Such behavior probably depends on the predator changing its energy requirements in relation to fish size and dietary protein levels influencing the size of the fish at first maturity (Al Hafedh et al., 1999). Ontogenetic diet shifts were often explained as a reflection of the changing abilities of fish; essentially, as fish grow they become more proficient at handling larger prey that are more profitable (Papaconstantinou, 1983; Colloca et al., 1994). In relation to juvenile–adult transitions Amorim and Hawkins (2005) reported that for *E. gurnardus*, both sound production and feeding behavior changed with fish size, suggesting that smaller grey gurnards compete for food by contest tactics whereas larger specimens predominantly scramble for food, probably because body size gives an advantage in locating, capturing and handling prey. The authors further suggest that sounds emitted during feeding may potentially give information on the motivation and ability of the individual to compete for food resources. These differences may imply variation of the hearing capabilities of fishes. Gaudie (1988) postulated a model of otolith function in which the saccular otolith acts as a system of levers through which sound waves are converted into shearing forces in the plain of the air cell in the macula. This model implies that the shape of the otolith measured as the ratio of macula to otolith area (M:O) or its approximation by sulcus acusticus area/sagitta area (S:O), is related to the frequency response and the auditory threshold of the otolith (Gaudie, 1988; Aguirre and Lombarte, 1999). Therefore, the shape of the otolith is a determinant of the particular sound frequencies for which the otolith acts as a transducer (Gaudie, 1988). It has been observed that the S:O ratio increases in species with a highly mobility pattern (Gaudie, 1988; Lombarte and Popper, 1994; Arellano et al., 1995). In some studies (Lombarte, 1992; Lombarte and Fortuño, 1992; Aguirre and Lombarte, 1999) authors reported that the depth distribution, feeding habits and spatial niches were the principle variable factors which could affect intra- and inter-specific variation in the otolith shape and sensory area associated with it.

At intra-specific level our results showed lower mean values of S:O ratio for juveniles than the adults within *A. cuculus* but not within *E. gurnardus*. At inter-specific level significant differences were seen between adults groups and not between juveniles ones. These results could be interpreted as follow: the large increase of the S:O ratio with growth in *A. cuculus*, indicating that the size

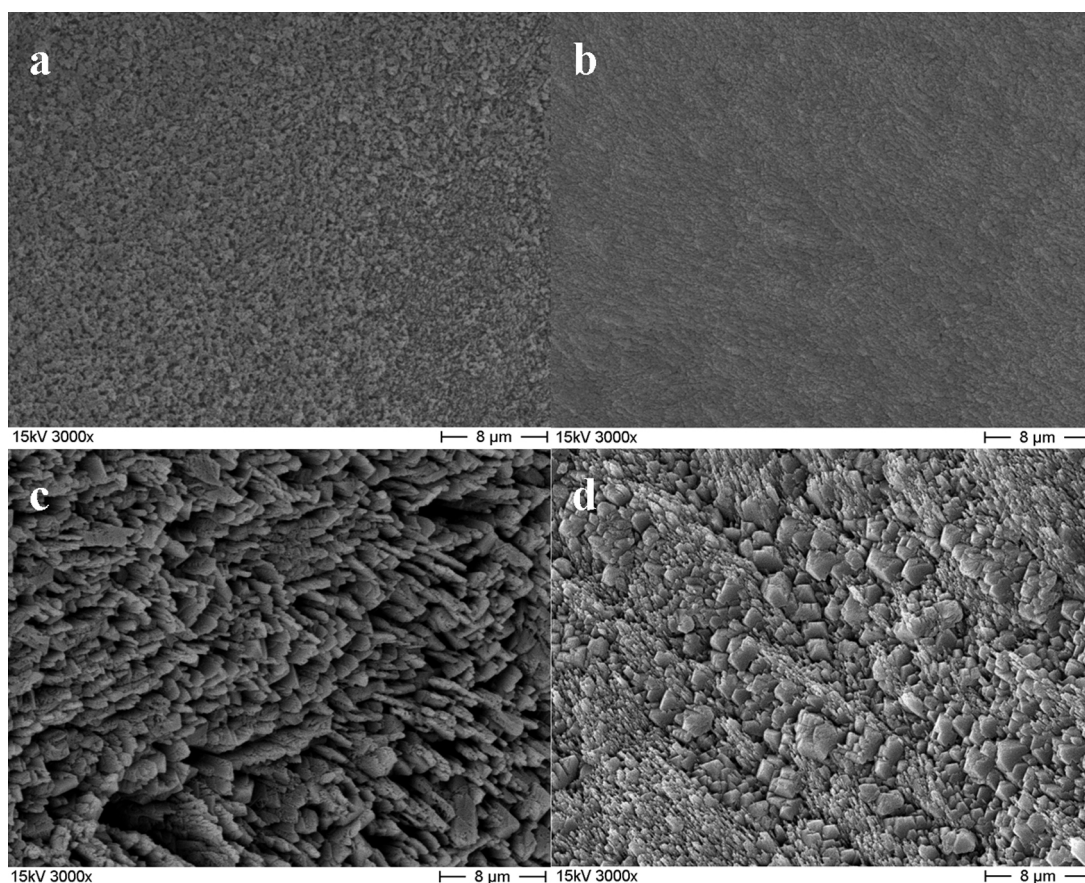


Fig. 5. *Aspitrigla cuculus*, crystalline structure on sulcus acusticus investigated by SEM: juveniles specimens a, b (a—ostium region; b—cauda region); adults specimens c, d (c—ostium region; d—cauda region).

was the most important predictor of the S:O ratio (Aguirre and Lombarte, 1999; Aguirre, 2003). The two species cohabit the same area but differ in terms of bathymetrical distribution. The deepest bathymetric distribution was reported for *A. cuculus* (Colloca et al., 1994; Piccinetti et al., 2012). Also comparing depth distribution between recruits and spawners of the two species only in the case of *A. cuculus* depth distribution detached differences between size classes while no bathymetric pattern was discovered for *E. gurnardus* (Manfredi et al., 2014). According to Lombarte and Fortuño (1992), the ratio is larger in species inhabiting deeper waters. Also the food and spatial niches may affect the sulcus acusticus variation. Hearing is used to locate food items, intra-specific recognition and predator avoidance (Luczkovich et al., 1999). *E. gurnardus* were found to feed more on epibenthic, benthopelagic and neto-benthic preys and it was an opportunistic predators as other gurnards (Montanini et al., 2010). Instead *A. cuculus* was found to feed almost exclusively on neto-benthic invertebrates highlighting a specialistic behavior (Colloca et al., 2010; Vallisneri et al., 2013). These differences in feeding strategy could be required different hearing capabilities also linked to ontogenesis.

Considering mean values of ostium and cauda region on sulcus area, caudal area seems to be more discriminant than ostium. These differences took in account morphological differences between ostium and cauda region: the ostium is generally funnel-shaped and shorter than the cauda. The cauda is generally tubular and curved, more or less bent towards the posterior region. Three dimensional techniques should be required in future research.

Changes during the growth in the otolith shape were analyzed in relation to juvenile–adult transitions. The crystal structures were examined in terms of both calcium carbonate crystal polymorphism and variations in crystal habit within those morphs.

Although no difference in compounds were showed by X-ray diffraction, the crystals on sulcus acusticus of sagittae were different in shape, size and arrangement at intra-specific level. Either ontogenetic or environmentally induced switching of crystal morph genes could have an important influence on the phylogenetic significance assigned to the variation in crystal morph (Gauldie, 1993). Otolith chemical composition may vary throughout the different stages in the fishes life history or when environmental changes occur (Volpedo and Cirelli, 2006). The crystalline structure of juvenile can be distinguished from the adults in the sulcus acusticus area where small, thin crystals sometimes melt together or embedded in the organic materials became larger and thick. Juveniles showed a relative uniform demineralization, which suggests that these otoliths are poorly mineralized and rich in organic matrix. The peculiar structure of the sulcus zone structure is probably linked with otolith growth due to opposition of material (Morales-Nin, 1986; Lombarte and Morales-Nin, 1995). Cermeño et al. (2006) suggested that the uniform distribution of the organic matrix could probably be corresponding to a high continuous growth rate metabolism and stable environmental conditions during the initial life phases related to spawning ground. Although some more detailed studies on the density and the variation in the matrix materials of otolith may be required in future to better understand their growth pattern. Inter and intraspecific shape and ultrastructure differences between ostium and cauda may be corresponding to the variability of the sensorial macula. It is hypothesized that such differences might have an effect in the frequency threshold heard by fish (Gauldie, 1988; Monteiro et al., 2005).

In conclusion, our study confirmed that the sulcus acusticus morphology can be used as a tool within population structure

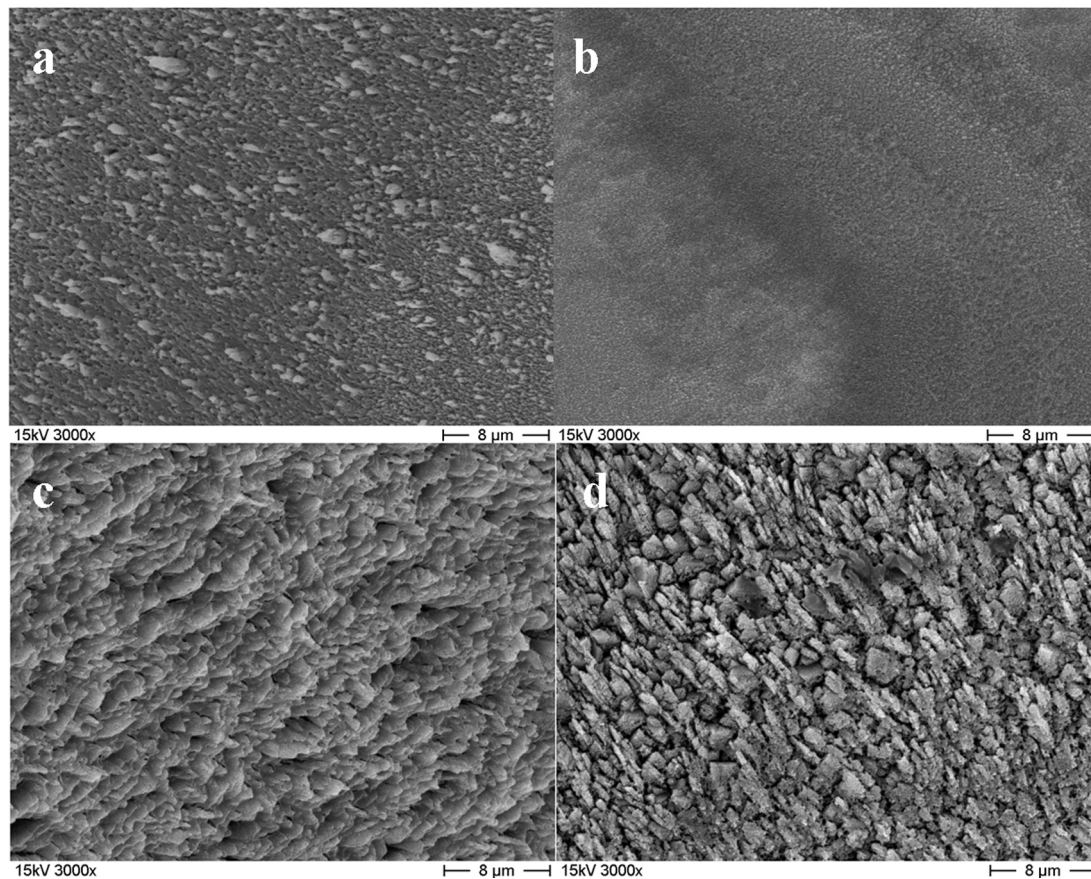


Fig. 6. *Eutrigla gurnardus*, crystalline structure on sulcus acusticus investigated by SEM: juveniles specimens a, b (a—ostium region; b—cauda region); adults specimens c, d (c—ostium region; d—cauda region).

(intra-specific comparisons) and among species (inter-specific comparisons). Changes in sulcus acusticus shape could occur in relation to fish growth and greater complexity in the crystalline structure of the sulcus acusticus. These differences might be related to: (1) phylogenetic influence that can be reflected in the sulcus acusticus morphology (Nolf, 1985; Torres et al., 2000); (2) physiological factors i.e. sexual maturation. The most significant changes take place near the specie-specific size at first maturity. At this size the metabolism of the fish undergoes important changes that provide variations in the growth, affecting the otolith morphology (Härkönen, 1986); (3) environmental factors i.e. depth range which might affect the pattern of crystallization and shape of otoliths as observed in the study of Jitpukdee and Wannitikul (2004); (4) eco-physiological factors i.e. feeding pattern: (a) feeding competition within species; (b) ontogenetic diet shifts as a reflection of the changing abilities of fish; (c) specie-specific feeding strategy linked to different ecological niches. These changes, relating to both endogenous and exogenous factors, are designed to improve the hearing which it changes and evolves during the life of the fish.

Acknowledgments

We thank Professor Corrado Piccinetti for his assistance in the bottom Trawl surveys. We also thank Dr. Maria Roberta Randi for precious assistance during SEM investigations.

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USO DEL SOLCO ACUSTICO DEGLI OTOLITI COME “MARCATORE” DISCRIMINANTE DI SPECIE ITTICHE BERSAGLIO

USING SULCUS ACUSTICUS OF OTOLITH AS DISCRIMINANT “MARKER” IN REFERENCE FISH SPECIES

Abstract - The variability of sulcus acusticus of sagittal otolith in reference species of triglids (red gurnard *Aspitrigla cuculus*, tub gurnard *Chelidonichthys lucerna* and grey gurnard *Eutrigla gurnardus*) from the Adriatic Sea, were analysed for inter-intraspecific discrimination purposes. Significant differences in sulcus morphometry and microstructure between species and between juveniles and adults at “critical size” species were found. Different ratio of sulcus area/otolith area, sulcus width/sulcus length and sulcus crystalline structure could be related to eco-morphological adaptations of the auditory system. This approach should be highly informative for fish phylogenetic, physiological and ecological studies.

Key-words: sulcus acusticus; sagittal otolith; crystalline structure; Triglidae.

Introduzione - Gli otoliti rappresentano dei “marcatori” naturali in quanto sembrano registrare alcune fasi del ciclo biologico e variazioni dell’habitat (Volpedo *et al.*, 2010). La sagitta gioca un ruolo importante nell’udito dei pesci, costituendo il principale recettore di senso per la percezione dei suoni: essa è rivestita dall’epitelio sensoriale contenente le cellule di senso dell’udito, provviste di una corona di ciglia che si conficcano nei cristalli del solco acustico della sagitta. Un movimento dell’acqua provoca un cambiamento della disposizione delle ciglia, che è comunicato al sistema nervoso centrale. Perciò il solco acustico si relaziona alle capacità uditive dei pesci ed è coinvolto nella percezione e nella localizzazione della sorgente sonora. Scopo del lavoro consiste nell’analisi delle variazioni inter e intra-specifiche del solco acustico delle sagitte mediante analisi della forma e della microstruttura di tre specie bersaglio.

Materiali e metodi - Un totale di 355 esemplari sono stati raccolti durante le campagne di pesca a strascico MEDITS e GRUND nel nord-centro Adriatico tra il 2007 e il 2010. Per ogni esemplare, sono stati registrati: lunghezza totale (LT, mm), peso (g), sesso e maturità sessuale (Relini *et al.*, 2008), definendo una taglia critica che discrimina i giovanili dagli adulti (Tab. 1). Sono state estratte le sagitte, trattate con perossido di idrogeno al 3% per 15 minuti e lavate due volte in acqua distillata per 5 minuti in ultrasuoni. Le immagini digitali (ottenute da fotocamera NIKON P5100 collegata a uno stereo-microscopio Leica MZ6) sono state elaborate con il software ImageJ 1.46h al fine di calcolare: a) il valore medio del rapporto AS(area solco)/AO(area otolite)%; b) il valore medio del rapporto hS(altezza massima solco)/LS(lunghezza massima solco)%. L’elaborazione statistica è stata svolta mediante il software R 2.14.1. Per l’analisi ultrastrutturale del *sulcus acusticus* al microscopio

elettronico a scansione (Hitachi S-2400), sub-campioni sono stati montati su stubs, metallizzati e fotografati.

Tab. 1 - Parametri biometrici.
Biometric parameters

Specie	n° dati	range LT (mm)	Taglia critica (mm)
<i>A. cuculus</i>	115	65 - 207	110
<i>C. lucerna</i>	100	87 - 272	180
<i>E. gurnardus</i>	140	56 - 195	104

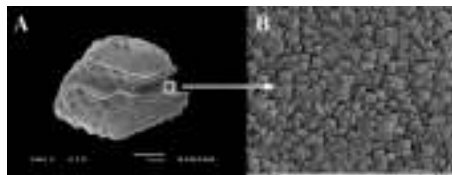


Fig. 1 - Otolite di *A. cuculus* (A) e dettaglio dei cristalli del solco acustico (B).
Otolith of A. cuculus (A) and details of the crystal of sulcus acusticus (B).

Risultati e conclusioni – I valori medi del rapporto AS/AO risultano sempre statisticamente significativi sia nel confronto intra che inter-specifico, con una significatività marcata per *A. cuculus* (Tab. 2). Questo risultato, evidenziando un aumento della complessità nella comunicazione acustica durante l'accrescimento, potrebbe essere correlato al comportamento trofico della specie quale “predatore specialista” e quindi particolarmente esigente e conflittuale rispetto alle altre specie simpatriche, più tipicamente “generaliste”. Il rapporto hS/LS discrimina meno, confermando tuttavia l'alta variabilità del solco per *A. cuculus*. L'analisi ultrastrutturale del solco evidenzia trattarsi di un complesso policristallino, costituito da cristalli di carbonato di calcio fase aragonite. Nei giovanili i cristalli presentano un abito tendenzialmente poco sviluppato, spessore inferiore al μm e sono fortemente permeati dalla matrice organica. Negli adulti i cristalli presentano facce ben formate con spigoli visibili e dimensioni superiori al μm . Le differenze riscontrate a livello del solco acustico appaiono in correlazione sia alle modificazioni fisiologiche, che ai fattori ambientali. In particolare, a ridosso della "taglia critica" che separa i giovanili dagli adulti, le modificazioni a carico del solco e della conseguente capacità uditiva, coincidono con la migrazione verso profondità maggiori, l'avvio della maturità sessuale e i cambiamenti quali/quantitativi della dieta (Vallisneri *et al.*, 2012).

Tab. 2 - Solco acustico AS: area solco; AO: area otolite; hS: altezza solco; LS: lunghezza solco.
Sulcus acusticus. AS: sulcus area; AO: otolith area; hS: sulcus width; LS: sulcus length.

	AS/AO%± DS	AS/AO%± DS	p.value	hS/LS% ± DS	hS/LS%± DS	p.value
CONFRONTI INTRA-SPECIFICI						
<i>Aspitrigla cuculus</i> giovanili-adulti	17.2 ± 2.3	20.1 ± 2.3	p<0.001	26.4 ± 1.7	28.9 ± 2.6	p<0.001
<i>Chelidonichthys lucerna</i> giovanili-adulti	14.8 ± 1.2	16.4 ± 2.4	p<0.05	24.2 ± 2.5	24.2 ± 2.1	n.s
<i>Eutrigla gurnardus</i> giovanili-adulti	16.5 ± 1.8	17.7 ± 2.4	p<0.05	28.6 ± 3.4	28.7 ± 2.7	n.s
CONFRONTI INTER-SPECIFICI						
<i>Aspitrigla cuculus-Chelidonichthys lucerna</i>	19.4 ± 2.6	15.1 ± 2.1	p<0.001	28.3 ± 2.6	24.2 ± 2.4	p<0.001
<i>Aspitrigla cuculus-Eutrigla gurnardus</i>	19.4 ± 2.6	17.4 ± 2.3	p<0.001	28.3 ± 2.6	28.6 ± 2.9	n.s
<i>Chelidonichthys lucerna-Eutrigla gurnardus</i>	15.1 ± 2.1	17.4 ± 2.3	p<0.001	24.2 ± 2.4	28.6 ± 2.9	p<0.001

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HEARING CAPABILITIES IN TARGET GURNARD SPECIES (TELEOSTEI, SCORPAENIFORMES): ONTOGENETIC AND ENVIRONMENTAL EFFECTS ON THE VARIABILITY OF THE SULCUS ACUSTICUS IN THE SAGITTAL OTOLITH

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Eco-morphological studies can help us to understand the interactions between organisms and their environment and to determine the adaptive character of morphological structures. The variability in the shape of the sagittal otolith, especially in the sulcus acusticus area, could be a morpho-functional characteristic which interacts with the processes of detection and transduction of sound and with different communication strategies. The aim of the work is to study the morphogenesis of the sulcus acusticus (divided into two sections, the anterior *ostium* and the posterior *cauda*) of the sagittae of two target species of trawl survey, *Aspitrigla cuculus* and *Eutrigla gurnardus*, belonging to the family Triglidae (Scorpaeniformes) and particularly known for their wide repertoire of vocalizations. A total of 152 otoliths were taken from samples collected between 2007-2010 from several scientific surveys in the Adriatic Sea at depths ranging from 37 to 238 m. To detect the ontogenetic changes in otolith morphology, morphometric measurements were compared between fish size class. To detect inter-specific comparisons, total length (TL) and allometry effects were avoided by normalizing all measurements to a standard TL. Nine morphological features were selected on the sulcus acusticus: area (mm²), perimeter (mm), length (mm), height (mm), vertical height of the collum (mm), ostial area (mm²), caudal area (mm²), ostial length (mm), caudal length (mm). External crystalline structures of the sulcus acusticus were investigated by SEM. Significant differences were observed in the sulcus acusticus both at inter- and intra-specific level among juveniles and adults, very prominent near the "critical size" that coincides with the beginning of sexual maturity, migration into deeper water, colonization of different trophic niches. Particularly discriminating between juveniles and adults were the differences in the allometric growth of the sulcus morphometry and growing organization of the crystalline arrangement with ultrastructural faces and edges always better defined.

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USING SAGITTAL OTOLITHS FOR ECOMORHOLOGICAL CHARACTERIZATION OF DEMERSAL FISH FROM ADRIATIC SEA

CARATTERIZZAZIONE ECOMORFOLOGICA DI SPECIE ITTICHE DEMERSALI DELL'ADRIATICO TRAMITE L'UTILIZZO DELLE SAGITTE

Abstract - The morphology of the sagittal otolith were studied in six fish species associated with different feeding pattern and ecological niche. Three percentage indexes were calculated: A%, L% and EL% for each species. Statistical analysis showed inter-specific differences in the morphological features (PCA, PERMANOVA and post-hoc pair-wise analysis on species groups) differentiating benthic species from those bento-pelagic and necto-benthic. Our results suggested that otoliths could be used as fish ecomorphological marker.

Key-words: otoliths, morphometry, feeding, demersal fisheries, Adriatic Sea.

Introduction - Otoliths are a powerful taxonomic feature for fish species identification because of their high inter-specific variability in shape. The morphology of otoliths is strictly linked with environmental factors, feeding habits, physiology. The aim of this study is to analyze whether the morphological features could be associated with diet and ecological niche in six demersal species for MEDITS trawl survey project: *Chelidonichthys lucerna*, *Merlangius merlangus*, *Merluccius merluccius*, *Mullus barbatus*, *Pagellus erythrinus*, *Trisopterus minutus capelanus*.

Materials and methods - A total of 231 sagittal otoliths were sampled from 6 fish demersal species (Tab. 1) obtained from trawl surveys from North Middle Adriatic Sea. The right and left sagitta of all specimens were removed, cleaned in the ultrasonic bath and kept dry. Digital images of all the sagittae were collected using a NIKON P5100 digital camera linked to a Leica MZ6 stereomicroscope. By ImageJ 1.47j software the following measurements were recorded from both right and left sagittae: LO (maximum length of sagitta) and WO (maximum width of sagitta) in mm; OA (otolith area) in mm². To detect inter-specific comparisons, total length of fish (TL) and allometry effects were avoided by normalizing all measurements to a given TL according to Leonart *et al.* (2000). Three percentage indexes were calculated: A%: otolith width/TL of fish%; L%: otolith length/TL of fish%; EL%: otolith width/otolith length%. PCA, PERMANOVA and post-hoc pair-wise analysis on species groups measurements and indexes were performed to test inter-specific differences. The information about feeding habits (IRI%) using to compare the results, were taken from Piccinetti *et al.* (2007). Elaborations were conducted with R software.

Results and conclusions - Two morphologic groups in sagitta shape were found: group 1 with lanceolated otolith including necto-benthic and bento-pelagic gadiforms (*M. merlangus*, *M. merluccius* and *T. minutus c.*) and group 2 with oval otolith including benthic species (*C. lucerna*, *M. barbatus* and *P. erythrinus*). PCA analysis discriminated within group (Fig. 1): within group 1 *M. merluccius* differed from *M. merlangus* and *T. minutus c.* both for the diet as active predator of bony fishes that for more slender body; within group 2 *P. erythrinus* differed

from *C. lucerna* and *M. barbatus* particularly for the diet that was based mainly on polychaetes. PERMANOVA found significant differences among species and pair-wise test comparison were all significant ($p < 0.001$) except for *C. lucerna* - *M. barbatus* and *M. merlangus* - *T. minutus* c. ($p > 0.05$), as evidence by the PCA plot. The present results support the hypothesis that sagitta morphologic features could be used to characterized the sagittae of fish and provides to be an efficient tool for distinguishing the fish ecotype, according to Volpedo and Echeverría (2003).

Tab. 1 - Biological parameters, otolith indexes. IRI%=index of relative importance in percentage of the most preyed taxon.

Parametri biologici principali, indici degli otoliti. IRI%=indice di importanza relativa in percentuale del taxon più predato.

Order/species	TL (mm)	depth (m)	Habitat	IRI%	n°otolith	A%	L%	EL%	Group	PERMANOVA
GADIFORMES										
<i>Merlangius merlangus</i>	96	41	bento-pelagic	Crustacea: 79.79	17	6.6	2.6	39.0	1	Group 1 vs. Group 2
<i>Merluccius merluccius</i>	190	112	necto-benthic	Teleostei: 70.84	35	5.2	2.1	39.5	1	
<i>Trisopterus minutus</i> c.	156	56	bento-pelagic	Crustacea: 96.21	60	5.7	2.2	39.0	1	
PERCIFORMES										
<i>Mullus barbatus</i>	112	47	benthic	Crustacea: 85.88	41	2.2	1.6	71.3	2	$p < 0.001$
<i>Pagellus erythrinus</i>	153	31	benthic	Anellida: 32.34	58	4.8	3.3	65.7	2	
SCORPAENIFORMES										
<i>Chelidonichthys lucerna</i>	220	35	benthic	Crustacea: 94.91	20	1.7	1.3	75.3	2	

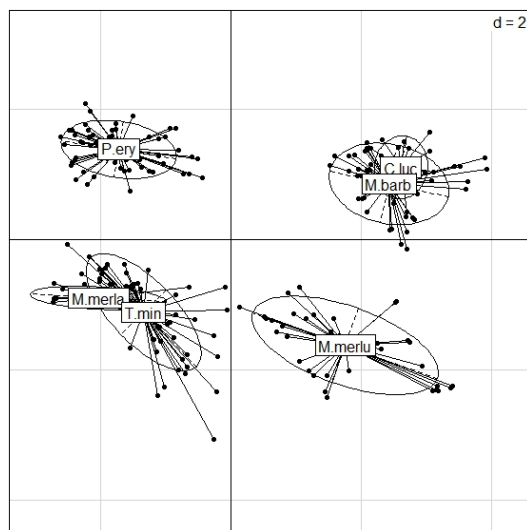


Fig. 1 - PCA plot.

Grafico della PCA.

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PRELIMINARY RESULTS ON ORGANIC AND MINERAL FRACTIONS IN OTOLITHS OF THREE FISH SPECIES FROM ADRIATIC SEA

RISULTATI PRELIMINARI SULLA COMPONENTE ORGANICA E MINERALE IN OTOLITI DI TRE SPECIE DI PESCI DELL'ADRIATICO

Abstract - The otolith microstructure is governed by the organic matrix and may be linked to the ontogenetic and phylogenetic differentiation of otolith shape. It is also a potential indicator of environmental, community, population and individual fish levels. This preliminary study showed intra-inter specific quantitative differences in the organic matrix contents in the otolith, suggesting changes in the degree of mineralization. In fact, in the same species, juveniles showed a greater contents of organic matrix respect to adults and differences between species. Adults showed differences between the different ecological types.

Key-words: otolith structure, *Chelidonichthys lucerna*, *Boops boops*, *Engraulis encrasicolus*.

Introduction - Fish otoliths are calcified biomineral composed principally of calcium carbonate and a small fraction of organic matrix (OM). The sagittal otolith has a species-specific shape that is likely the result of a different assemblage of the mineral building blocks through the diverse use of the organic matrix components making diverse frameworks. The aim of this preliminary study is to highlight quali-quantitative ontogenetic differences against organic and mineral content in the sagitta of two species of the Adriatic Sea: *Chelidonichthys lucerna* (Linnaeus, 1758) (benthic type) and *Boops boops* (Linnaeus, 1758) (necto-benthic type). The same analyses was carried out also at inter-specific level, adding to the species mentioned above, the species *Engraulis encrasicolus* (Linnaeus, 1758) (pelagic type) in order to evaluate a possible environmental influence linked to biomineralization process.

Materials and methods - Samples were collected from trawl surveys carried out in the north-central Adriatic Sea from 2008 to 2012. The sagittal otoliths of all specimens were removed from otic capsule, cleaned from adhering tissues with 3% (v/v) H₂O₂ for 15 minutes and then sonicated in MilliQwater in the ultrasonic bath for 5 minutes. Finally they were air dried and stored. X-ray powder diffraction (XRD) patterns were collected using a PanAnalytic X'Pert Pro equipped with X'Celerator detector powder diffractometer using Cu Ka radiation generated at 40 kV and 40 mA. The content in organic matter of the otoliths was determined by thermo gravimetric analysis (TGA) using a SDT Q600 simultaneous thermal analysis instrument (TA instrument). The analysis was performed under nitrogen flow from 30 to 120 °C with a heating rate of 10 °C min⁻¹, an isothermal at 120 °C for 5 min, and another cycle from 120 to 600 °C with the same heating rate. Results were reported as means of several replicas (generally three for each group of individuals) using a total of 40 otoliths. We have taken into account random and instrumental errors by propagation of uncertainty (Taylor, 1997).

Results and conclusions - For all samples, the XRD patterns showed only the diffraction peaks of aragonite (Fig. 1a). No transition between CaCO_3 crystalline forms were found at ontogenetic level between juveniles and adults of *C. lucerna* and *B. boops*. The otoliths thermograms showed a first weight loss in a range around 130-400 °C followed by another one in the range between 410 and 500 °C. Around 530 °C aragonite converted into calcite in all samples, as showed by XRD diffractograms (Fig. 1b). The weight percentage values of the organic matrix, expressed in terms of weight loss (water + OM) ranged between 2.1 and 3.4% (w/w) (Tab. 1). The juveniles had greater loss than adults both for *C. lucerna* and *B. boops* (errors are not overlapped at all, Taylor, 1997), showing that the organic matrix could have different roles depending on the ontogenesis. At inter-specific level, comparing only adult individuals, *E. encrasicolus* (pelagic type) showed greater weight loss than *C. lucerna* (benthic type) and *B. boops* (necto-benthic type) showing that the chemical composition of otoliths may also be influenced by environmental factors. In conclusion, these preliminary results confirmed the data reported for other species (Morales-Nin, 1986; Cermeño *et al.*, 2006) and suggest that the biomineral formation is influenced by genetic and environmental factors in unknown proportion (Parmentier *et al.*, 2002).

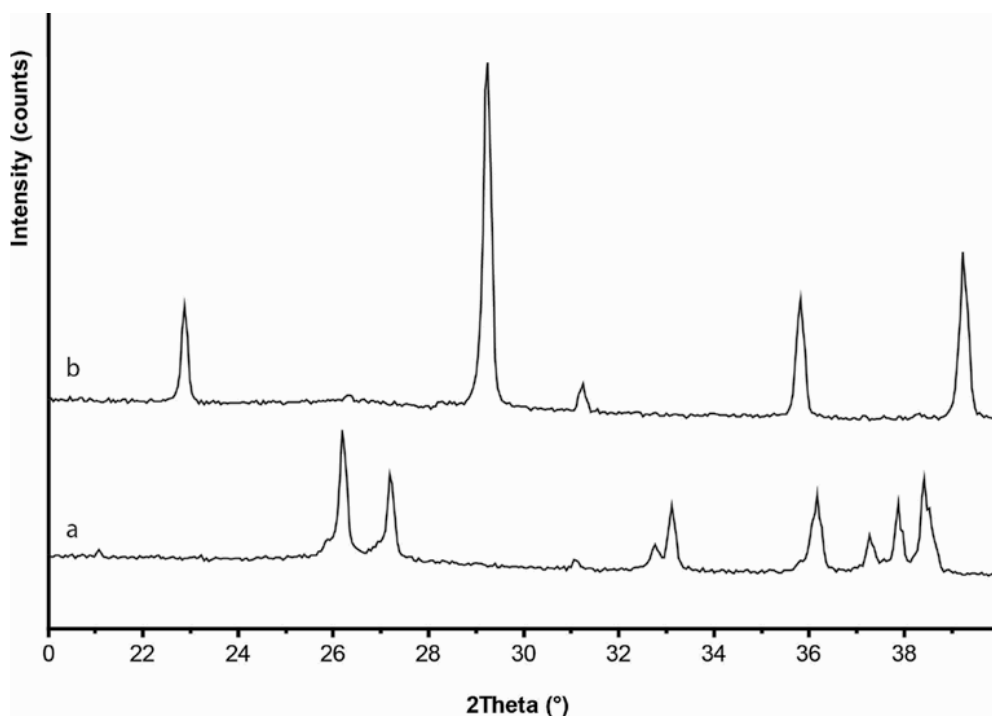


Fig. 1 - Diffractograms from otolith of *B. boops* adults: a) Untreated sample in which only the diffraction peaks of aragonite are present; b) XRD thermal treated powder (up to 600 °C) the diffraction peaks showing the presence of the calcite.

Figura di diffrazione a raggi X di otoliti di esemplari adulti di B. boops: a) diffrattogramma dell'aragonite; b) diffrattogramma di polveri trattate termicamente fino a 600 °C, i picchi indicano la presenza di calcite.

Tab. 1 - List of studied species including size, ecotype, samples and total weight loss % (w/w) corresponding to the weight percentage of organic matrix (OM) of the otoliths.

Elenco delle specie studiate per taglia, ecotipo, numero campioni e perdita in peso percentuale corrispondente al peso percentuale della matrice organica (OM) degli otoliti.

Species class	TL mean (mm)	Environment	N. otoliths	OM %(w/w)
<i>C. lucerna</i> juvenile	100	benthic	16	2.943 ± 0.006
<i>C. lucerna</i> adult	230	benthic	4	2.702 ± 0.005
<i>B. boops</i> juvenile	92	necto-benthic	4	2.51 ± 0.01
<i>B. boops</i> adult	176	necto-benthic	4	2.081 ± 0.004
<i>E. encrasicolus</i> adult	130	pelagic	12	3.39 ± 0.01

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THE ORGANIC MATRIX OF OTOLITH FROM TRIGLIDAE (OSTEICHTHYES, SCORPAENIFORMES): QUANTI-QUALITATIVE ANALYSIS

The present study is the first attempt to analyze at quanti-qualitative level the organic matter of otoliths in sound producers bony fishes of Triglidae family. Otoliths are paired metabolically inert concentric deposits in which alternating layers of protein and calcium carbonate (CaCO_3) grow around a nucleus and are formed by daily growth increments of CaCO_3 , used for balance and/or hearing in all teleost fishes. The chronological properties of otoliths are unparalleled in the animal world, allowing accurate estimates of fish age and growth and environmental reconstruction based on incorporated elements (CAMPANA and THORROLD, 2001). The role of organic matrix is not fully understood, although different studies have demonstrated that it plays a crucial role in determining the precipitation of the CaCO_3 polymorph (MANN, 2001) and controlling the shape of the otolith (MILLER *et al.*, 2010). We collected sagittal otoliths from target gurnard species (*Chelidonichthys cuculus*, *Chelidonichthys lucerna*, *Eutrigla gurnardus*, *Lepidotrigla cavillone*) sampled in north-middle Adriatic by MEDITS trawl fisheries. The otoliths were removed from otic capsule, cleaned from adhering tissues with 3% (v/v) H_2O_2 for 15 minutes and then sonicated in MilliQwater in the ultrasonic bath for 5 minutes. The quantitative content of the organic matter in the otoliths was determined by thermo gravimetric analysis (TGA) using a SDT Q600 simultaneous thermal analysis instrument (TA instrument). The qualitative content of the organic matter was determined, after sonication in a denaturing buffer and determination of the protein content by Lowry DC method (Biorad); the sample has been analyzed by SDS-PAGE and bands have been detected by Silver Staining (Sigma). At quantitative level, the preliminary analysis of organic matter for all species showed a very small fraction of the whole otolith ranging from 2.52% to 2.54% in terms of weight percentage in adults and from 2.81% to 3.50% in juveniles, confirming also ontogenetic differences in organic matrix implication (CERMEÑO *et al.*, 2006). At qualitative level, the preliminary analysis of the obtained gels for adult *L. cavillone*, showed the presence of three major bands under the thresholds corresponding at around 45, 30 and 20 kDa; another less obvious band is visible around 75 kDa. The band observed around 45 kDa may correspond to otolith matrix protein (OMP), one of the major components of fish sagittae according to MOTTA *et al.* (2009).

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FIRST STUDY ON THE PROPERTIES OF THE ORGANIC MATRIX IN THE OTOLITHS OF SOME BONY FISH SPECIES

PRIMO STUDIO SULLE PROPRIETÀ DELLA MATRICE ORGANICA DEGLI OTOLITI DI PESCI OSSEI

Abstract - The aim of the present study is to analyse the properties of the organic matrix from the otoliths of some bony fish species with the aim of investigating intra- and inter-specific differences in otolith biomineralization processes. In the urea soluble fractions a unique large band around 50-55 kDa was observed in all samples. Acid protein fractions were observed in two out of three species, indicating that the organic matrix has different roles depending on species and the time of its formation.

Key-words: otolith, protein matrix, SDS-page, Silver staining, Adriatic Sea.

Introduction - Otoliths are calcium carbonate structures (usually aragonite) located in the inner ear of bony fish, and laid down on an organic matrix. Mineralogy, microstructures and chemical composition of the calcium carbonate of otoliths have been thoroughly investigated, but little is known about the organic molecules (Dauphin and Dufour, 2003). This organic matrix is composed of proteins, glycosaminoglycans and lipids (Borelli *et al.*, 2001). The organic matrix plays a key role in controlling the formation of the different layers and shape of otoliths (Gauldie, 1999). The aim of this study is to provide more insight into the biochemical properties of otolith soluble organic matrix of some demersal bony fishes from Adriatic Sea. In addition we have identified a suitable technique for extraction of protein matter.

Materials and methods - A total of 295 otoliths (sagittae) were collected from 2009 to 2012 by trawling in the northern and central Adriatic Sea. In particular, 150 otoliths from *Lepidotrigla cavillone* (Lacepède, 1801), 50 from *Lepidotrigla dieuzeidei* (Blanc and Hureau, 1973), 45 from *Trigloporus lastoviza* (Bonnaterre, 1788) and 50 from *Helicolenus dactylopterus* (Delaroche, 1809) were used. In a first investigation, right and left otoliths were previously cleaned with 3% H₂O₂ and then in ultrasonic bath with MilliQ. The samples were ground with an agate mortar into fine powders, then mixed and decalcified with EDTA 100 mM according to Motta *et al.* (2009). At a later stage, we followed the protocol described by Borelli *et al.* (2001) with some modifications (i.e. protein precipitation was enhanced by using TCA, trichloroacetic acid, 100% w/v). Protein fractions (soluble in acetic acid 0.5 M or in urea 8 M), were loaded into a polyacrylamide gel (12%), separated by SDS page and detected by Silver staining (Sigma). The acid soluble fractions and urea soluble fractions were estimated by Lowry DC-Biorad and Bradford QuickStart-Biorad methods, respectively.

Results and conclusions - A first evidence was that ultrasonic treatment on samples (for both cleaning and protein extraction steps) should be bypassed, in order to avoid protein degradation. Table 1 shows the protein yields, computed from known weight fractions of otoliths. For *L. cavillone* and *L. dieuzeidei*, higher amounts of proteins

were extracted by acetic acid protocol (0.16 and 0.11 µg proteins/mg of otoliths, respectively), than by urea extraction protocol (0.057-0.058 µg/mg). In contrast, urea was slightly more efficient as solubilizing agent for *T. lastoviza* (0.057 vs 0.04 µg/mg). Very low yields were instead obtained from *H. dactylopterus* otoliths by both methods (< 0.002 µg/mg). Separation of the total amount of each fraction has revealed that a 50-55 kDa band was clearly evident in the acid fraction of *T. lastoviza* (13 µg protein/lane), whereas it became evident in *L. cavillone* acid fraction only when higher amount of total proteins was loaded (30 µg/lane). No clear signal was instead seen in acid *L. dieuzeidei* fraction (22 µg/lane). The total amount of *H. dactylopterus* proteins obtained from acid extraction was instead too low to get information about its composition. In the urea soluble fractions a unique large band around 50-55 kDa was revealed for *L. cavillone*, *L. dieuzeidei* and *T. lastoviza* pellet (11 µg/lane). In spite of a very low amount of sample loaded into the gel (1.6 µg/lane), a slight signal was also seen in the lane of *H. dactylopterus* sample. Interestingly, a common clear band at the top of the separating gel was seen for all samples (both acetic acid and urea soluble fractions): these signals may indicate the presence of high molecular weight proteins (>300/350 kDa), unable to enter into the pores of polyacrylamide gels (12%). Currently it is not possible to indicate whether the 50-55 kDa band represents a unique protein or whether it represents the sum of more distinct proteins with similar molecular size. More information may be obtained in the future by 2D map. Finally, we observed that solutions turned into yellow, as a result of a pH decrease, by mixing *L. dieuzeidei* and *T. lastoviza* acid protein fractions to bromophenol blue-containing sample loading buffer. This effect was not seen in *L. cavillone* extract. The differences in acid protein fractions showed variation in otolith matrix composition linked to the time of its formation and specie-specific ontogenetic changes according to Morales-Nin (1986). Further studies have to focus on protein content variation with fish age, especially because the organic matrix acts as a guide to the deposition and growth of otoliths.

Tab. 1 - Protein yields.

Rese proteiche.

	<i>L. cav</i>	<i>L. die</i>	<i>T. las</i>	<i>H. dac</i>
Otoliths weight (mg)	191.0	192.0	298.0	1040.0
Acetic acid soluble fraction (ug)	30.0	22.0	13.0	0-0.1
Urea soluble fraction (ug)	11.0	11.0	17.0	1.6
Protein Yield by Acetic Acid extraction (ug protein/ mg otoliths)	0.16	0.11	0.04	0.00
Protein Yield by Urea extraction (ug protein/ mg otoliths)	0.058	0.057	0.057	0.002

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ELLIPTIC FOURIER ANALYSIS OF OTOLITHS OF TRIGLIDAE IN THE NORTH-MIDDLE ADRIATIC SEA

ANALISI ELLITTICA DI FOURIER DEGLI OTOLITI DEI TRIGLIDI IN ALTO-MEDIO ADRIATICO

Abstract - The sagittal otoliths of specimens belonging to seven species of triglids collected in the north-middle Adriatic Sea were investigated by means of the Elliptic Fourier Analysis method (EFA). The EFA method was proved to be a suitable tool for the separation of species showing intra-interspecific differences. Thus, it could provide useful information in phylogenetic and eco-morphological studies.

Key-words: otolith, shape analysis, Elliptic Fourier Analysis, Triglidae, Adriatic Sea.

Introduction - Fish otolith shape analysis is an important way for describing and characterizing mathematic otolith outlines. It is used with multiple goals, for example, species phylogeny and stock discrimination (Lombarte *et al.*, 2006). In particular the Elliptic Fourier Analysis (EFA) represents one among the most valuable and time-efficient method since data are automatically normalised in relation to the first harmonic and consequently they become invariant to size, rotation, and starting point (Iwata and Ukai, 2002). In this study the EFA method was applied on otoliths collected from 7 species of triglids (*Aspitrigla cuculus*, *Chelidonichthys lastoviza*, *C. lucerna*, *Eutrigla gurnardus*, *Lepidotrigla cavillone*, *L. dieuzeidei* and *Trigla lura*) (Teleostei, Scorpaeniformes) distributed in the north-middle Adriatic Sea. The aim of this study is to verify the existence of intra and interspecific differences associated with endogenous and exogenous factors.

Materials and methods - A total of 240 specimens were selected from samples collected during bottom trawl surveys carried out in 2007 and 2008 along the Italian coasts from the Gulf of Trieste to the Tremiti Islands. For each specimen total length (TL, mm), weight (W, g) and sex were recorded. The left sagitta was removed, cleaned in ultrasounds bath and kept dry for later analysis. For each species otoliths selected from adult (males and females) individuals and from juvenile (undetermined) ones were analyzed. Digital images were collected using a NIKON P5100 digital camera linked to a Leica MZ6 stereomicroscope. Each sagitta was photographed with the *sulcus acusticus* facing up and the *rostrum* to the right. The SHAPE program was used to extract the contour shape of the sagitta and to assess the variability of shapes by means of the study of principal component analysis (PCA). The statistical analysis of the collected data was carried out by means of the R software (R Development Core Team, 2010).

Results - About 99% of variation in otolith shape was explained by a maximum of 20 harmonics. The first 4 discriminated over 80% of the variance. In most samples, the first component discriminated better the different widths of otoliths; the second was better related to different shape of *excisura ostii*; the third and fourth were better related to different shape of *rostrum* and *antirostrum*. The interspecific comparison of adult specimens showed the Genus *Lepidotrigla* and *C. lastoviza* phylogenetically close, while *L. dieuzeidei* was discriminated by the Genera *Aspitrigla* and *Eutrigla*.

The interspecific variability of juvenile specimens showed that *C. lucerna* and *T. lyra* were widely discriminated. This finding is probably due to individual genetic factors (Gauldie and Crampton, 2002) and to the different depths of their trophic niches. In fact, during their life cycle, triglids in Adriatic show a differential migration pattern to greater depths and away from the Italian to the Croatian coast (Montanini *et al.*, 2008). The intraspecific variability of adult specimens was higher for *C. lucerna* and *E. gurnardus* while it was lower for the Genera *Aspitrigla* and *Lepidotrigla*. Intraspecific comparison between juveniles and adults showed that in all species there was an increase in otolith shape variability linked to growth and to environmental conditions.

Conclusions - According to these results, the EFA method was proved to be a suitable tool for supporting phylogenetic and eco-morphological investigations and assessing affinities among the investigated triglids species. It allowed to find similarity between *Lepidotrigla* sp. and *C. lastoviza*; distance between *C. lucerna* and *T. lyra* and an increase in the variability of otolith shape from juveniles to adults. However, in order to facilitate correct biological interpretation of data, the EFA method should be correlated with appropriate sampling plans (Farias *et al.*, 2009; Stagoni *et al.*, 2009).

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ANALISI ELLITTICA DI FOURIER DEGLI OTOLITI DEL GENERE *LEPIDOTRIGLA* (TELEOSTEI: TRIGLIDAE) NEL MARE ADRIATICO

ELLIPTICAL FOURIER ANALYSIS OF OTOLITHS OF GENUS LEPIDOTRIGLA (TELEOSTEI: TRIGLIDAE) IN ADRIATIC SEA

Abstract - Shape analyses were carried out on otoliths of genus *Lepidotrigla* in order to discriminate species difference. Samples were random collected in Adriatic sea during Grund 2007 scientific trawl survey. Fishes were measured for length, weight, otoliths shape indices and outline. On data were performed univariate and multivariate analyses to detect difference by species.

Key-words: otoliths, shape, fourier analysis.

Introduzione - La morfometria geometrica è uno strumento relativamente nuovo applicato alla biologia, in particolare l'analisi ellittica di Fourier (EFA) (Tracey, 2006). L'EFA si è dimostrata robusta nei confronti dell'effetto taglia (crescita allometrica). Al contrario le analisi morfometriche convenzionali (es. indici di forma) vanno condotte sui dati trasformati, per mitigare o annullare la correlazione tra parametri misurati e taglia dell'esemplare, come gli otoliti dei pesci (Leonart, 2000). Si è cercato quindi di applicare tale procedura allo studio della forma degli otoliti del genere *Lepidotrigla*, rappresentato in Adriatico da due sole specie *L. cavillone* (Lacepède, 1801) e *L. dieuzeidei* (Blanc e Hureau, 1973), dalla morfologia esterna molto simile, per verificare la specie-specificità dell'otolite.

Materiali e metodi - Durante la campagna di pesca Grund 2007 in Alto e Medio Adriatico, sono stati analizzati un totale di 75 esemplari suddivisi in: 50 di *L. cavillone*; di cui 25 a nord (LC1) e 25 a sud (LC2) della congiungente Ancona-Zara; 25 di *L. dieuzeidei* (LD) a sud di tale linea. Il campionamento è random. Ogni esemplare è stato misurato (1 mm) e pesato (0.1 g). Gli otoliti (sagitte) sono stati lavati, asciugati e pesati (0.1 mg); la sagitta sinistra è stata fotografata al microscopio (Leica MZ6 - Nikon P5100). Le immagini sono state elaborate con ImageJ (Rasband, 2008) per calcolare gli indici di forma aspect-ratio (AR), circularity (Circ), roundness (Round) e con Shape (Iwata, 2002) per ricostruire l'outline con EFA (20 armoniche, normalizzate). I dati sono stati elaborati con R (R Development Core Team, 2008).

Risultati - Il range di taglia è omogeneo per tutti i campioni (80-120 mm); le mediane sono di 104 mm (LC1), 88 mm (LC2), 97 mm (LD); le medie sono 101,8 mm (LC1), 90,6 mm (LC2), 95,2 mm (LD). L'ANOVA sulla distribuzione di lunghezze dei 3 campioni (test di Levene $F_{2,72}=0,929$ $p=0,3996$) ha evidenziato una differenza significativa ($F_{2,72}=8.8439$, $p<0.001$), il test di Tukey per confronti multipli indica una differenza tra LC1 e LC2 ($p<0.001$), ma non tra LD e LC1 o LD e LC2. Perciò i due gruppi di *L. cavillone* sono stati trattati separatamente nelle analisi successive. Gli indici di forma degli otoliti sono tutti correlati alla lunghezza degli esemplari e sono stati quindi standardizzati secondo il metodo proposto da Leonart (2000). L'ANOVA sugli indici di forma standardizzati non ha evidenziato differenze significative tra i 3 campioni. Dall'analisi EFA risulta che per tutti i campioni già le prime 5 armoniche

spiegano oltre l'84% della varianza dei dati. La ricostruzione dell'outline con le prime 8 armoniche (oltre 93% della varianza) e la relativa analisi PCA hanno evidenziato che la prima componente è collegata al fattore taglia (non discriminante), la seconda è collegata alla geometria del rostro-antirostro e discrimina nettamente LD da LC1 e LC2 (tra loro invece interdistribuiti). La terza componente è relativa all'invasione tra rostro e antirostro (non discriminante).

Conclusioni - Negli ultimi anni lo studio degli otoliti si è sviluppato su vari fronti: microstruttura, accrescimenti annuali, confronto e validazioni dei metodi di "ageing", dinamica di popolazione, udito ed equilibrio, allometria, microchimica, ricostruzioni ecologiche e identificazione delle specie (Campana, 2005). In questo ultimo campo l'EFA si è dimostrata particolarmente adatta allo studio della forma degli otoliti, riuscendo nel nostro caso, a discriminare efficacemente le due specie (*L. cavillone* e *L. dieuzeidei*) a differenza dell'analisi univariata sugli indici di forma. Particolare importanza ha il fatto che escludendo dall'analisi EFA la prima armonica si può eliminare a priori l'"effetto taglia", semplificando l'interpretazione dei risultati (Crampton, 1995; Rohlf e Archie, 1984). Ricostruire l'"outline" medio di un campione di otoliti utilizzando un numero variabile di armoniche consente invece di condurre analisi mirate: ciò permette di individuare variazioni del contorno degli otoliti anche molto lievi. Applicando tale metodologia su un numero maggiore di esemplari e utilizzando adeguati piani di campionamento sarebbe interessante verificare se esistano differenze intraspecifiche associate a differenti fattori (sexo, profondità, popolazioni, ecc...). Più in generale tale tecnica di analisi potrebbe risultare utile nell'identificazione delle specie presenti nei contenuti stomacali, al fine di ricostruire le reti trofiche.

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COMPARATIVE MORPHOLOGY OF TRIGLIDAE OTOLITHS FROM THE NORTH-MIDDLE ADRIATIC SEA

MORFOLOGIA COMPARATIVA DEGLI OTOLITI DEI TRIGLIDI IN ALTO-MEDIO ADRIATICO

Abstract – The sagittal otoliths of Triglidae family from the Adriatic Sea (north-eastern Mediterranean) were described using morphological and morphometric characters. The morphological descriptions were based on the otolith shape, outline and sulcus acusticus features. The morphometric parameters determined were expressed in terms of 5 shape indices. Otolith shape intra-inter specific changes have been described providing informations about species identification, fish size, sex and phylogenesis.

Key-words: otolith reading, shape, Triglidae.

Introduction - Triglidae (Teleostei, Scorpaeniformes) are known for their sound producing ability in agonistic contexts related to territorial defense, reproduction and competitive feeding (Amorim *et al.*, 2004). In the Adriatic Sea (north-eastern Mediterranean) triglids are represented by 7 species: *Aspitrigla cuculus* (*ASPICUC*), *Chelidonichthys lastoviza* (*TRIPLAS*), *C. lucerna* (*TRIGLUC*), *Eutrigla gurnardus* (*EUTRIGUR*) *Lepidotrigla cavillone* (*LEPTCAV*), *L. dieuzeidei* (*LEPTDIE*), *Trigla lyra* (*TRIGLYR*), poorly discussed for the Mediterranean Sea (Vallisneri *et al.*, 2010). Otolith shape and *sulcus acusticus* features, related to genetic and environmental factors, might be an important key for species identification. The aim of this study was to analyze interspecific and intraspecific shape differences in sagittal otoliths of triglid species.

Materials and methods – Samples of seven Triglidae species were collected during several bottom trawl surveys in the north-middle Adriatic Sea, from the Gulf of Trieste to the Tremiti Islands in 2007 and 2008. For each specimen total length (TL, mm), weight (W, g) and sex were recorded. A total of 634 sagittal otoliths were removed, washed and weighed to 0.01 mg. The sagittae were photographed under a microscope (Leica MZ6-Nikon P5100). The images were processed with ImageJ (Rasband, 2008) to calculate five shape indices (aspect ratio, roundness, rectangularity, ellipticity, circularity). Shape indices for which “species-length” interactions were significant ($P < 0.001$) were normalised. The data were processed with R (R Development Core Team, 2010). For the same fish length range, a sample of sagittae were analyzed ultrastructurally by scanning electron microscopy.

Results - 1) UNIVARIATE ANALYSIS (ANOVA): Interspecific comparison, analyzed by univariate analysis, were tested by Kruskal-Wallis and Tukey tests. About shape indices, major shape variation were respectively: for “aspect ratio” *LEPTDIE-TRIGLUC*; *LEPTCAV-TRIGLUC*; *LEPTCAV-TRIGLYR*; for “roundness” *TRIGLUC-LEPTCAV*; for “rectangularity” *ASPICUC-LEPTCAV*; for “ellipticity” *LEPTDIE-TRIGLUC*; *LEPTCAV-TRIGLUC*; *TRIPLAS-TRIGLUC*; *LEPTCAV-TRIGLYR*; *LEPTDIE-TRIGLYR*; for “circularity” *LEPTDIE-LEPTCAV*; *LEPTDIE-TRIPLAS*; *LEPTCAV-TRIPLAS*. Otoliths morphologically more

different, defined by three shape indices, were those belonging to *L. cavillone* and *C. lucerna*. The results were in agreement with the analytical key about the family (FAO, 1987) that placed these two species at the extremes. 2) MULTIVARIATE ANALYSIS (MANOVA): Intraspecific comparison were analyzed by multivariate analysis. The otolith outline shape indices changed with size (between juveniles and adult) and sex for some species. In particular, intraspecific comparison showed significant differences between juveniles and adults for *A. cuculus*, *C. lucerna*, *L. dieuzeidei* and between females and males for *L. dieuzeidei*. Therefore, about the intraspecific relationship, *L. dieuzeidei* was the species more diversified. 3) SCANNING ELECTRON MICROSCOPY (SEM): otolith shape of triglids were approximately oval. Preliminaries analyses showed interspecific variations in the morphology and ultrastructure of the *sulcus acusticus*, about shape, size, direction of crystalline arrangement and crystalline surface. Intraspecific variation appeared relate to growth and environmental factors. These results were in agreement to Tuset *et al.* (2003) and Jitpukdee (2009) for other families.

Conclusions - These results suggested: similarity between species belonged to the same genus; differences between species phylogenetically distant (e.g. *L. cavillone* and *C. lucerna*); intraspecific differences between juveniles and adults (e.g. *A. cuculus*, *C. lucerna*, *Lepidotrigla dieuzeidei*); similarity between species belonged to different genera (*Lepidotrigla* spp. and *C. lastoviza*). These methods (geometric morphometrics and ultrastructure analysis) constitute an important instrument for species identification using sagittal otoliths collected in feeding remains of bony fish predators, in fossiliferous layers, in archaeological sites.

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ATLANTE DEGLI OTOLITI DI SPECIE ITTICHE DEL NORD-MEDIO ADRIATICO

ATLAS OF THE FISH OTOLITHS IN THE NORTHERN-MIDDLE ADRIATIC SEA

Abstract – *Fish otoliths of 104 species of the Northern-Middle Adriatic Sea have been digitally photographed using image analysis procedures for reference collection. Shape and size, characteristics of the species, were analyzed by stereomicroscope and SEM. One of the main goal of image collections was to identify species remains recovered from fish stomachs.*

Key-words: *otolith identification, image analysis, data-base, North-Middle Adriatic Sea.*

Introduzione - Lo studio morfologico degli otoliti ha molteplici applicazioni: 1) valutazione delle relazioni trofiche nelle reti alimentari (mediante analisi dei contenuti stomacali di specie marine ittiofaghe); 2) studi tassonomici (identificazione della specie e correlazioni filogenetiche, Piera *et al.*, 2005; sito web (2006); 3) valutazione delle risorse ittiche (con identificazione dell'età, della dinamica di popolazione, degli stocks, Vallisneri *et al.*, 2008); 4) studi di morfologia funzionale ed ecomorfologia (mediante valutazione delle correlazioni tra caratteri morfologici e biometrici degli otoliti e caratteri ambientali); 5) confronto tra la fauna fossile e l'attuale (paleontologia e paleoecologia); 6) determinazione di animali ritrovati in scavi archeologici (archeozoologia). Tuttavia, a tuttora non si dispone di un atlante o di un database esaustivo di immagini ottiche ed elettroniche digitali di otoliti delle specie ittiche (Lombarte *et al.*, 2006; Piera *et al.*, 2005), in particolare del Nord-Medio Adriatico, ma solo parziali raccolte (Costa, 1991).

Materiali e metodi - Per ciascuna specie ittica è stata redatta una scheda che riporta: nome scientifico, nome comune, famiglia, ordine, area. È stato messo a punto un sistema informatico di acquisizione di immagini digitali di otoliti (sagittae) realizzate allo stereomicroscopio ed al microscopio elettronico a scansione per l'analisi dei dettagli. Il materiale iconografico raccolto, con un migliaio di fotografie, costituisce una banca dati di riferimento, oggettiva, immediata e di facile consultazione. Ciascun otolite è stato lavato in una soluzione contenente ipoclorito di sodio diluito all'1% per essere ripulito da eventuali residui di tessuto. Successivamente sono state realizzate fotografie che riprendono: 1) la superficie prossimale recante il solco acustico (un incavo lungo la superficie); 2) la superficie distale in cui è visibile il nucleo. L'otolite (immerso in una pellicola d'acqua) è stato fotografato su entrambe le superfici. Per ottenere una buona rappresentazione del contorno dell'otolite, l'immagine è stata contrastata il meglio possibile (con lo sfondo nero) tramite il programma grafico "Image J". Le immagini sono in TIFF, di preferenza nella scala dei grigi e 2592×1944 pixels di dimensione.

Risultati - È stato realizzato un archivio informatico e cartaceo contenente una scheda che riporta immagine e dati relativi agli otoliti, nonché alla biometria delle specie ittiche dell'alto-medio Adriatico. In totale sono stati fino ad ora analizzati gli otoliti di 104 specie, appartenenti a 48 famiglie ed a 15 ordini: Anguilliformes (Congri-

dae, Ophichthidae), Atheriniformes (Atherinidae), Aulopiformes (Chlorophthalmidae), Clupeiformes (Clupeidae, Engraulidae), Gadiformes (Gadidae, Lotidae, Macruridae, Merluccidae, Phycidae), Lampriformes (Trachipteridae), Lophiiformes (Lophiidae), Mychtophiformes (Mychtophidae), Ophidiiformes (Ophidiidae), Osmeriformes (Argentinidae), Perciformes (Blennidae, Callionymidae, Carangidae, Centracanthidae, Cepolidae, Gobiidae, Labridae, Mugilidae, Mullidae, Pomatomidae, Scianidae, Scombridae, Serranidae, Sparidae, Sphyraenidae, Trachinidae, Trichiuridae, Uranoscopidae), Pleuronectiformes (Bothidae, Citharidae, Cynoglossidae, Pleuronettidae, Scopthalmidae, Soleidae, Sphyraenidae), Scorpaeniformes (Peristediidae, Scorpaenidae, Sebastidae, Triglidae), Syngnathiformes (Centriscidae), Zeiformes (Caproidae, Zeidae). Gli esemplari considerati hanno tutti raggiunto la taglia di maturità sessuale per garantire un confronto equo escludendo modificazioni morfologiche correlate all'ontogenesi degli otoliti. Il profilo morfologico e la biometria degli otoliti evidenziano la specie-specificità ed un andamento che tende a rispecchiare la filogenesi, in accordo con Lombarte *et al.* (2006). Il profilo degli otoliti, relativamente ai dati raccolti, si può definire secondo 11 differenti figure geometriche: 1) fusiforme, 2) triangolare, 3) oblungo, 4) quadrato, 5) ovale, 6) rotondo, 7) con processo, 8) con insenatura 9) a "T", 10) a "L", 11) a "8". Pare evidente un profilo simile nell'ambito di taxa filogeneticamente vicini; per esempio il genere *Solea* è generalmente caratterizzato da otoliti con profilo 6 e di taglia relativamente piccola (come molti della famiglia Soleidae); il genere *Trisopterus*, da profilo 1 e di taglia relativamente grande (come molti della famiglia Gadidae). Le specie della comunità demersale di acque profonde, sulla base dei dati finora raccolti, sembrano possedere generalmente otoliti di taglie relative maggiori rispetto alle specie demersali di acque basse o a quelle della comunità pelagica, in accordo con la letteratura (Lombarte *et al.*, 2006). Per esempio, il rapporto fra taglia otolite e taglia somatica di *Solea* si aggira attorno a 2%, mentre quello di *Merlangius* attorno a 7%.

Conclusioni – Si sottolinea l'importanza della realizzazione di collezioni fotografiche di riferimento, per le diverse aree geografiche, nello studio degli otoliti. L'archiviazione di immagini digitali al microscopio ottico ed elettronico consente, infatti, di realizzare un confronto rapido e sicuro per tutte le esigenze della ricerca.

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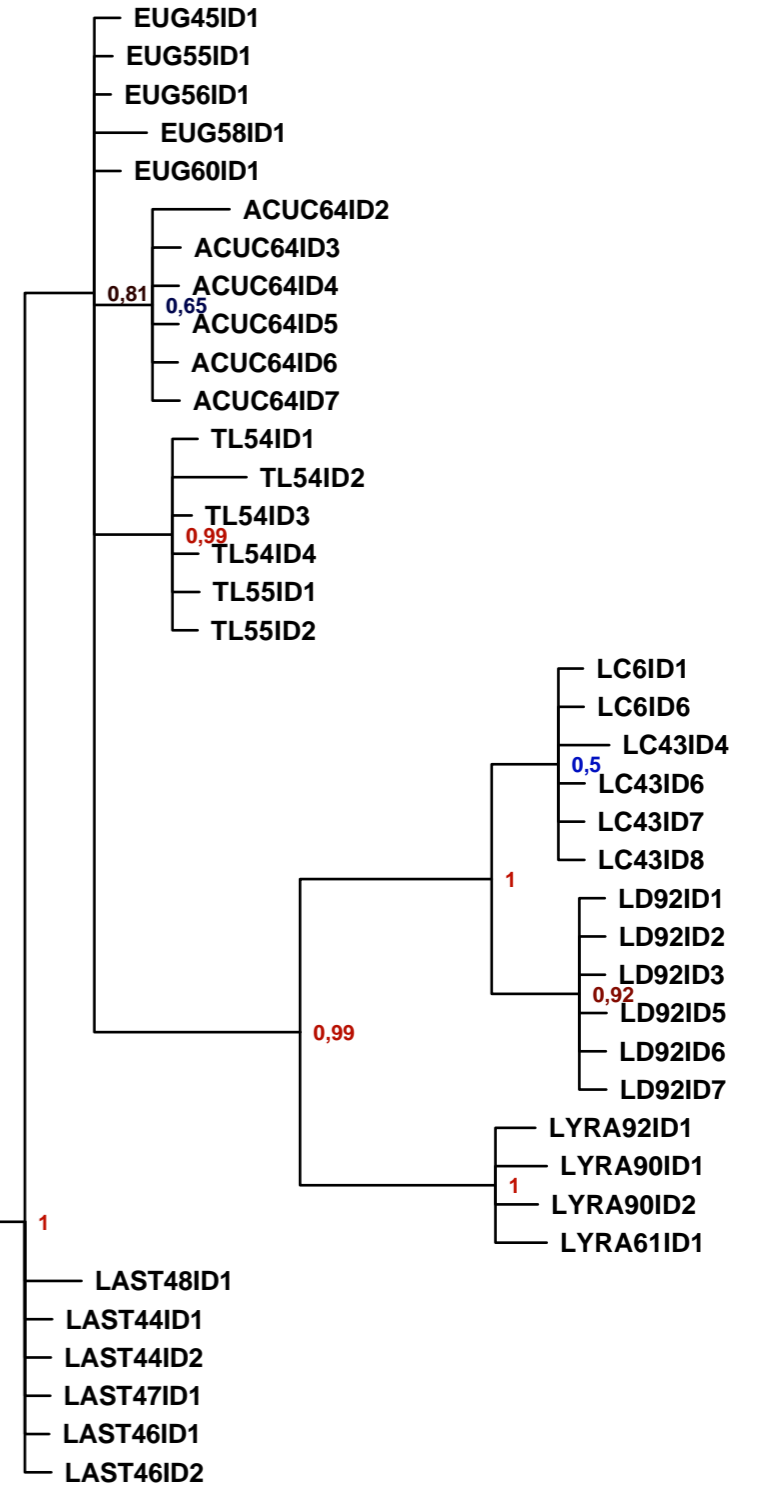
Chapter V. Preliminary data on mDNA phylogeny

SUMMARY OF CHAPTER V.

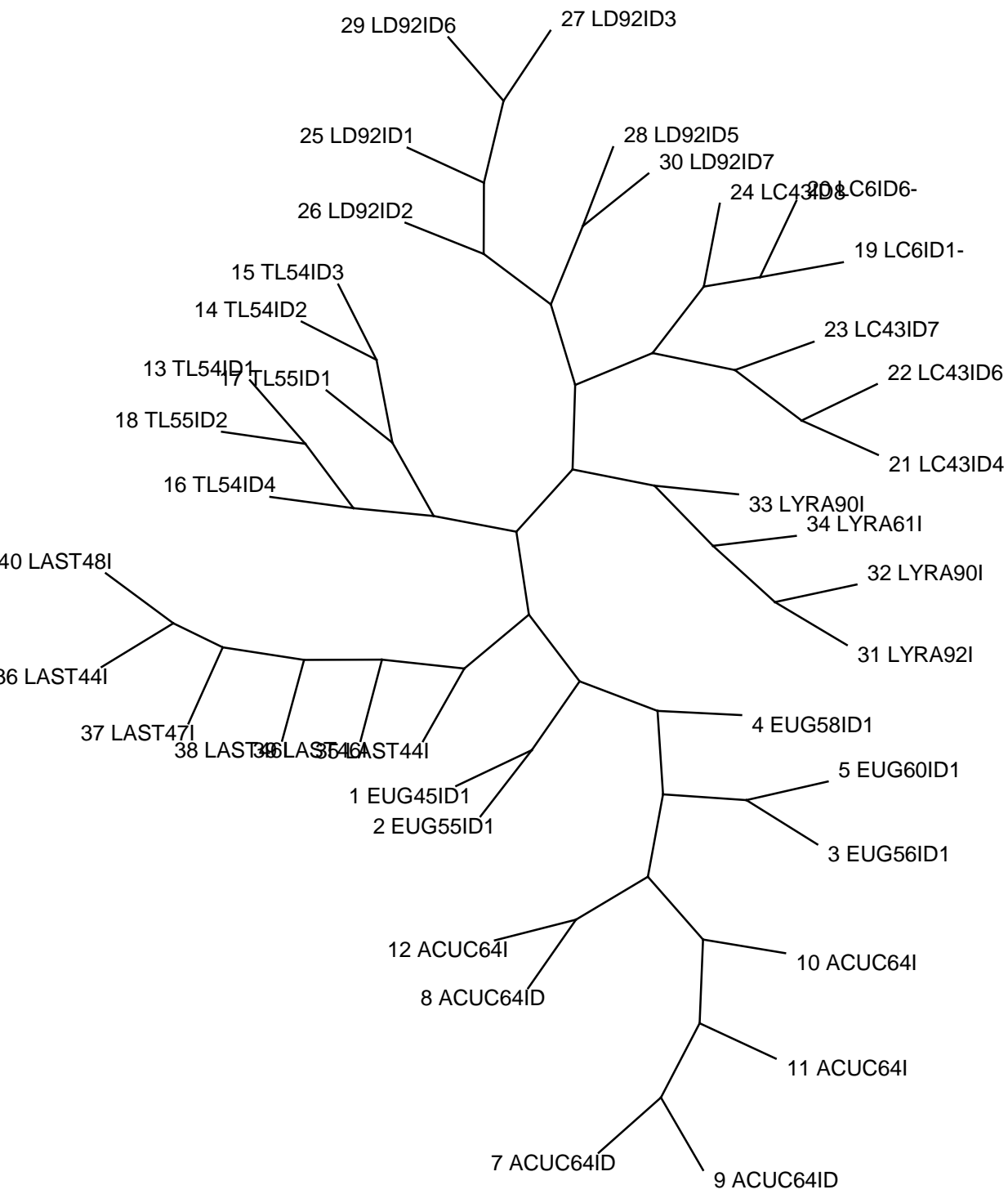
Preliminary molecular approach were conducted on mDNA on the 7 species from north-middle Adriatic Sea. As highlighted by the Italian Society of Marine Biology, many taxonomic names (genus) of Triglidae family changed in the last decades. Two methodologies were applied: 1) it was directly applied on Adriatic gurnard samples studying 16SrRNA gene extract from muscle tissues; 2) it was focused on mitochondrial gene cytochrome oxidase I (COI) from standardised DNA barcode library. About method 1, small sections of muscle (25 mg) were dissected from fresh samples and preserved in 95% ethanol. Before to proceed with the extraction protocol, tissue samples were incubated at 37°C for 1 hour to remove all ethanol residuals and after cutting, tissue were briefly sonicated. Afterwards genomic DNA were extracted using NucleoSpin Tissue (Macherey-Nagel) following supplier guidelines. A fragment between 500 – 600 bp of the mitochondrial 16SrRNA gene were amplified by PCR using primers from Palumbi (1991). Purification and sequencing were performed as services by Macrogen Inc. (Seoul, Korea). Sequences were edited using Bioedit Sequence Alignment Editor v7.0.9.0 (Hall, 1999), and inspected by eye against the original chromatogram. Multiple sequence alignments were performed with Clustal W (Thompson et al., 1994) as implemented in MEGA version v5 (Tamura et al., 2011). We created Neighbor-Joining tree (MEGA5, Tamura et al. 2011) using the Maximum Composite Likelihood (Tamura et al. 2004). The analyzes need to be completed, revised and discussed (Fig.1, 2 and 3). The main objective is to compare molecular tree with otoliths shape tree and feeding habits of gurnard species in order to discriminate the contribution on the variability of sagittal otolith among and within species and to use otolith as discriminate marker.

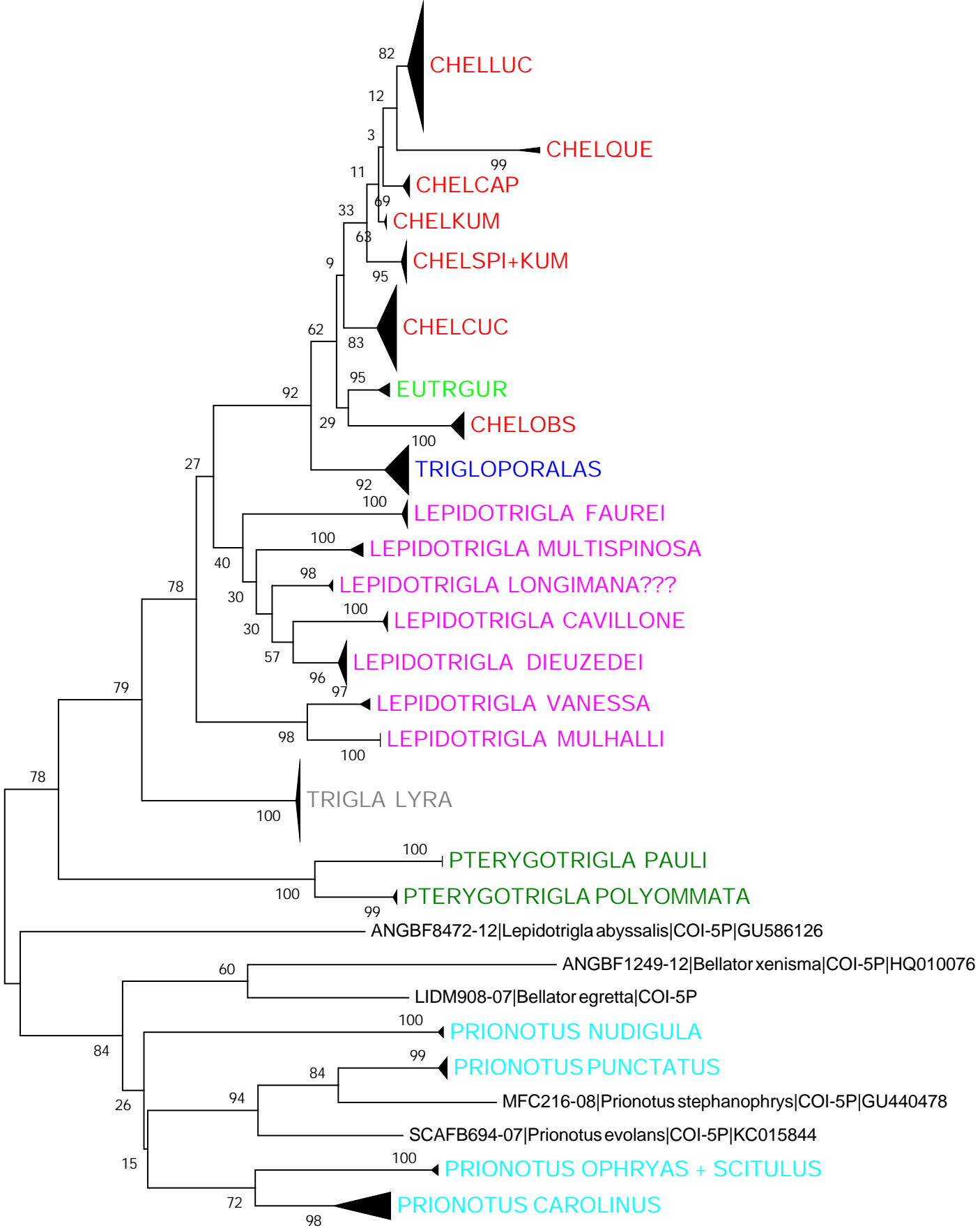
Scorpaena_scrofa_16S

Scorpaena_notata_16S



0.3





0.05

Chapter VI. Conclusions

This study supports the importance of investigating the bio-ecology of increasingly exploited and poorly known species, such as gurnards from Adriatic Sea, to quantify their ecological role into marine community. It also focuses more on the effect of fish body size, depth and latitude distribution, food resource partitioning to investigate inter and intra-specific structuring factor of Adriatic stocks. Frequency of length distribution, length-weight linear and non-linear relationships, sexual maturity plots, feeding habits were compare to other Mediterranean Seas. In addition studies on sagittal otolith were conducted to overlap with the analysis described above and have a broader picture of the state of this resource. According to literature, considered gurnards species showed a “critical size” species related reached in the first stages of life, coinciding with the start of sexual maturity, the tendency to migrate to greater depths, a change of diet from crustaceans to fish and an increase of variety of food items eaten. Distribution of prey items, predator size range and depth distribution were the “main dimensions” that influence the breadth of trophic niche and the relative difference amongst Adriatic gurnards. Several feeding preferences were individuated and a possible impact among bigger-size gurnards and other commercial fishes (anchovy, gadoids) and Crustacea (such as mantis prawn and shrimps) were to be necessary considered. They in fact prey intensively on these species with marked effects on food webs, and this alone justifies the importance of a “multispecific approach” in terms of analyses and management. About otolith (sclerochronological studies, geometric morphometrics, EFA, ultrastructure analysis by SEM and chemical investigations) several conclusions could be extract. Adriatic gurnard species showed a very fast growth despite other results in other areas; intra-specific differences and increase in the variability of otolith shape, sulcus acusticus shape, S:O ratios, sulcus acusticus external crystals arrangement were shown between juveniles and adults and they are linked to growth (individual genetic factors) and to environmental conditions (e.g. depth and trophic niche distribution). However, in order to facilitate correct biological interpretation of data, these results should be correlated with molecular studies, above all for compare morphological distance to genetic ones. Future work is needed to assess all available data, to fill all gaps in the light of appropriate sampling plans. As a general

conclusion, the goal of this scientific research for fishery management applications is to improve knowledge as to fish population biology and ecology in view of an ecosystem-based management of commercially important stocks. An appropriate management of fisheries resources can only be achieved with the continuous supply of information on the structure and biology of populations in different geographical areas, in order to understand the intra and inter-specific relationships and consequently predict the temporal fluctuations.

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