

**THESIS WORK**

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# **Feeding behaviour of common bottlenose dolphins (*Tursiops truncatus*) in the Amvrakikos Gulf, Greece**

MASTER THESIS

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# 1. INTRODUCTION

## 1.1. Background

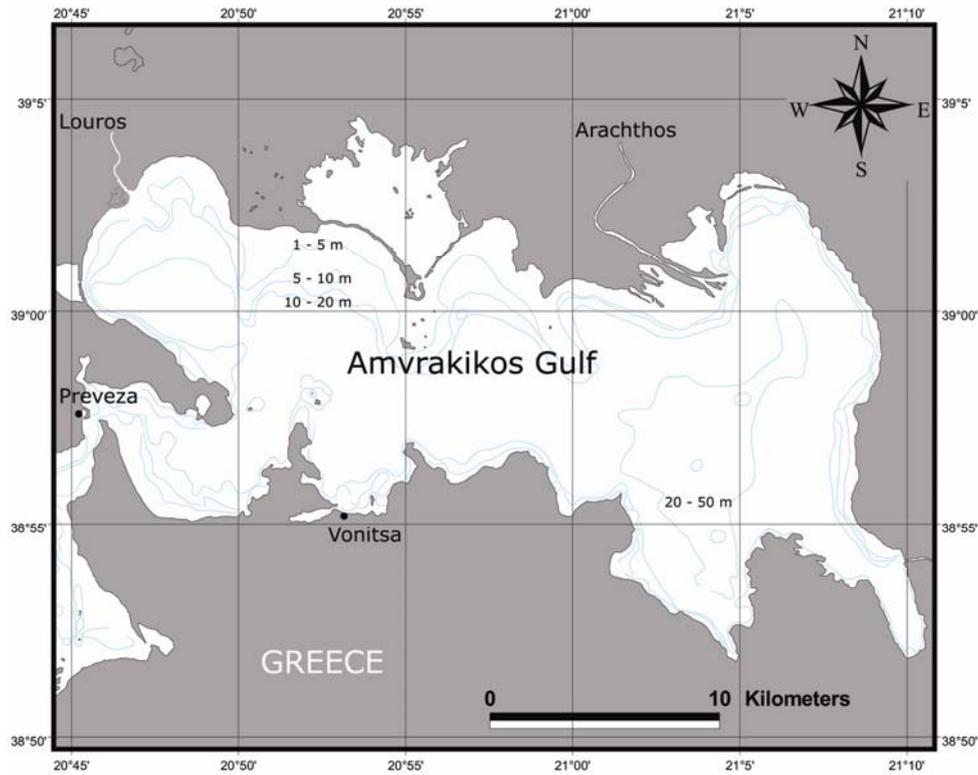
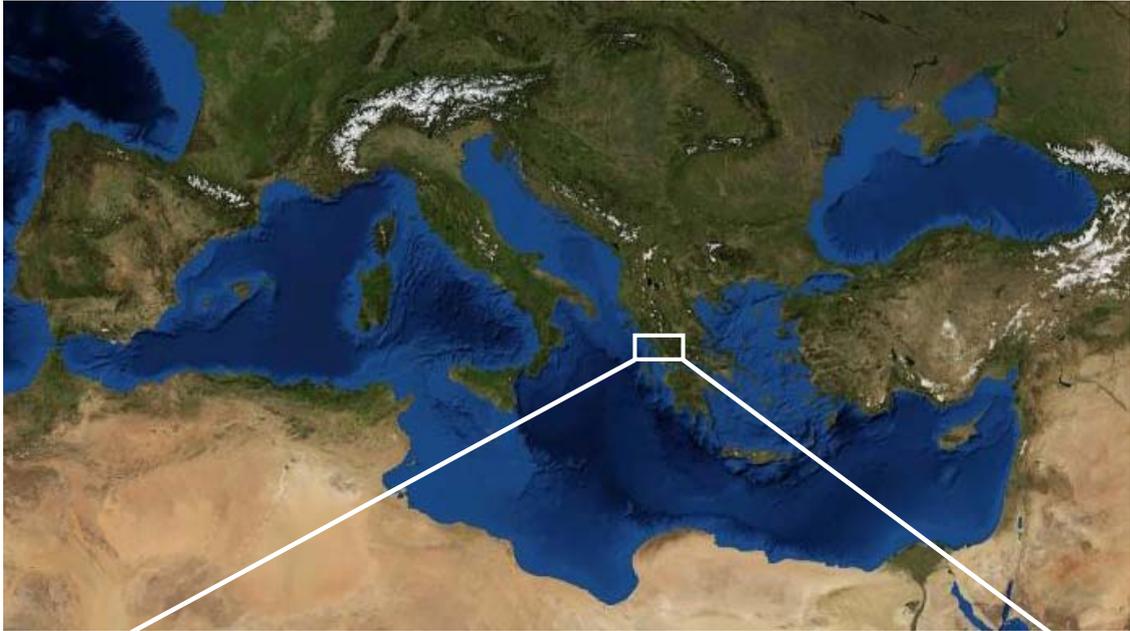
The feeding behaviour of common bottlenose dolphins (*Tursiops truncatus*) can be influenced by a number of ecological variables. These dolphins can adapt their feeding strategies according to food type and local conditions (Shane *et al.*, 1986), therefore the knowledge of prey kind and habitat characteristics can help us to understand why a given feeding strategy has evolved.

### 1.1.1. Study area

The Amvrakikos Gulf is a semi-closed sea lying along the north-eastern Hellenic coast of the Ionian Sea, western part of Greece. It is situated between latitude 38° 50' and 39° 50' North and longitude 20° 45' and 21° 13' East (Fig. 1.1).

The Gulf has a surface of 405 km<sup>2</sup>, a coastline length of 256 km. The sea floor is mostly 30 m deep (maximum depth 60 m) and covered with mud or sand. It receives freshwater and sediment fluxes from two rivers, namely from Louros and Arachthos (Fig. 1.1). The rivers discharge into its northern reach and strongly affect the water quality of the Gulf. The waters are murky (Secchi disk readings ranging between 2-12 m) and eutrophic. The only communication with the open Ionian Sea is a narrow (at the narrowest point is 370 m) and shallow (at the shallowest point is <5 m) channel, the Preveza channel. The Gulf acts as a typical dilution basin from winter to early summer due to freshwater inputs. During summer, evaporation becomes more important. Salinity fluctuates widely but remains low throughout the year (17-35‰) resulting in strong stratification of the upper layers (Bearzi *et al.*, 2008a).

Due to the isolated character of the Gulf, water quality is strongly influenced by environmental factors and several anthropogenic activities. In the delta area, there are a number of aquaculture farms, as well as in the gulf itself. 5 shell farms and 24 fish farms (lagoons excluded) function within the basin; the latter dominating along the western coastline and north-eastern part (Appendix A, Fig. 1).



**Figure 1.1.** Location of the Amvrakikos Gulf (created in ArcView3.2) within the Mediterranean region (NASA World Wind 1.4).

Fish farm and agricultural activities together with discharges of domestic sewage from coastal towns and villages contribute to nutrient enrichment of the water. The high values of ammonia, silicate, and phosphate, highlight the eutrophied character of the area (Frigos *et al.*, 1997). The local trophic web is reduced and the ecosystem is based mainly on the high

levels of the above mentioned nutrients which enhance primary productivity, therefore facilitating the build up of primary and secondary consumers such as zooplankton, cephalopods and fish. These conditions result in high concentration of small epipelagic fishes - mainly European pilchard (*Sardina pilchardus*), round sardinella (*Sardinella aurita*) and European anchovy (*Engraulis encrasicolus*) - which in turn increases the susceptibility to other predators such as bottlenose dolphins. According to Bearzi (2008a), mean dolphin density was 0.37 animals/km<sup>2</sup>, which is considered among the highest observed in Greek and Mediterranean coastal areas (Bearzi *et al.*, 2008b). In addition to this, the area provides home for many piscivorous seabirds, including the vulnerable Dalmatian pelican (*Pelecanus crispus*), hosts a number of migratory birds and the endangered loggerhead sea turtle (*Caretta caretta*), which make the habitat even more valuable from a conservation standpoint. The Amvrakikos Gulf has been listed under the RAMSAR Convention for the protection of wetlands of national importance (Athanasopoulos *et al.*, 2003) and became a National Park in 2008. In spite of the conservation status, the Amvrakikos Gulf is strongly affected by numerous human activities. These impacts together with the environmental characteristics of the basin make this closed system even more unique and vulnerable compared to the open Ionian Sea.

### **1.1.2. Study species**

The common bottlenose dolphin (*Tursiops truncatus*, Montagu, 1821) (hereafter ‘bottlenose dolphin’) (Fig. 1.2.) is the only cetacean species observed in the Amvrakikos Gulf. The genus *Tursiops* (Delphinidae) can be found in the major oceans of the world from approximately 60° N latitude in the Atlantic Ocean and 40° N latitude in the Pacific Ocean to about 55° S latitude in the southern oceans. Presently, two species are internationally recognized *Tursiops truncatus* and *Tursiops aduncus*, or the Indo-Pacific bottlenose dolphin. Only the former is known to occur in the Mediterranean Sea and North Atlantic Ocean. The latter has a smaller distribution and is found only along the coastline in the Indian and south-eastern Pacific Oceans (Reeves *et al.*, 2002).



**Figure 1.2.** Common bottlenose dolphins, photographed in the Amvrakikos Gulf, showing the characteristic morphology of the species: robust body, short beak, fading brown/grey coloration, white/pinkish belly.

The bottlenose dolphin appears to have two genetically distinct forms, referred to as “coastal” and “offshore” (Wells *et al.*, 1999). In the Mediterranean, the two ecotypes have not been reported. The species is often regarded as predominantly ‘coastal’ or ‘inshore’ but this distinction may be misleading as they can be encountered in continental shelf and shallow plateaux waters at any distance from the coast (Bearzi *et al.*, 2008b). In the basin, as anywhere else, they can occur in a wide variety of habitats. Although they are thought of as the most common cetacean species in coastal waters of the Mediterranean Sea, their distribution is increasingly scattered and fragmented into small units. The bottlenose dolphin is considered a species of special interest for conservation under the European Union’s Habitats Directive. Furthermore, the Mediterranean ‘subpopulation’ was qualified as ‘Vulnerable’ according to the International Union for Conservation of Nature (IUCN) Red List criteria (Bearzi *et al.*, 2008b), thus monitoring and research on this species are relevant in areas where they occur in high densities.

In Greek waters, they are mainly found over bottoms that do not exceed 250 m depth and usually limited to the first 6 km from the shore. It is common in coastal areas, straits, bays and gulfs, of the entire Aegean plateau, where it can be found further offshore in shallow waters in between islands. It is well present in the north and south Ionian Sea and in the Cretan Seas, although only locally along the coast that run parallel to the Hellenic Trench (Frantzis, 2007).

Investigations of geographically distinct populations have clarified striking and subtle intraspecific behavioural differences commonly interpreted as specific adaptations to local ecological conditions (Shane, 1990b; Ballance, 1992; Defran & Weller, 1999; Bearzi *et al.*, 2008b).

### 1.1.3. Diet and feeding behaviour

Bottlenose dolphins possess high level of behavioural flexibility (Reynolds *et al.*, 2000) and they are able to adjust their feeding habits according to habitat type and food availability. The species is described as opportunistic or euriphagous feeder (Leatherwood, 1975; Barros & Odell, 1990; Corkeron *et al.*, 1990; Gonzalvo *et al.*, 2008) that consumes a great variety of species and is capable of exploiting the readily available food sources from the environment.

The diet of bottlenose dolphins can be investigated in many ways, for instance analysing stomach contents, using fatty acids as tracers or stable isotope markers of a prey item which reflected in the tissues of the predator. However, there are some alternative ways of determining the species consumed by dolphins. One is direct observation of prey items and another is the identification of fish scales collected right after predatory events by the dolphins. Microscopic fish scale structure and features allow for the identification of fish up to major groups and species levels (Esmaeili *et al.*, 2007), as shown by a number of studies (Mosher, 1969; Rowland, 1969; Hill *et al.*, 1979; Johal & Dua, 1994; Patterson, *et al.*, 2002; Kaur & Dua, 2004). The possibility of identifying fish species based on scales allows to obtain information on prey captured by dolphins during surface feeding.

Stomach content studies showed that bottlenose dolphins prey upon both pelagic and benthonic species involving cephalopods and fishes (Barros & Odell, 1990; Gales *et al.*, 1992; Barros & Wells, 1998; Barros *et al.*, 2000; Blanco *et al.*, 2001; Gannon & Waples, 2004). In Mediterranean coastal waters, bottlenose dolphins target primarily demersal prey. Reported prey items include demersal species such as European hake (*Merluccius merluccius*), European conger (*Conger conger*), red mullet (*Mullus barbatus*), striped red mullet (*Mullus surmuletus*), common cuttlefish (*Sepia officinalis*), common octopus (*Octopus vulgaris*) and a variety of other bony fishes and molluscs (Bearzi *et al.*, 2008b). In north-eastern Sardinia, Italy, bottlenose dolphins were documented feeding on species, such as garpike (*Belone belone*), flathead mullet (*Mugil cephalus*), salema (*Sarpa salpa*) and European pilchard, which were aggregated around fish farms (Díaz López, 2006).

Like other opportunistic predators, bottlenose dolphins can use a variety of foraging techniques, adapted to prey type and to the physiography of the environment (Reynolds *et al.*, 2000; Santos *et al.*, 2007). These strategies are diverse and complex and may include cooperative hunting strategies or individual techniques. Working in groups to encircle a large school of fish and trapping it against the surface (also known as ‘carouseling’) (Neumann,

2001) can be one way of capturing epipelagic fish species. Dolphins can chase the fish also against shoreline to trap them in shallow water where they are easy prey (Shane *et al.*, 1986). On the other hand, bottlenose dolphins in Argentina feed individually in a single-line formation near shore and move in a line-abreast formation far from shore apparently to feed on shoaling fish (Würsig & Würsig, 1979). Among some rare strategies is ‘beach-feeding’, that includes chasing fish onto mud banks and then stranding to capture them. It is one of the most daring foraging strategies observed in bottlenose dolphins in the northern Gulf of California, and on the Atlantic coast of the United States, in the salt marshes of Georgia, South Carolina, Louisiana and Texas (Shane *et al.*, 1986; Silber & Dagmar, 1995). Observations of Gazda *et al.* (2005) revealed distinctive behavioural roles during group feeding of bottlenose dolphins in Cedar Key, Florida, where in each group, one individual (as ‘driver’) was herding the fishes in a circle towards the remaining ‘barrier’ dolphins. These foraging strategies can perhaps mirror the best how this species can work in a well organised group, with individual role specialization, which is rare in mammals. There are some well documented cases about coordinated group hunts in mammals, for instance, in African lionesses (*Panthera leo*) and in chimpanzees (*Pan troglodytes*) in the Tai National Forest, Ivory Coast (Gazda *et al.*, 2005). Cooperative hunting strategies among delphinids are remarkable, and have been described in species, including bottlenose dolphins, killer whales (*Orcinus orca*) and humpback whales (*Megaptera novaeangliae*) (Packer & Rutten, 1988).

The foraging and feeding strategies of bottlenose dolphins are flexible enough to allow consumption of a wide range of prey types (Gales *et al.*, 1992). The high level of their learning ability helps these animals to utilize alternative food sources. In many areas, scarcity of potential prey could be one reason why dolphins feed in association with fishing operations. Bottlenose dolphins frequently accompany shrimp boats in Texas bays. They feed on fishes that are caught incidentally and discarded from trawl nets intended for shrimps (Fertl & Würsig, 1993). In the Mediterranean, bottlenose dolphins are often observed to interact with fisheries. These interactions were studied and described by many authors (see review in Bearzi *et al.*, 2008b). While these interactions could provide easy prey, the animals are exposed to higher risk of entrapment, by-catches and deliberate killing. Bottlenose dolphins in the waters surrounding the island of Kalamos, Greece, were observed foraging near fish farm cages (Bearzi *et al.*, 2004). Dolphins did not prey on the caged fish. They were attracted by the presence of other potential prey items concentrated around fish farms due to the increased nutrient levels derived from the cages.

These studies have shown that even within a species feeding habits can differ remarkably, supporting the fact that the food sources and local conditions of the environment play important role in shaping dolphins' feeding habits. Knowledge of the diet and feeding strategies of bottlenose dolphins is essential to increase our knowledge about the species' biology.

## 1.2. Aims and objectives

Observing and analysing feeding behaviour allow insight into possible causes why a particular feeding strategy is adapted to the local conditions of the habitat where the animals live. The present study explores the diet and feeding behaviour of bottlenose dolphins during surface foraging in a semi-closed, eutrophic marine environment.

The objectives of the study are:

- 1) Characterization and analysis of surface feeding behaviour.
- 2) Investigation of spatial distribution of surface feeding and to explore the presence of feeding hotspots in the study area.
- 3) Identification of fish scale samples obtained after surface feeding events of bottlenose dolphins.

According to the preliminary observations, I have postulated three hypotheses:

H 1: Surface feeding represents a remarkable part of the behavioural budget of the local dolphin population.

H 2: Surface feeding ‘hotspots’ may exist within the study area.

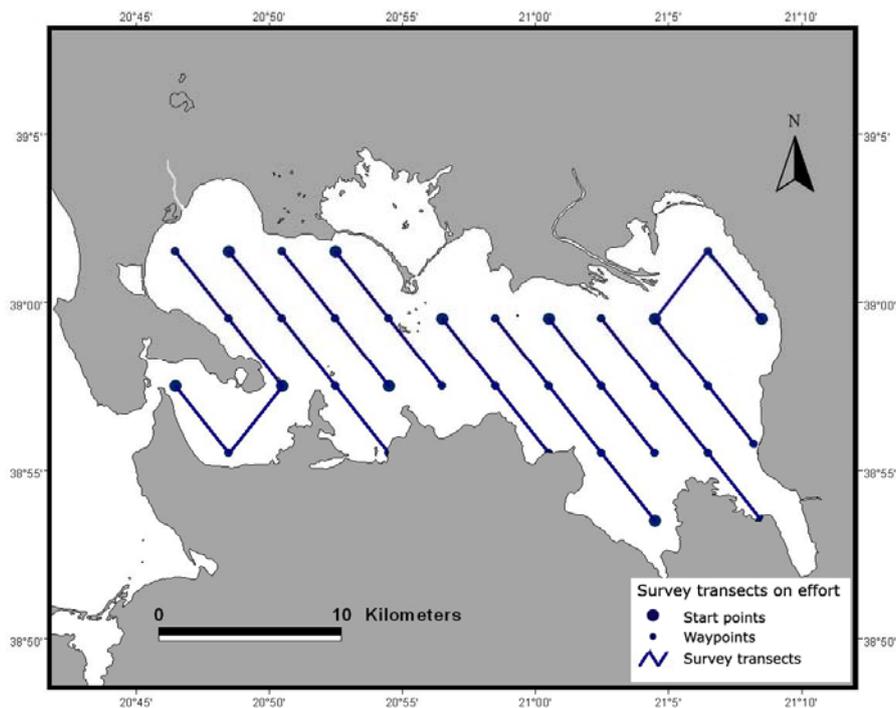
H 3: Dolphins prey primarily on sardines during surface feeding events.

## 2. MATERIALS AND METHODS

### 2.1. Data collection

#### 2.1.1. Daily surveys

Daily boat surveys were conducted from January 2007 to December 2007 in the Amvrakikos Gulf, western part of Greece. The study area is situated between 38° 50' and 39° 05' latitude North and 20° 45' and 21° 10' longitude East. Boat surveys were carried out in the morning and early afternoon from a 5.8 m inflatable boat, equipped with fibreglass keel and powered by 100 HP four-stroke outboard engines. The onboard equipment contains (note that the following list involves only accessories used in navigation and behavioural sampling, excluding the equipment used in other part of the research): Global Positioning System (GARMIN GPS map 178C Sounder) (to follow the survey transects, to record navigational data, locations and movements of sighted dolphins (Appendix A, Fig. 2) or the positions of any other encountered marine animals); a hand computer (NetPad) to record behavioural data of dolphins at 5 minute intervals; beeping watches (set for 5 min intervals); stopwatches (for measuring dive time); a binocular Nikon 7x50, tape recorders (for collection of navigation data, data of encountered animals and additional behavioural data if needed). Daily surveys started and finished in the port of Vonitsa. The study area was covered by 9 survey transects with 34 waypoints (Fig. 2.1). Navigation could be defined “On Transect” (i.e. navigation following a pre-defined route) or “Off Transect” (i.e.: navigation outside of the pre-defined survey tracks- navigation *ad libitum*). Navigation data were collected during surveys using a tape recorder, including information on time, GPS positions, boat speed, sea state, sighting condition. Information on any kind of encounter (fish school, sea turtles, marine birds, etc.) was also recorded. Survey data were defined ‘on effort’ (positive sighting conditions) when the following terms applied: 1) daylight and good visibility (no fog or rain); 2) sea state  $\leq 1$  Douglas with no swell; 3) at least two experienced observer consistently scanning the sea surface 4) eye elevation of approximately 2m for both observers; and 5) “standard” navigation speed (14-17 knots or 28-36 km/h). Binoculars were not used during navigation.



**Figure 2.1.** Map of the study area with survey transects and waypoints (created in ArcView3.2).

A daily survey was interrupted and navigation went off effort, if 1) dolphins were sighted; 2) sea or weather conditions deteriorated; or 3) other factors forced the crew to return to the port (e.g.: late hour, running out of fuel, etc. Bearzi *et al.*, 2008a). After completing a daily survey, navigation data were transcribed from the tape recorder and entered into the database.

### 2.1.2. Dolphin sightings and observations

During daily surveys, if dolphins were sighted the collection of navigation data stopped and behavioural observation and data collection started. All observations were conducted with flat sea or wavelets that did not break (sea state  $\leq 1$  Douglas) and with good visibility adequate for finding and observing dolphins. Once dolphins were sighted, time, GPS position, sea state, bow and dolphin angle were recorded. Then dolphins were approached carefully and were followed parallel within a respect distance during the observation, trying to prevent any disturbance (Bearzi *et al.*, 1999). Initial estimates of school size and composition, as well as information on time, location, and sea state were then noted. From this point, movement of the focal group was recorded by means of the GPS at 1 min interval. The focal group was defined according to Shane (1990a) and Bearzi (1999) as “any group of dolphins observed in apparent association, moving in the same direction and often, but not always,

engaged in the same activity”. When a group splits, one of the daughter groups was followed, based on a random choice that was independent of group size and/or activity (Bearzi *et al.*, 2005). Behavioural data were collected by means of the hand computer *NetPad* at standard 5-min intervals. Group follow approach (Mann *et al.*, 2000) was applied by continuously observing the behaviour of all members of the focal group. According to the occurrence of different behavioural events (e.g.: leap, bow, chin or tail slap, rostral nudge etc.), the regularity of behavioural states (i.e. a predefined categories of events and activities, see below) was recorded. The following categories were entered at the end of the 5 minute: date, time, longest dive (exceeding 60 seconds), distance from boat (any boat including the research boat), directionality, regularity of behavioural states (surface foraging, social interaction, aerial behaviour, percussive, and stationary behaviour), bird number and behaviour. If dolphins were within the range of any fish farm it was entered as well. During behavioural sampling, surface foraging was recorded whenever some kind of foraging or feeding activity occurred; by Shane’s definition (1990a) ‘obvious feeding activities performed close to the water surface’. Dolphins often performed fast subsurface swim or inverted swim pursuing fish near the surface. Fish sometimes cleared water while pursued by the dolphins (Bearzi *et al.*, 1999). Dolphins often surfaced with prey items in their mouth (Fig. 2.2). Visible prey parts could be detected (e.g.: fish scales due to the predatory events). Whenever it was possible, fish scale collection (described below) was carried out, for purpose of prey identification.



**Figure 2.2.** Bottlenose dolphins pursuing and catching fish at the surface.

The observation was continued till the sufficient behavioural data were collected, but was interrupted and immediately finished, if any sign of disturbance occurred, environmental

conditions worsened, or the group was lost for more than 10 minutes. After accomplishment of the observation, all behavioural data were downloaded from the NetPad and entered into the database.

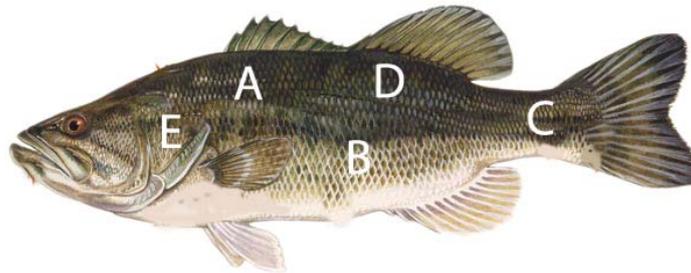
### 2.1.3. Fish scale sampling and collection

Fish scales were collected following surface feeding events of dolphins by means of a snare of 37x26 cm (mesh size of 1 mm) mounted on top of a 1.2 m wooden pole. During surface feeding by the dolphins, drifting fish scales could be visually detected and collected by slowly approaching the area where feeding events had occurred. The scales were preserved in ethanol 80% and stored into labelled vials. Date, time, position, and number of the scales were recorded on the vial.

A catalogue of scales of local fish species was created for scale identification purposes. Fishes were obtained from local fishermen operating in the Amvrakikos Gulf and from fish markets in the area (only species caught in the Gulf were collected). The final catalogue included 11 fish species, both pelagic and demersal (Table 2.1. and Appendix C; P49-60). Photographs were taken from each sampled individual and the size of the fish was measured. A minimum of 10 scales were removed from five body parts of a given fish, to account for the morphological variability of scales (Fig. 2.3. and Table 2.2). These scales were also preserved in ethanol 80% and stored in labelled vials.

**Table 2.1.** List of fish species included in the catalogue.

Scientific name	Common name	Living conditions	Scale type
<i>Boops boops</i>	Bogue	demersal	ctenoid
<i>Dicentrarchus labrax</i>	European seabass	demersal	ctenoid
<i>Diplodus annularis</i>	Annular seabream	benthopelagic	ctenoid
<i>Engraulis encrasicolus</i>	European anchovy	pelagic/ neritic	cycloid
<i>Liza ramado</i>	Thinlip mullet	pelagic/ neritic	ctenoid
<i>Mullus barbatus</i>	Red mullet	demersal	ctenoid
<i>Pagrus pagrus</i>	Common seabream	benthopelagic	ctenoid
<i>Sardina pilchardus</i>	European pilchard	pelagic/ neritic	cycloid
<i>Sardinella aurita</i>	Round sardinella	pelagic/ reef-associated	cycloid
<i>Sparus aurata</i>	Gilthead seabream	demersal	ctenoid
<i>Trachurus trachurus</i>	European horse mackerel	pelagic-neritic	cycloid



**Figure 2.3.** Sampling zones on the fish body.

**Table 2.2.** Definition of sampling zones.

Zone A	dorsal, anterior, above the lateral line
Zone B	ventral, below the lateral line
Zone C	caudal, below the lateral line
Zone D	dorsal, posterior, above the lateral line
Zone E	caudal to operculum, below the lateral line

#### 2.1.4. Survey and sampling effort

In 2007, 136 days were spent at sea with 7,065 km of total effort distributed within an area of about 400 km<sup>2</sup>. A total of 101 sightings were carried out, from which I used only the sightings conducted “*On Transect*”, resulting 55 sightings. Only these were used in statistical analysis to ensure standard sampling conditions. Twelve months of boat-based behavioural sampling accumulated 85 h 32 min of direct observation and 1012 behavioural samples (5-min each). Monthly distribution of behavioural sampling effort varies with an average of 84.33 samples ( $n = 12$ ; range = 46-141; SD = 29.82; SE = 8.61).

A total of 69 fish scale samples (vials) were collected on 35 days in 2007. The samples were collected in each month except February and November. Each sample represents a predatory event in a given feeding spot, but a sample can contain more than a single scale. The number of scales included in a vial ranged between 1-14 (mean=3.87; SD=3.10; n=69).

## 2.2. Data analysis

### 2.2.1. Classification of feeding behaviour

In this study, foraging and feeding are often used interchangeably; however, foraging involves the pursuit and capture of prey while feeding refers to actual consumption. Due to data collection, surface foraging (henceforth SF) was recorded whenever some kind of foraging or feeding activities were performed close to the water surface. Thus SF in our case comprehends both foraging and feeding activities performed at the surface.

Behavioural samples were divided into two categories: ‘*Feeding*’ and ‘*Non-Feeding*’. The category ‘*Feeding*’ contains all the 5-min samples where SF occurred. The category ‘*Non-Feeding*’ contains all the 5-min samples where no SF occurred. The analysis was performed with the category of ‘*Feeding*’. Since behavioural states could be combined within a 5-min sample, as they can be performed by different group individuals, therefore ‘*Feeding*’ was subdivided into five categories according to the occurrence of different behavioural states and directionality (as it is represented in Table 2.3). Definition of ‘*Feeding categories*’ is described in Table 2.4. Frequencies of ‘*Feeding categories*’ were calculated to see how often SF occurred together with other behavioural states and directionality.

**Table 2.3.** ‘Feeding categories’ – combination of SF with other behavioural states and directionality.

	Directionality			Feeding	Social behaviours			
	No	Poor	Straight			Surface active behaviours		
	No	Poor	Straight	Surface Foraging	Social Interactions	Aerial Behaviour	Percussive Behaviour	Stationary Behaviour
<b>PF</b>								
<b>S/F</b>								
<b>T/F</b>								
<b>S/T/F</b>								
<b>O</b>								

Note: PF: Pure feeding; S/F: Social/Feeding; T/F: Travel/Feeding; S/T/F: Social/Travel/Feeding; O: Other category.

**Table 2.4.** Definition of ‘Feeding categories’.

<b>Pure feeding (PF)</b>	Moving with poor or no directionality within the 5-min sample No social interactions, like physical contact (e.g., touching, rubbing, biting, etc.) or socio-sexual behaviours (e.g., ventral-ventral swim, rostro-genital contact) occurred No surface active behaviours (e.g.: leaps of aerial behaviour, tail slaps of percussive behaviour) were performed No stationary behaviour occurred
<b>Social/Feeding (S/F)</b>	Moving with poor or no directionality
	Within a given 5-min sample, SF and social behaviours (like social interactions, aerial or percussive behaviours) occurred simultaneously by different focal group individuals or consecutively by the whole focal group
<b>Travel/Feeding (T/F)</b>	Within a given 5-min sample, SF occurred while the focal group was having straight directional movement (e.g.: travelling can be interrupted by short bouts of SF; which can be performed simultaneously by different focal group individuals or consecutively by the whole focal group)
<b>Social/Travel/Feeding (S/T/F)</b>	Within a given 5-min sample, the focal group had straight directional movement; SF and different types of social behaviours occurred simultaneously by different focal group individuals or consecutively by the whole focal group.
<b>Others (O)</b>	Within a given 5 min sample, the focal group had no directionality, SF, different type of social behaviours and stationary behaviour were performed simultaneously by different focal group individuals or consecutively by the whole focal group.

## 2.2.2. Analysis of feeding behaviour

Behavioural data were available on nominal or ordinal scale; therefore non-parametric statistics were used for data analysis due to non-normal distributions of data. Statistical analysis was performed using Statistica Version 5.1. Statistical significance was tested at the  $p < 0.05$  level. A chi-square ( $\chi^2$ ) test of independence was used to determine whether the number of foraging bird specimens depends on the regularity of SF. In further analysis, SF data were used on nominal scale allowing the calculation of frequencies. To see how SF is combined with other behavioural states in different seasons, feeding budgets were created according to ‘Feeding categories’. Seasons were defined following Defran & Weller (1999)

and Bearzi *et al.* (1999) as: spring (March-May), summer (June-August), autumn (September-November), and winter (December-February). Seasonal differences were tested by using  $\chi^2$ -test of homogeneity. To investigate seasonal fluctuation of SF activity, frequencies of total 'Feeding' were calculated for each sighting (one sighting = one day). Data were then grouped into four seasons (mentioned above). Kruskal-Wallis test was run to determine differences in SF activity among seasons. Positions and frequency distribution of SF were plotted and analysed by using GIS software (ArcView 3.2). The same software was used to plot additional information (i.e. fish farm positions, total movement of dolphins, positions of surface feeding and fish scale sampling). The additional maps are involved in Appendix A; P39-41. Map units and distance units were set as km. The projection chosen was the World Mercator projection in the spheroid WGS 84. SF positions were plotted by using information from *GPS* (focal group positions at 1 min intervals). To use the navigational data in ArcView3.2, the GPS positions were converted to GIS positions, using:

$$\text{Degree} + (\text{Minute} + \text{Second} / 100) / 60$$

To analyse SF activity within the study area, a grid table was created (cell size 2'x 2' / 3704 m latitude by 2889 m longitude) by using the *CR\_Tools* extension to ArcView. To avoid biases, cells (representing sampling areas) with insufficient behavioural sample size were excluded from the analysis, using a defined minimum value (i.e.: median minus lower quartile of the total behavioural samples). Therefore only cells with  $\geq 15$  behavioural samples were used for the analysis, resulting in a set of 18 cells. Data were then standardized (i.e.: No. of SF / No. of Behavioural samples); and frequency of SF was calculated in each cell and plotted to the map. To test whether cells were spatially autocorrelated, and hence not independent, the Global Moran's I index was computed for SF activity by using the *MapStat* extension to ArcView. Moran's index was not significantly different from zero ( $I = 0.25$ ,  $p > 0.05$ ) showing that cells were not spatially autocorrelated. To examine whether the occurrence of SF was independent from cells (=areas), a contingency table was created (using SF data on nominal scale) and  $\chi^2$ -test of independence was applied. Cells with significantly higher SF occurrence were determined as "preferred areas".

### 2.2.3. Preparation and analysis of fish scales

One scale of unknown fish species was randomly extracted from a vial for species identification. Scales were hydrated with distilled water for 3 h, placed in 10% potassium hydroxide (KOH) solution for a maximum of 30 min, gently brushed, then mounted between two micro slides (Agazzi *et al.*, 2004). The scales were analysed with a Mitutoyo Profile Projector PJ 300 (magnification 20x) and photographed with a Nikon D50 digital camera (Nikkor 18-55 mm lens). The preparation of scales derived from known species was done in the same way, resulting in a catalogue of scale photographs (Appendix C; P49-60). The photographs of scales of unknown species were then compared with the catalogue and could be identified by using a set of pre-defined features (Table 2.5).

**Table 2.5.** Scale terminology and discriminative features.

<b>Type</b>		ctenoid/ cycloid
<b>Shape</b>		circular/ spheroid/ oval/ rhomboid/ square/ fan- or shell-like
<b>Appearance</b>		thin/ robust smooth/ rough surface flexible/ brittle (dry scale)
<b>Fields</b>	Anterior Field (AF) – rostral section/cephalic to focus	extended/ flattened; even/ uneven/ wavy margin; articulated (bi-/ tri-/ multi-lobed) or disarticulated
	Lateral Fields (LFs) - ventral and dorsal section of the scale	extended/ elongated/ compressed; even/ uneven margin; articulated/ disarticulated
	Posterior Field (PF) – caudal section/caudal to focus	pointed/ rounded/ flattened/ irregular/ variable; even/ uneven/ crenellated margin
<b>Focus</b>	the first part, often central, of the scale to appear in growth (Patterson, 2002)	distinct/ indistinct; position (central/ shifted towards the posterior or anterior field); area around the focus (circular/ reticulated/ granular)
<b>Circuli</b>	lines of growth that appear like elevated markings on the surface, usually appearing as lines that more or less follow the outline of the scale (Patterson, 2002)	tightly compacted/ compact/ less compacted; parallel/ concentric with margin; continuous/ discontinuous; appearance of circuli around the focus (compact/ loose; circular/ semi-circular); presence/ absence in the PF

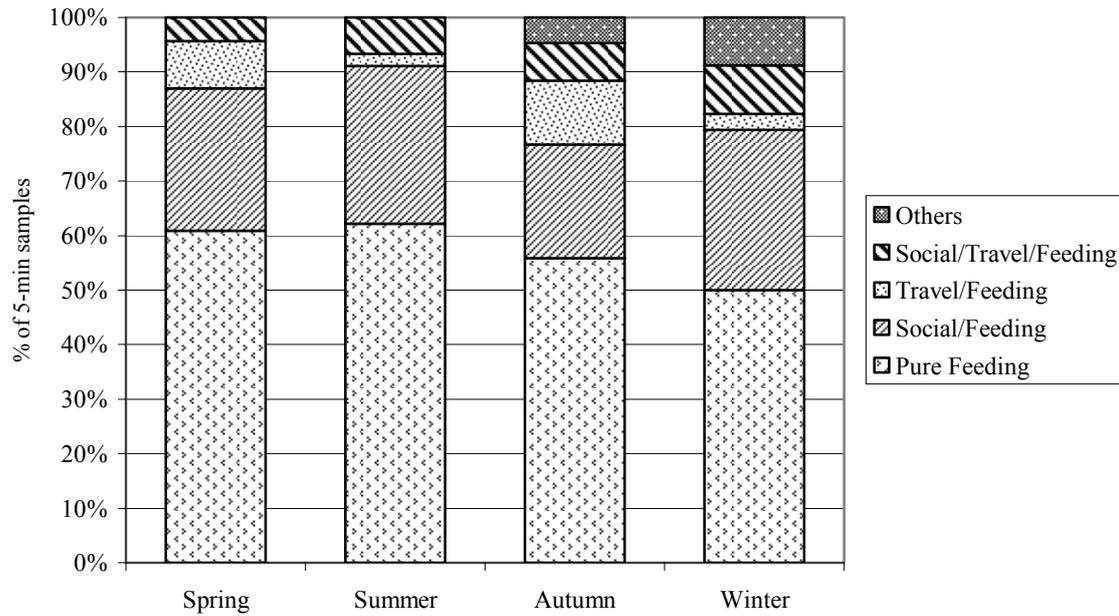
<b>Radii</b>	grooves that radiate from the focus to the scale margin (Patterson, 2002)	absence/ presence; variable/ constant number
<b>Fracture lines</b>	distinct fracture cracks that appear in variable directions, not necessarily pointing towards the focus	absence/ presence; location (AF/ LFs/ PF)
<b>Ctenii</b>	toothlike structures on the posterior portion of some scales (Patterson, 2002)	absence/ presence; shape (thorn-like/ arrowlike; thin/ robust); widespread/ concentrated

### 3. RESULTS

#### 3.1. Classification and characterization of feeding behaviour

Surface foraging (SF) occurred in 145 behavioural samples (71 regular; 74 occasional SF). This represented 14.33 % of the total behavioural budget and termed as '*Feeding*' category. The rest (85.67%) of the behavioural budget fell into the '*Non-Feeding*' category. SF occurred in feeding association with marine bird species (predominantly seagulls and terns). SF was recorded in 97.93% of the time with foraging bird species and only 2.07% of the time was observed without birds. A significant association was found between bird number and the regularity of SF ( $\chi^2 = 14.05$ ;  $df = 5$ ;  $p < 0.05$ ); showing that the number of foraging bird specimens depends on the regularity of SF. Division of '*Feeding*' into five '*Feeding categories*' and calculation of frequencies, revealed how SF was combined with different behavioural states and directionality. The results showed that SF occurred most of the time alone without combination of any other behavioural states (i.e. '*Pure Feeding*' - 57.24%) while the focal group had poor or no directionality within a 5-min sample. However, it was found that different types of social behaviours often occurred with SF (i.e. '*Social/Feeding*' - 26.21%) simultaneously by different focal group individuals or consecutively by the whole focal group within a given 5-min sample. During this time the focal group had poor or no directionality as well. The remaining three categories contributed less to total '*Feeding*' (Social/Travel/Feeding - 6.90%; Travel/Feeding - 6.21%; Other - 3.45%).

A slight variation was detected in frequency distribution of feeding categories among seasons (Fig. 3.1), indicating that combination of SF with other behavioural states varied. '*Pure Feeding*' proved to be dominant in all seasons. '*Social/Feeding*' was also present with high values throughout the year; however a fall could be detected in autumn. While '*Travel/Feeding*' occurred in relatively moderate proportion in spring and autumn, its participation was lesser in summer and winter. The presence of '*Social/Travel/Feeding*' appeared to be constant. The category '*Others*' occurred only in cold seasons, showing that the combination of SF with social behaviours and stationary behaviour was present only in autumn and winter.



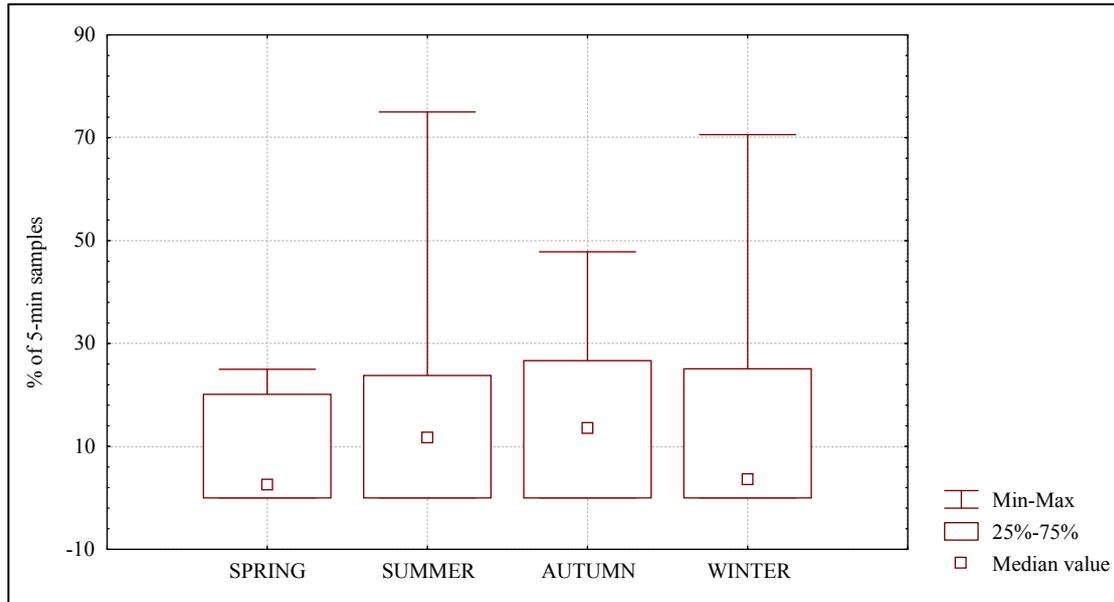
**Figure 3.1.** Frequency distribution of feeding categories in different seasons.

There were significant differences in frequency distribution of feeding categories between spring and winter ( $\chi^2=14.46$ ;  $df=4$ ;  $p<0.01$ ); summer and autumn ( $\chi^2=12.67$ ;  $df=4$ ;  $p<0.05$ ); summer and winter ( $\chi^2=10.56$ ;  $df=4$ ;  $p<0.05$ ) (Table 3.1).

**Table 3.1.** Differences in frequency distribution of feeding categories between seasons.

Seasons	$\chi^2$	df	<i>P</i>
Spring-Summer	4.48	4	n.s.
Spring-Autumn	6.47	4	n.s.
Spring-Winter	14.46	4	<0.01
Summer-Autumn	12.67	4	<0.05
Summer-Winter	10.56	4	<0.05
Autumn-Winter	8.436	4	n.s.

Feeding activity appeared with similar frequency values in both cold and warm season (16.18% of total behavioural samples in cold season (i.e. Sep-Feb); 11.95% of total behavioural samples in warm season (i.e. Mar-Aug). Although SF activity seemed to be stable throughout the year, there was found some fluctuation in monthly distribution, with an average of 15.15% ( $n=12$ ; Range=0-35;  $SD=10.94$ ;  $SE=3.16$ ), but no clear seasonal pattern was detectable in SF activity. Investigation of seasonal variation in feeding revealed that SF activity did not changed significantly ( $KW=2.6344$ ;  $p>0.05$ ) among seasons (Fig. 3.2).



**Figure 3.2.** Occurrence of 'Surface feeding' in seasons.

### 3.2. Spatial distribution of feeding

Spatial distribution of SF has shown that feeding activity differed within the study area. 18 cells were selected and defined as sampling sites, according to the predefined minimum value ( $\geq 15$  behavioural samples in each cell). Feeding activity in different cells is represented in Table 3.2.

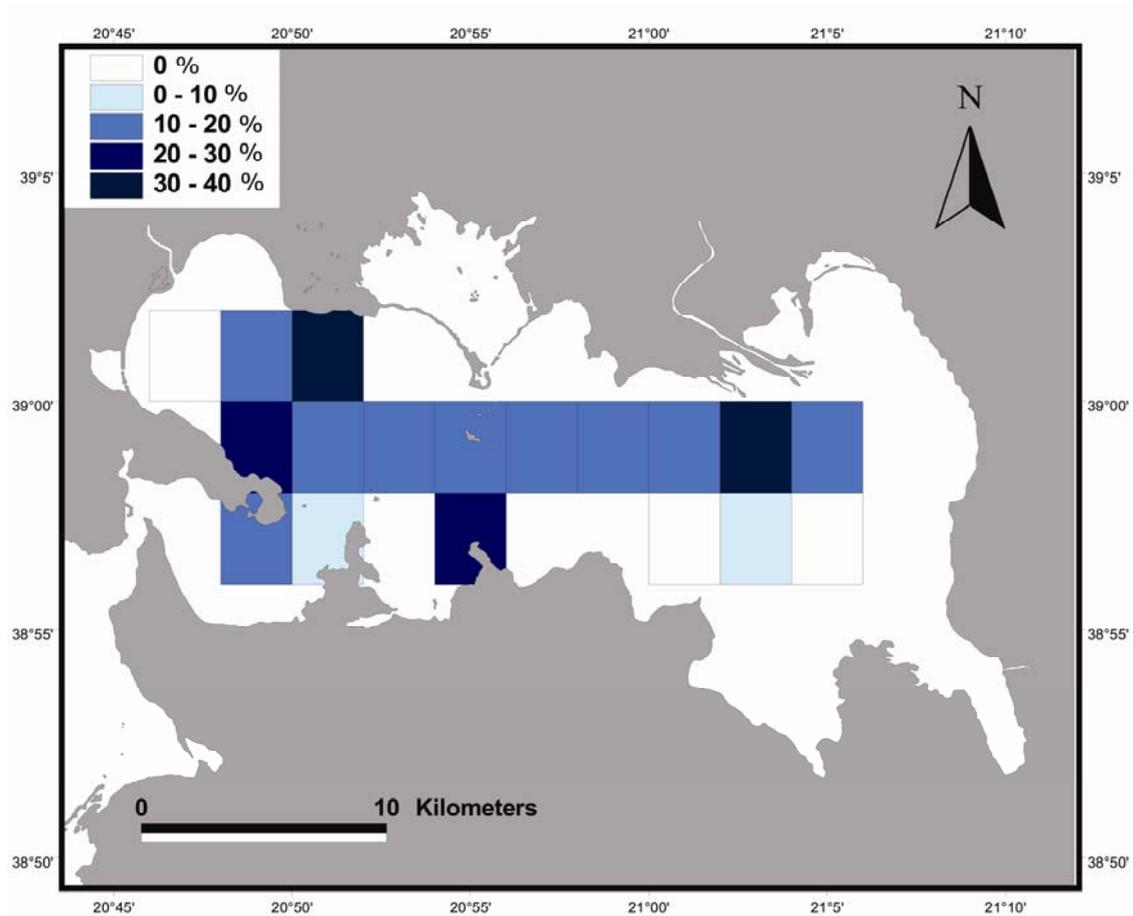
**Table 3.2.** Feeding activity in cells.

No. of Cell	% of SF	No. of Cell	% of SF	No. of Cell	% of SF
1.	18.84	7.	23.26	13.	19.61
2.	8.11	8.	17.86	14.	33.33
3.	22.22	9.	15.15	15.	14.29
4.	0.00	10.	14.52	16.	0.00
5.	4.55	11.	16.42	17.	12.90
6.	0.00	12.	12.04	18.	38.89

Note: Numbering of the cells starts from the left lower corner on the map (Fig. 3.3)

Significant association ( $\chi^2= 40.78$ ;  $df=17$ ;  $p<0.01$ ) was found between the occurrence of SF and different areas (i.e. cells) of the Gulf. There were places where SF occurred with higher frequencies while other sites were avoided in terms of feeding. Dolphins' surface feeding

behaviour was found to occur predominantly in mid-shallow waters (10-20m) in the middle-line of the Gulf; while the eastern (inner) part of the basin was not utilized as feeding site (Fig. 3.3).



**Figure 3.3.** Occurrence of 'Surface feeding' in different locations within the study area.

Three cells were found in this region with the lowest frequency values, two of them showed no feeding activity at all. Cells with the highest feeding activity appeared in the northern part of the study area. One 'preferred' cell was found with 38.89% ( $p < 0.01$ ) in the north-western part and another one (33.33%;  $p < 0.01$ ) towards east not far from the estuary of the River Arachthos. Further four cells proved to be significantly higher in terms of feeding activity; one was situated in the western site (23.26%;  $p < 0.05$ ); one in the south (22.22%;  $p < 0.05$ ); in the north-eastern site (19.61%;  $p < 0.05$ ); and in the south-west (18.84%;  $p < 0.05$ ). Feeding activity in the rest of the cells proved to be evenly distributed ( $\chi^2 = 19.50$ ;  $df = 11$ ; n.s.) within an extended area in the middle-line of the Gulf.

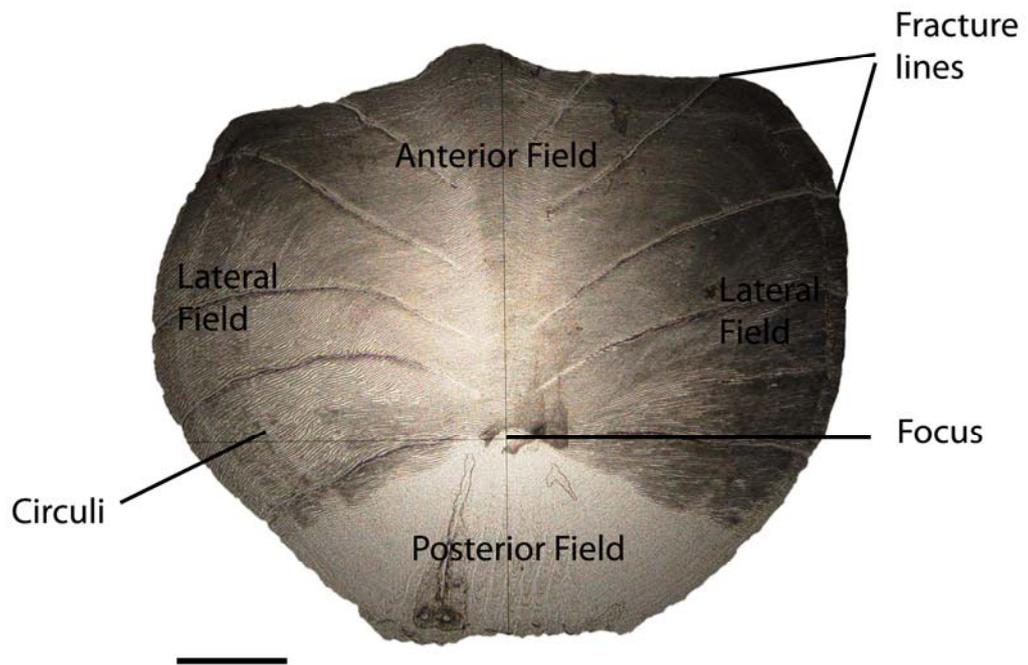
### 3.3. Fish scale identification

The catalogue of fish scales belonging to several fish species collected in the Amvrakikos Gulf is shown in Appendix C; P49-60. All the scale samples could be identified based on pre-defined scale features (Table 2.5). The gross morphological analysis of fish scales showed that all the scales (n=69) collected following surface feeding events were of Clupeidae and they involved exclusively European pilchard and/or round sardinella. While sardine scales were easily distinguishable from the other species living in the Amvrakikos Gulf, the scales of the two sardine species could not be reliably discriminated because they shared the same morphological characteristics (Table 3.3; Fig. 3.4-3.5). Comparisons of sardine scales from the same sampling zone are shown in Appendix B; P42-48. Scales from different body parts did not maintain the same size and morphological proportions. Some degree of variability also occurred within the pre-defined sampling zones.

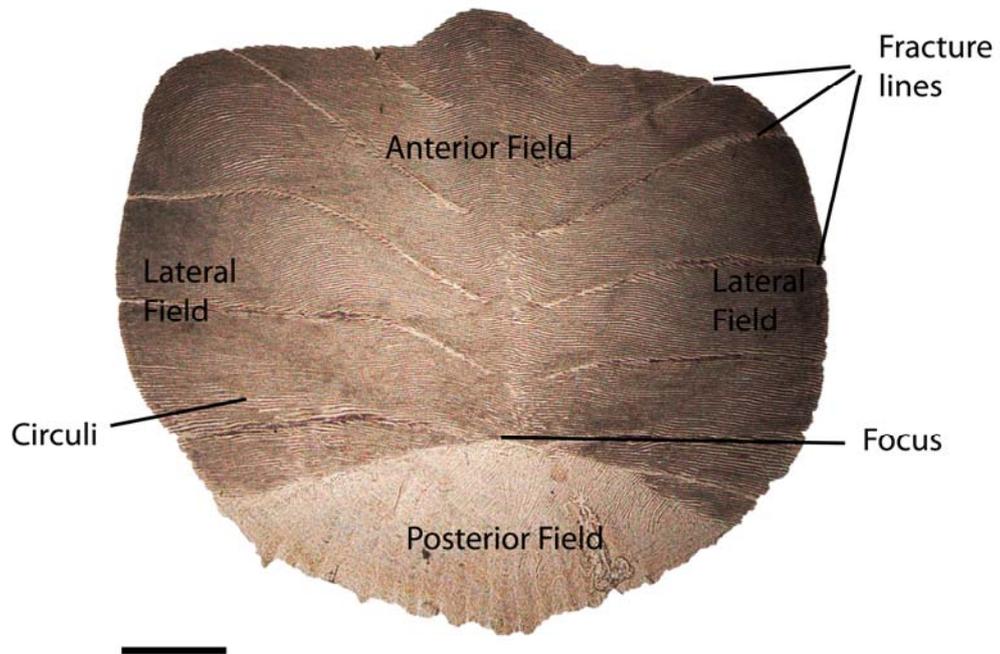
**Table 3.3.** Scale features of *Sardina pilchardus* and *Sardinella aurita*.

<b>Type</b>		cycloid
<b>Shape</b>		circular to disklike
<b>Appearance</b>		thin smooth surface flexible but rapidly curve and fracture as dries
<b>Fields</b>	<b>AF</b>	extended; even margin; trilobed
	<b>LFs</b>	extended; even margin; disarticulated
	<b>PF</b>	pointed but variable in shape, crenellated margin; subjected to fracturing
<b>Focus</b>		variable, generally indistinct; shifted towards the PF
<b>Circuli</b>		tightly compacted; straight to curved, generally concentric with margin; continuous between A and LFs; the continuity of circuli stops slightly below the imaginary line (dorso-ventral axis) of the focus; absent in the PF; indistinct around the focus
<b>Radii</b>		absent
<b>Fracture lines</b>		present; transverse grooves and variable fracture lines can occur in each field
<b>Ctenii</b>		absent

Notes: AF: Anterior Field; LFs: Lateral Fields; PF: Posterior Field.



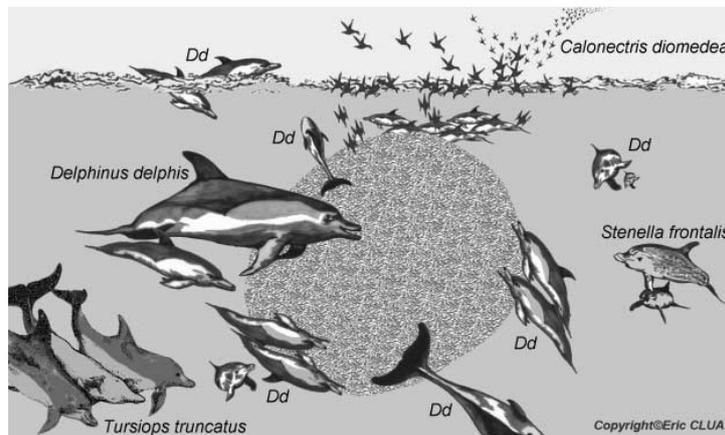
**Figure 3.4.** Scale characteristics of the scale of *Sardina pilchardus*; scale bar = 1.0 mm.



**Figure 3.5.** Scale characteristics of the scale of *Sardinella aurita*; scale bar = 1.0 mm.

## 4. DISCUSSION

This study has confirmed preliminary observations suggesting that the feeding behaviour of bottlenose dolphins living in the Amvrakikos Gulf is strongly influenced by prey kind and availability. The Amvrakikos Gulf is among the most productive coastal areas of Greece and it supports dense populations of small epipelagic fish (Bearzi *et al.*, 2008a). Our field observations revealed that local bottlenose dolphins often and regularly engage in surface foraging activities. Surface feeding was strongly linked with the occurrence of foraging seabirds (i.e. seagulls and terns). Birds tended to take advantage of densely packed and easily-obtainable fish that were schooled and driven to the surface by the dolphins. Over 97% of the observation time, when dolphins engaged in surface feeding seagulls and/or terns were also present. In addition, a significant relationship was found between the number of bird specimens and the regularity of surface feeding. Bird aggregations likely arise because they can detect which feeding patch has a highest density of prey (Sutherland, 1983). In the Amvrakikos Gulf, the gathering of foraging birds seemed to be triggered by dolphin feeding at the surface. Similar feeding aggregations of dolphins and seabirds (Fig. 4.1.) were documented in the eastern tropical Pacific by Au & Pitman (1986) and in the Azores by Clua & Grosvalet (2001).



**Figure 4.1.** Feeding aggregation of dolphins and seabirds (from Clua & Grosvalet, 2001).

When surface foraging occurred, it typically involved dolphin groups rather than single individuals. Cooperative feeding can promote fish schooling and facilitate prey capture (Bearzi M., 2005). The analysis of fish scale samples showed that two species of the family Clupeidae, European pilchard and round sardinella, were the exclusive target of dolphins during surface feeding. Such evidence is probably related to the high local abundance of these

epipelagic species. Due to the relatively small size of sardines, they can be captured more effectively if dolphins hunt in groups, thus promoting cooperative feeding. During predatory events, the dolphins were observed moving erratically, performing repeated short dives in varying directions in one location and they were seen actively pursuing and mouthing the fish. A similar kind of surface feeding was described for bottlenose dolphins in Texas and Florida by Shane (1990b) and in California by M. Bearzi (2005). The same type of foraging strategy targeting epipelagic schooling prey has been reported for a number of other small cetaceans, including short-beaked common dolphins (*Delphinus delphis*) in Mercury Bay, New Zealand (Neumann & Orams, 2005) and in the eastern Ionian Sea, Greece (Bearzi *et al.*, 2005).

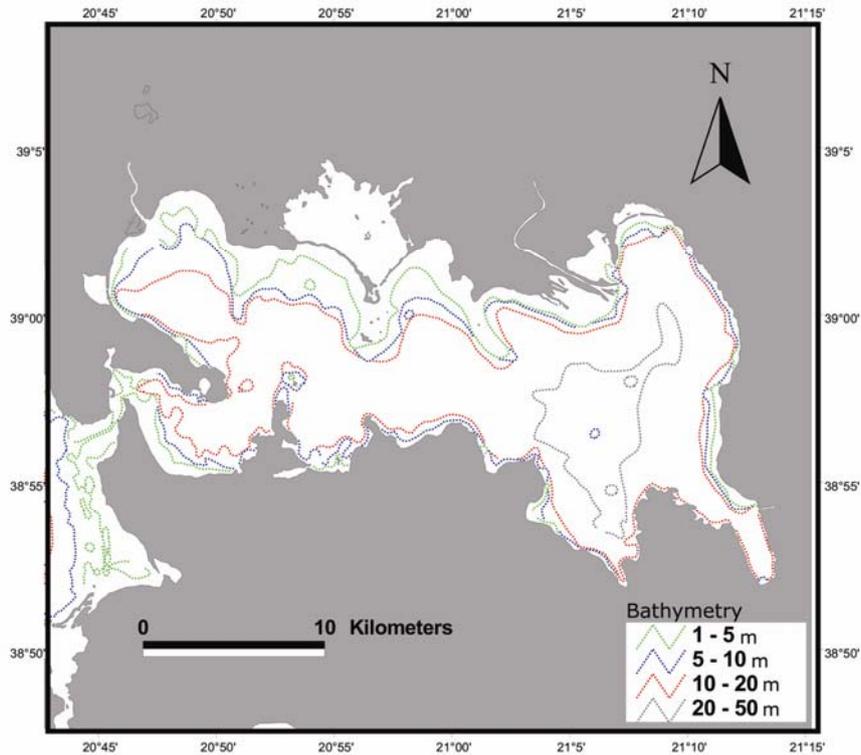
During field observations, 57% of surface foraging occurred as a 'pure' activity, with no other behavioural states being recorded in a given 5-min sample. However, social behaviours were recorded to occur in a feeding context on 26% of the observations. Aerial behaviour (e.g. leaps) and percussive behaviour (e.g. chin or tail slaps) could be performed either before, after or during surface feeding by different subgroups or individuals. Different forms of the above mentioned surface active behaviours can be used by dolphins as a type of non-vocal communication to attract the attention of others or synchronise their activity (Lusseau, 2006).

The behaviour of dolphins in the Amvrakikos Gulf appears similar to the behaviour of another bottlenose dolphin population living in the North Evoikos, Greece, which spent considerable time in surface feeding and socialising, indicating an abundance of pelagic prey (Zafiropoulos & Merlini, 2003). Frequently surface feeding and socialising in the Amvrakikos Gulf was suggestive of low feeding efforts and high energy levels of the animals. In contrast, in the South Evoikos Gulf, Greece, where food resources appeared to be limited, bottlenose dolphins spent most of their time travelling and surface feeding or socialising were uncommon (Zafiropoulos & Merlini, 2003). Bottlenose dolphins living in the nearby open waters of the eastern Ionian Sea, near the island of Kalamos, were rarely observed feeding at the surface (Bearzi *et al.*, 2005) and in most other parts of the Mediterranean bottlenose dolphins almost exclusively target demersal prey (Bearzi *et al.*, 2008b). Differences in feeding behaviour may relate to primary production, which in turn affects prey kind and availability. The eastern Ionian Sea is oligotrophic and epipelagic fish stocks have been overexploited by extensive fishing (Bearzi *et al.*, 2006).

Seasonal changes in feeding activity and diet composition are normally driven by the need of the animals to maintain a sufficient food intake (Gannon *et al.*, 1998). In this study, there was no significant difference in surface feeding activity among the four seasons,

suggesting absence of seasonality. Fluctuations in prey kind or availability in other areas were related to seasonal behavioural changes. For instance, bottlenose dolphins off Texas engaged in feeding activities more in fall and winter than in spring and summer (Shane *et al.*, 1986). In the Amvrakikos Gulf, surface feeding occurred with similar frequency values during the cold and the warm season. Moreover, no seasonal changes were found in the type of prey captured during surface foraging, based on evidence provided by fish scales. While it is not known whether epipelagic fish stocks actually fluctuate seasonally, the high proportion of surface feeding across seasons and the fact that dolphins stay within the Gulf year round, showing high levels of site fidelity (Bearzi *et al.*, 2008a), strongly suggests a relatively stable availability of their prey. The observed differences in the frequency distribution of feeding categories among seasons can reflect some degree of behavioural change, with groups travelling more in spring and autumn, and socialising more during summer. An analysis of the whole behavioural repertoire of the animals, which could not be done in this study, may provide additional insight into behavioural fluctuations.

The spatial distribution of surface feeding suggested the presence of foraging hotspots within the Gulf. The feeding behaviour of delphinids has been predominantly described in relation to bottom topography, water depth, and other environmental variables (e.g. Hanson & Defran, 1993; Harzen, 1998; Hastie *et al.*, 2004). However, the accessibility and behaviour of prey largely explain feeding activity of these predators (Acevedo-Gutiérrez & Parker, 2000). In this study, surface feeding occurred primarily within an extended area in the middle of the Gulf, where water depth is 10-20m (Fig. 4.2). Some places appeared to have a higher occurrence of feeding, but these differences may be a result of sampling bias, which could be avoided with increasing the number of samples in those areas. On the other hand, deeper waters of the south-eastern part of the Gulf, where anoxic nearbed conditions dominated (Nicolaidou & Papadopoulou, 1989; Poulos *et al.*, 2008), were avoided in terms of feeding. Lower occurrence of feeding in these areas likely relates to prey scarcity. In these deep waters (30-50m) (Fig. 4.2), increases in water temperature, decreases in dissolved oxygen and water stratification can result in bottom anoxia. Seabed currents in the Gulf are virtually absent, particularly in the summer (Nicolaidou & Papadopoulou, 1989), and oxygen supply is further restricted. These conditions can be a limiting factor for the occurrence of living organisms. Hypoxic conditions can therefore result in lower density of fish prey, thence reducing feeding by dolphins.



**Figure 4.2.** Bathymetry of the Amvrakikos Gulf.

Increasing hypoxia in recent times is mostly due to human activities (Tuvikene *et al.*, 2001). The Gulf can be considered as an extreme habitat due to its isolated characteristic and the impact of several human activities. It is strongly affected by runoffs derived from agriculture and domestic sewage, which makes this habitat prone to degradation. These facts should be taken into consideration and encourage a better management.

To get a clearer picture about the relationship between environmental conditions, prey availability and surface feeding, direct information on the spatial distribution of prey would be needed. Future work should focus on collecting detailed information on the distribution patterns of prey within the study area, to allow direct comparison between preferred feeding sites and prey distribution. Further investigations would be needed to elucidate whether estuaries of the two rivers have any effect on epipelagic fish assemblage, thus affecting surface feeding by the dolphins.

Furthermore, it is important to acknowledge some limitations of this study. Due to the lack of information on underwater foraging behaviour of bottlenose dolphins in the Amvrakikos Gulf, this study could only focus on feeding behaviour at the surface while other aspects of foraging behaviour remain unexplored. Collecting drifting scales following predatory events proved to be a good approach to gain insight into the diet of local dolphins

during surface feeding. However, this technique is appropriate only for species that lose scales. Fish that do not easily lose scales (e.g. mullets) could not be identified with this method. Nevertheless, the high local abundance of sardines and the constant presence of their scales in the samples provide solid evidence about their dominance in the diet of dolphins during surface feeding.

The morphological analysis of scales allowed to discriminate among major fish groups and to distinguish among species living in the Amvrakikos Gulf. In addition, the analysis revealed that scales of the two sardine species – European pilchard and round sardinella – have almost identical morphological features preventing a reliable discrimination between these species. If differences in scale morphology exist, they will need to be highlighted by a microstructural examination and more sophisticated microscopes and imagery.

In conclusion, this study showed that bottlenose dolphins living in the Amvrakikos Gulf often and regularly engage in feeding activities close to the surface and when they do so they prey primarily on sardines. Such foraging strategy - infrequently observed elsewhere in the Mediterranean - seems to have evolved as an adaptation to local conditions and a particularly high abundance and year round availability of epipelagic prey.

## 5. SUMMARY

Bottlenose dolphins show high level of behavioural flexibility in their feeding behaviour. Feeding habits can change remarkably according to habitat and prey type and it is important to understand how the species can adapt its feeding strategy to the local conditions.

The Amvrakikos Gulf, in western Greece, represents a unique ecological habitat with highly productive waters and has among the highest densities of bottlenose dolphins of any Mediterranean areas. The aim of this study was to explore the diet and behaviour of the local bottlenose dolphin population during surface foraging and to investigate how food availability and habitat characteristics shape their feeding strategies.

Field research was conducted from January 2007 to December 2007. Data were collected during boat-based survey trips performed in a total of 136 days, with 7,065 km of total effort distributed within an area of about 400 km<sup>2</sup>. Behavioural sampling resulted in 85 h 32 min of direct observation and 1012 behavioural samples of 5-min each. A total of 69 fish scale samples were collected in order to identify dolphin prey items during surface feeding events. Results showed that surface feeding represents 14 % of the total behavioural budget of local dolphins. Surface feeding occurred in significant association with foraging birds.

The main prey targeted by dolphins was epipelagic fish of the family Clupeidae. Two species of sardines were identified as exclusive targets by the local dolphins, namely European pilchard and round sardinella, which are present in high densities within the Gulf. The occurrence of surface feeding fluctuated during the year but no significant differences were found among seasons, indicating that prey may be abundant year-round. Analysis of the spatial distribution of feeding activity suggested that the study area contains preferred feeding sites, with surface feeding more evenly distributed in the middle-line of the Gulf. Areas of lower importance included the eastern part of the Gulf where bottom anoxia and other factors may result in prey scarcity.

Contrary to other Mediterranean areas, where bottlenose dolphins focus on demersal prey and rarely target small epipelagics, in the Amvrakikos Gulf surface feeding was found to represent an important part of the dolphin's daily activity, and sardines an important prey item. The local bottlenose dolphins therefore seem to have developed a highly specialized foraging behaviour determined by prey availability and local conditions.

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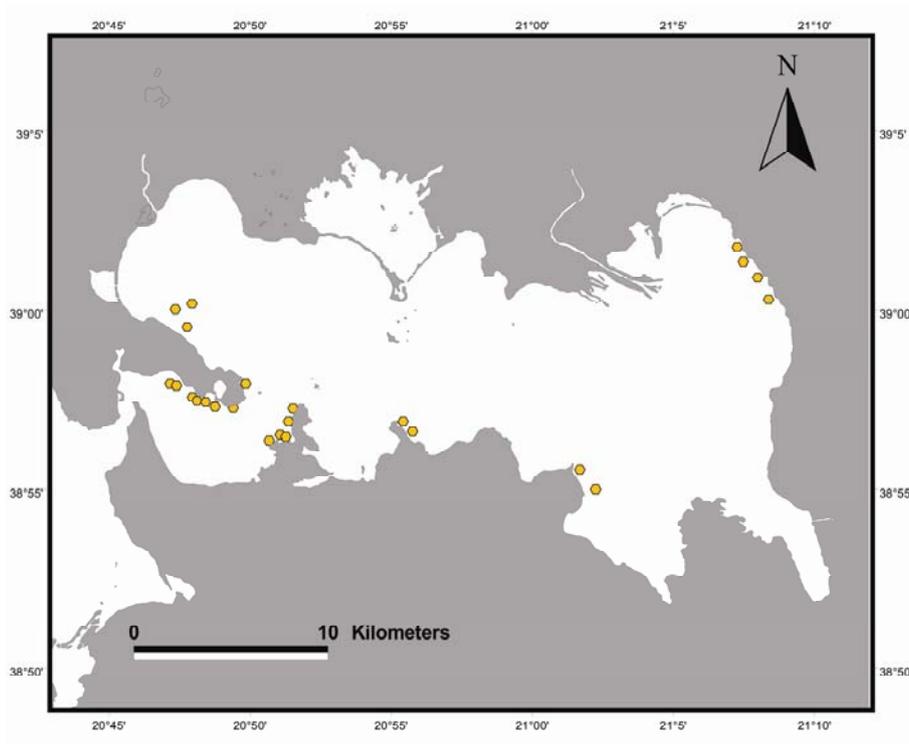
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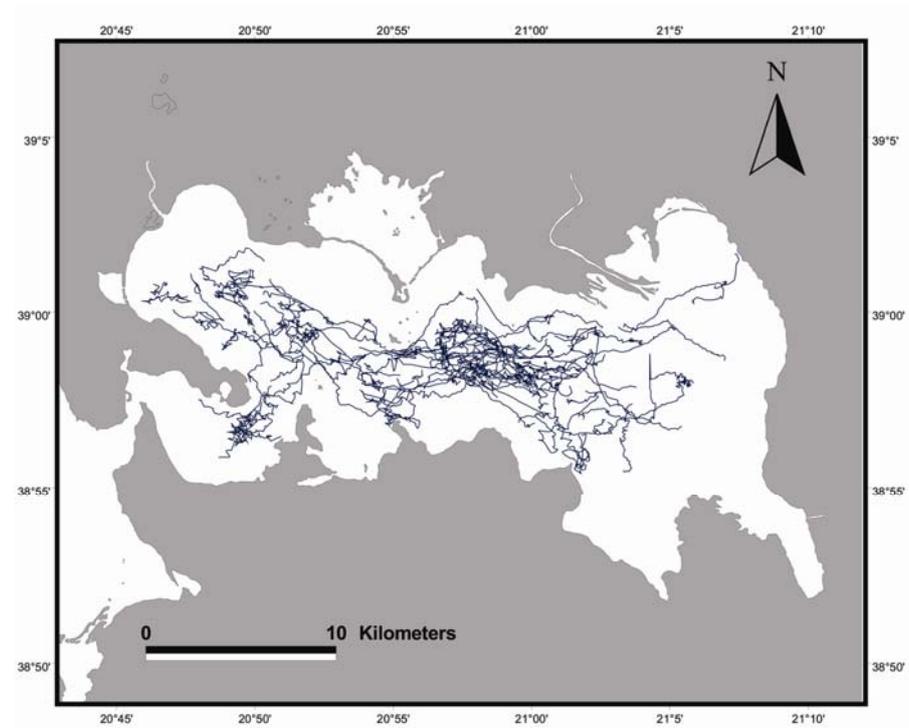
But my greatest thank goes to my parents and grandparents who ensured me a loving and understanding background, encouraging me to follow the right and straight path. They have given me an independent will and also the chance of making my own decisions to choose the way my heart told me. Special thanks to my aunty, my brother and sister who were always standing by my side and were there for me when I needed them. Without you I would not have been able to make it.

## **APPENDIX A**

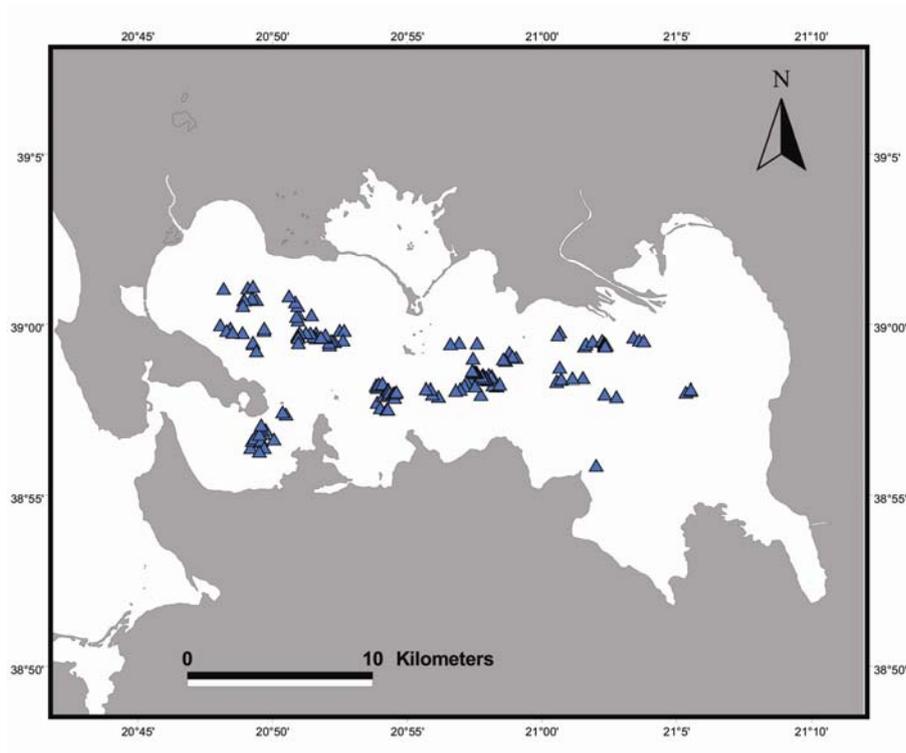
### **Additional Maps**



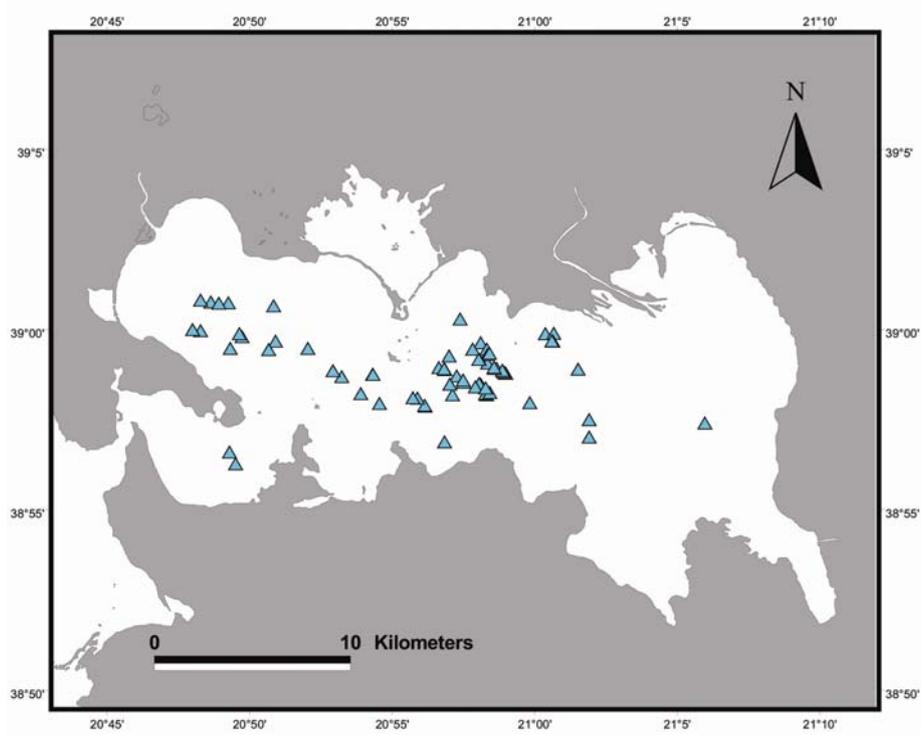
**Figure 1.** Fish farm positions in the Amvrakikos Gulf.



**Figure 2.** Movement pattern of bottlenose dolphins in 2007.



**Figure 3.** Positions of surface feeding in 2007.



**Figure 4.** Positions of fish scale sampling in 2007.

## **APPENDIX B**

### **Comparison of scales between *Sardina pilchardus* and *Sardinella aurita***



**Figure 1.** Scale of *Sardina pilchardus* derived from sampling zone A (scale bar = 1.0 mm); sampling zone A of *Sardina pilchardus* (scale bar = 10.0 cm).



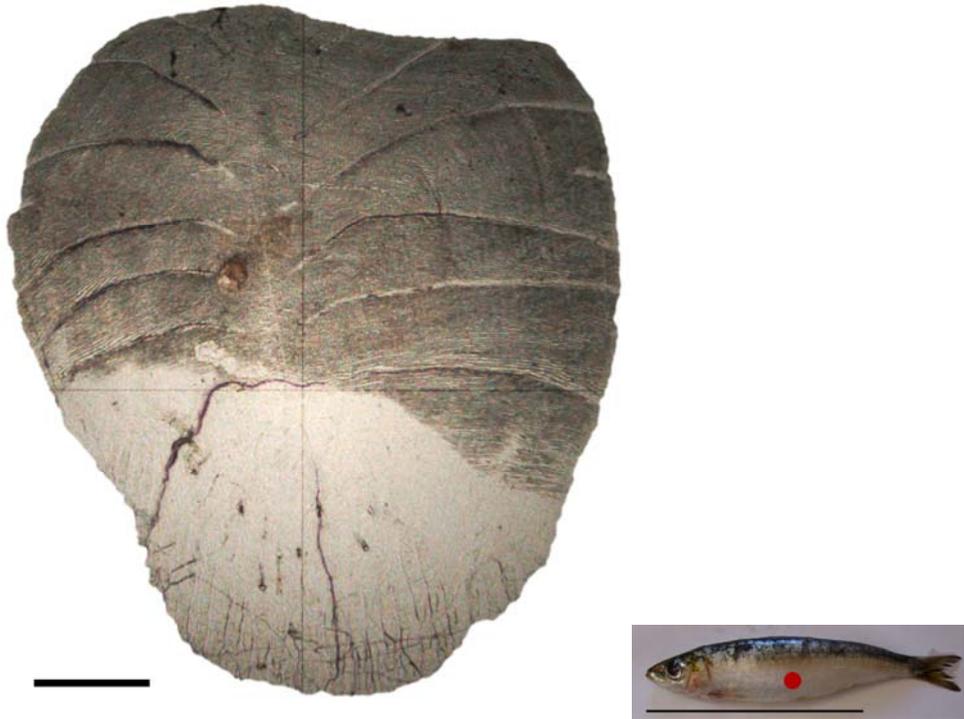
**Figure 2.** Scale of *Sardinella aurita* derived from sampling zone A (scale bar = 1.0 mm); sampling zone A of *Sardinella aurita* (scale bar = 10.0 cm).



**Figure 3.** Scale of *Sardina pilchardus* derived from sampling zone B (scale bar = 1.0 mm); sampling zone B of *Sardina pilchardus* (scale bar = 10.0 cm).



**Figure 4.** Scale of *Sardinella aurita* derived from sampling zone B (scale bar = 1.0 mm); sampling zone B of *Sardinella aurita* (scale bar = 10.0 cm).



**Figure 5.** Scale of *Sardina pilchardus* derived from sampling zone B (scale bar = 1.0 mm); sampling zone B of *Sardina pilchardus* (scale bar = 10.0 cm).



**Figure 6.** Scale of *Sardinella aurita* derived from sampling zone B (scale bar = 1.0 mm); sampling zone B of *Sardinella aurita* (scale bar = 10.0 cm).



**Figure 7.** Scale of *Sardina pilchardus* derived from sampling zone C (scale bar = 1.0 mm); sampling zone C of *Sardina pilchardus* (scale bar = 10.0 cm).



**Figure 8.** Scale of *Sardinella aurita* derived from sampling zone C (scale bar = 1.0 mm); sampling zone C of *Sardinella aurita* (scale bar = 10.0 cm).



**Figure 9.** Scale of *Sardina pilchardus* derived from sampling zone D (scale bar = 1.0 mm); sampling zone D of *Sardina pilchardus* (scale bar = 10.0 cm).



**Figure 10.** Scale of *Sardinella aurita* derived from sampling zone D (scale bar = 1.0 mm); sampling zone D of *Sardinella aurita* (scale bar = 10.0 cm).



**Figure 11.** Scale of *Sardina pilchardus* derived from sampling zone E (scale bar = 1.0 mm); sampling zone E of *Sardina pilchardus* (scale bar = 10.0 cm).

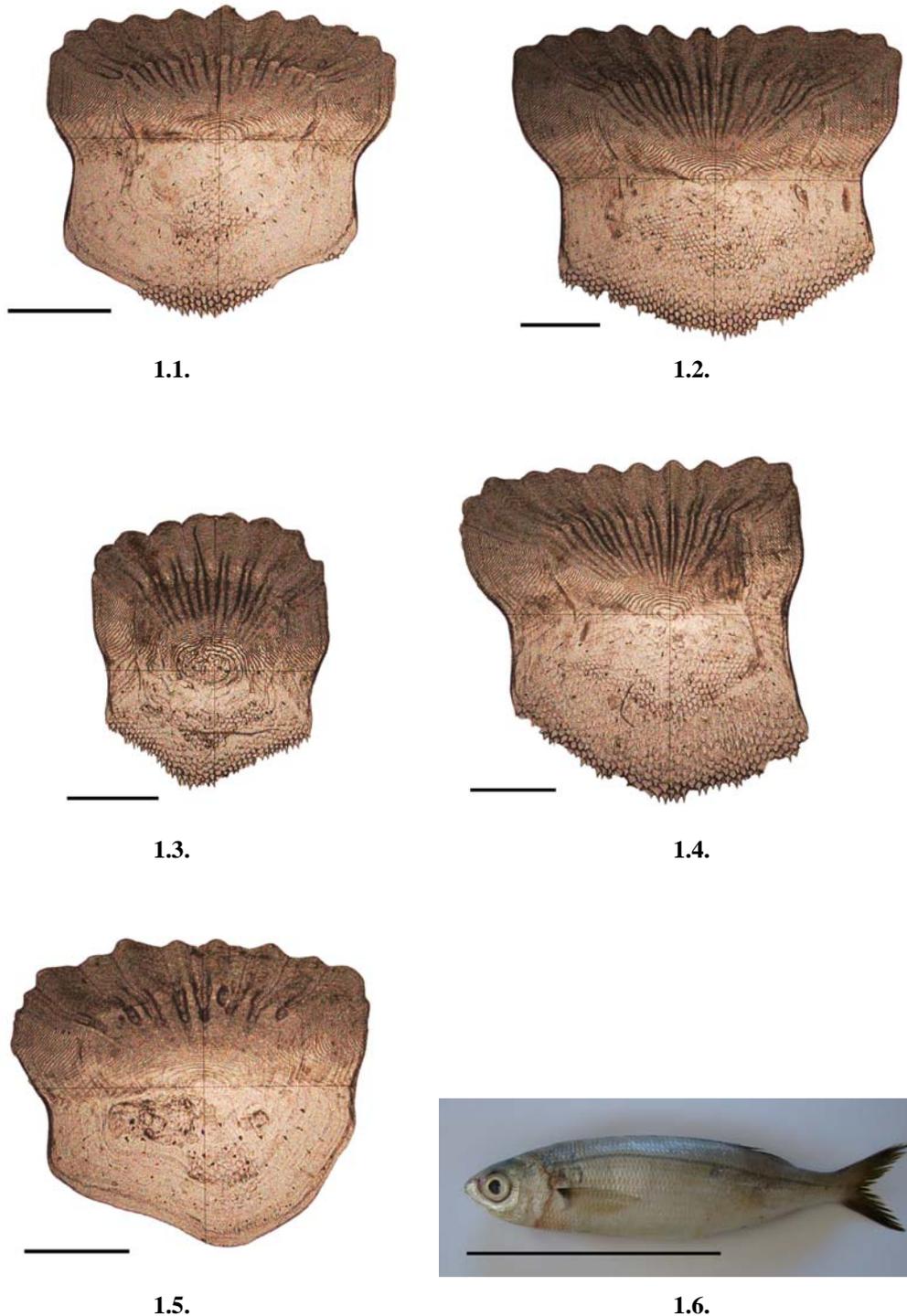


**Figure 12.** Scale of *Sardinella aurita* derived from sampling zone E (scale bar = 1mm); sampling zone E of *Sardinella aurita* (scale bar = 10.0 cm).

## **APPENDIX C**

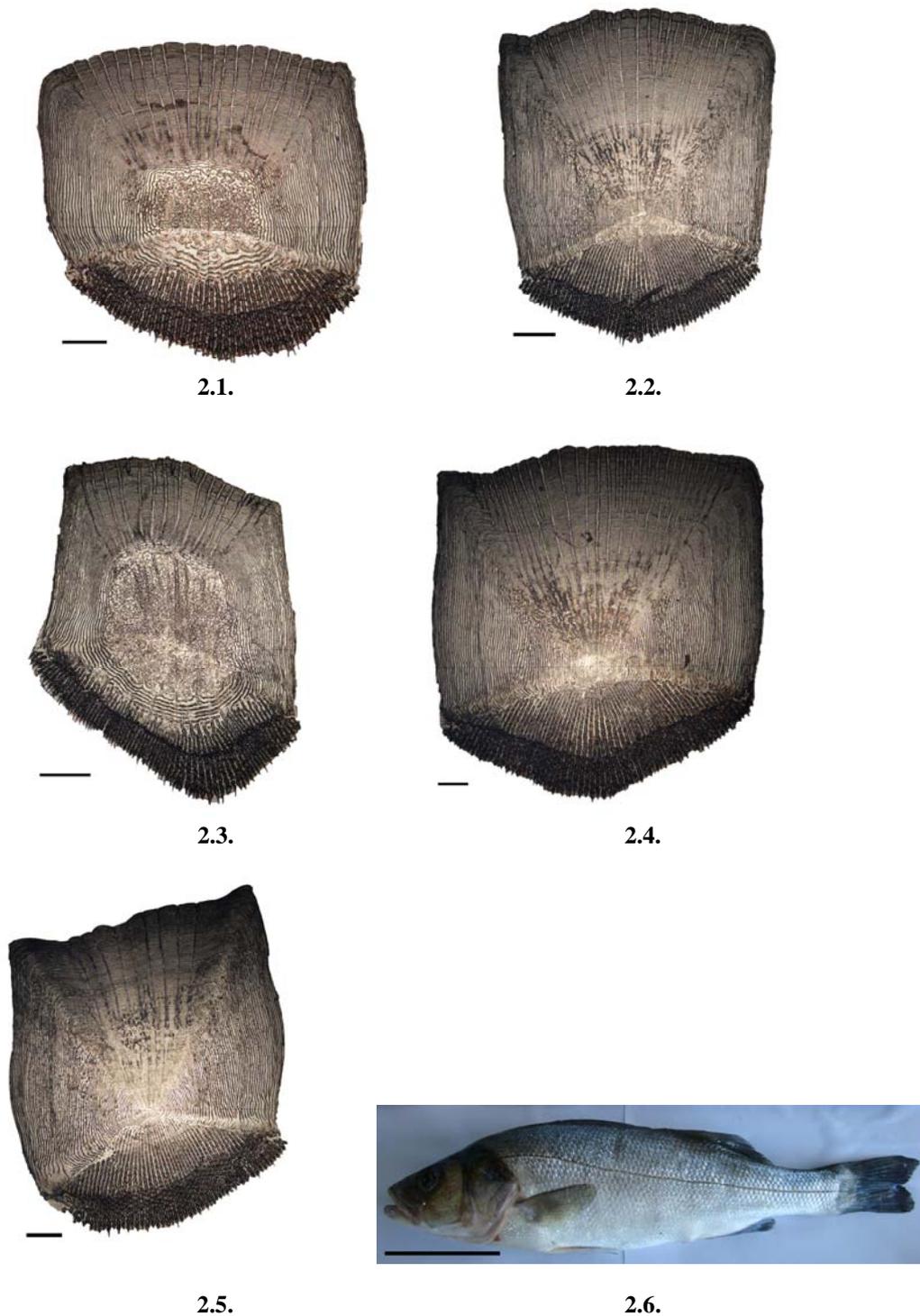
### **Fish Scale Catalogue**

**I. Species:** *Boops boops* (Linnaeus, 1758) / Bogue  
**Family:** Sparidae  
**Genus:** Perciformes



**Figure 1.1. - 1.5.** Scales of *Boops boops*; scale bar =1.0 mm.  
1.1. Scale derived from zone A (dorsal, anterior, above the lateral line); 1.2. Scale derived from zone B (ventral, below lateral line); 1.3. Scale derived from zone C (caudal); 1.4. Scale derived from zone D (dorsal, posterior, above lateral line); 1.5. Scale derived from zone E (caudal to operculum);  
**Figure 1.6.** The species – Bogue (*Boops boops*); scale bar = 10.0 cm.

**II. Species:** *Dicentrarchus labrax* (Linnaeus, 1758) / European seabass  
**Family:** Moronidae  
**Genus:** Perciformes

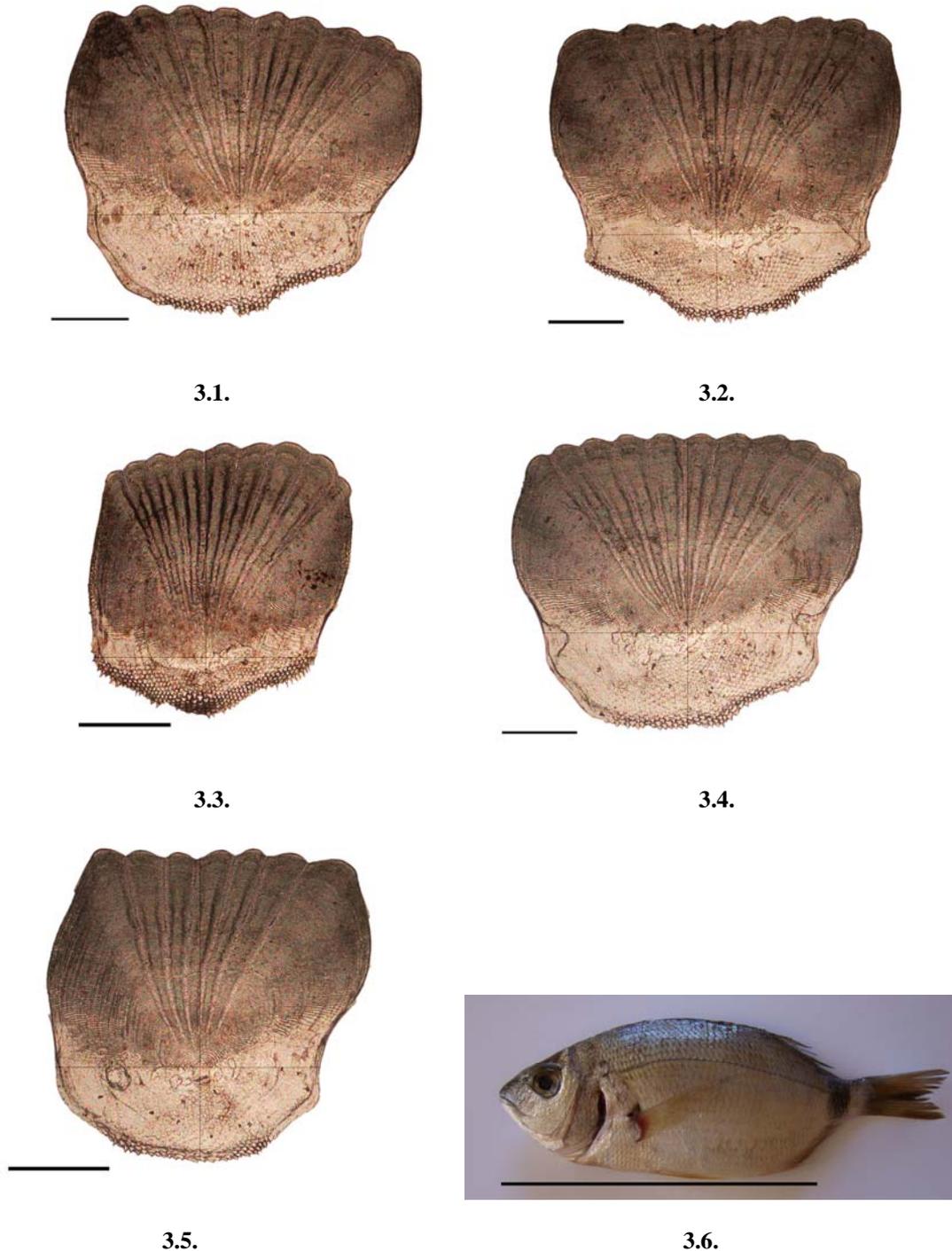


**Figure 2.1. - 2.5.** Scales of *Dicentrarchus labrax*; scale bar =1.0 mm.  
2.1. Scale derived from zone A (dorsal, anterior, above the lateral line); 2.2. Scale derived from zone B (ventral, below lateral line); 2.3. Scale derived from zone C (caudal); 2.4. Scale derived from zone D (dorsal, posterior, above lateral line); 2.5. Scale derived from zone E (caudal to operculum);  
**Figure 2.6.** The species – European seabass (*Dicentrarchus labrax*); scale bar = 10.0 cm.

**III. Species:** *Diplodus annularis* (Linnaeus, 1758) / Annular seabream

**Family:** Sparidae

**Genus:** Perciformes

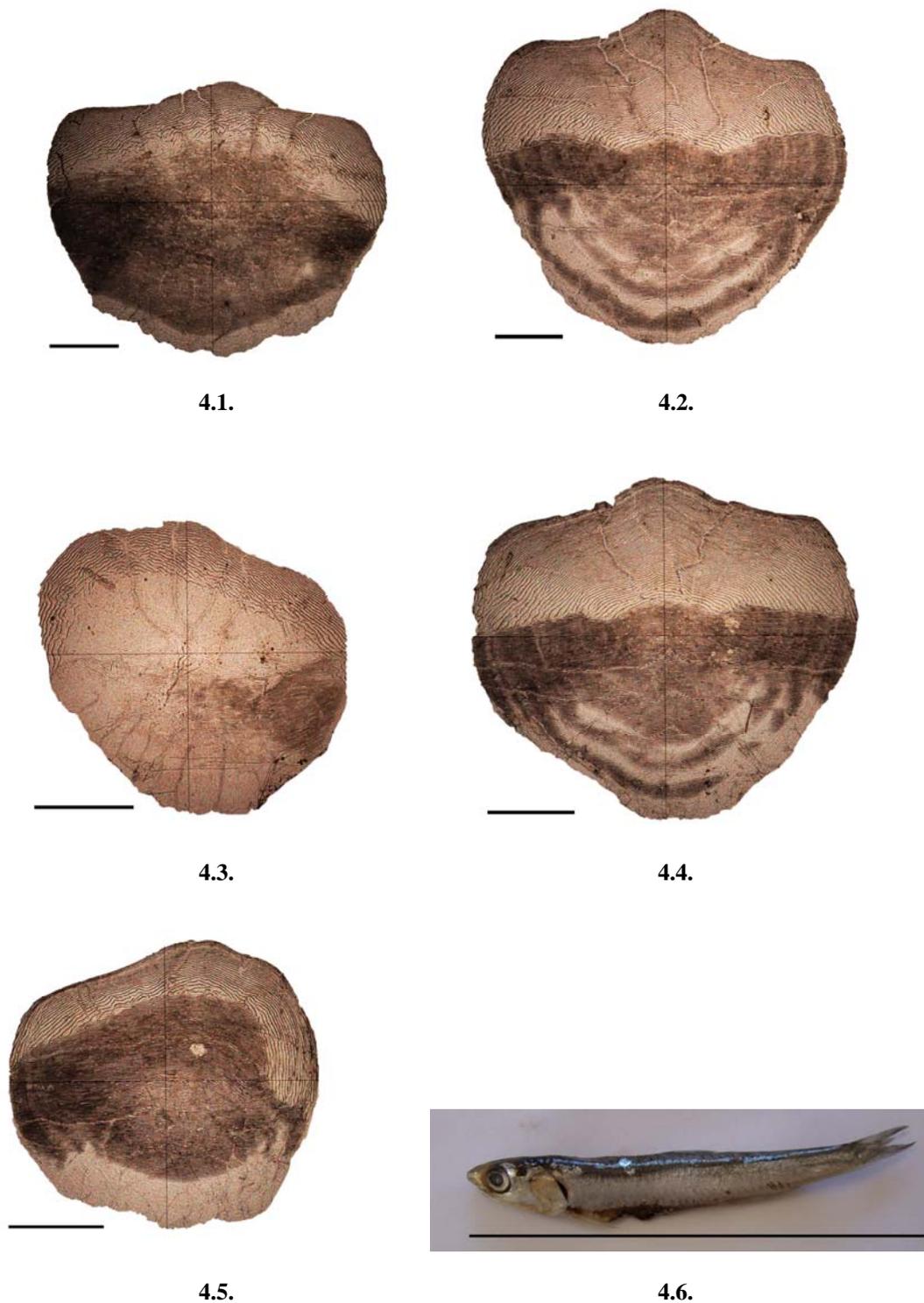


**Figure 3.1. - 3.5.** Scales of *Diplodus annularis*; scale bar =1.0 mm.

3.1. Scale derived from zone A (dorsal, anterior, above the lateral line); 3.2. Scale derived from zone B (ventral, below lateral line); 3.3. Scale derived from zone C (caudal); 3.4. Scale derived from zone D (dorsal, posterior, above lateral line); 3.5. Scale derived from zone E (caudal to operculum);

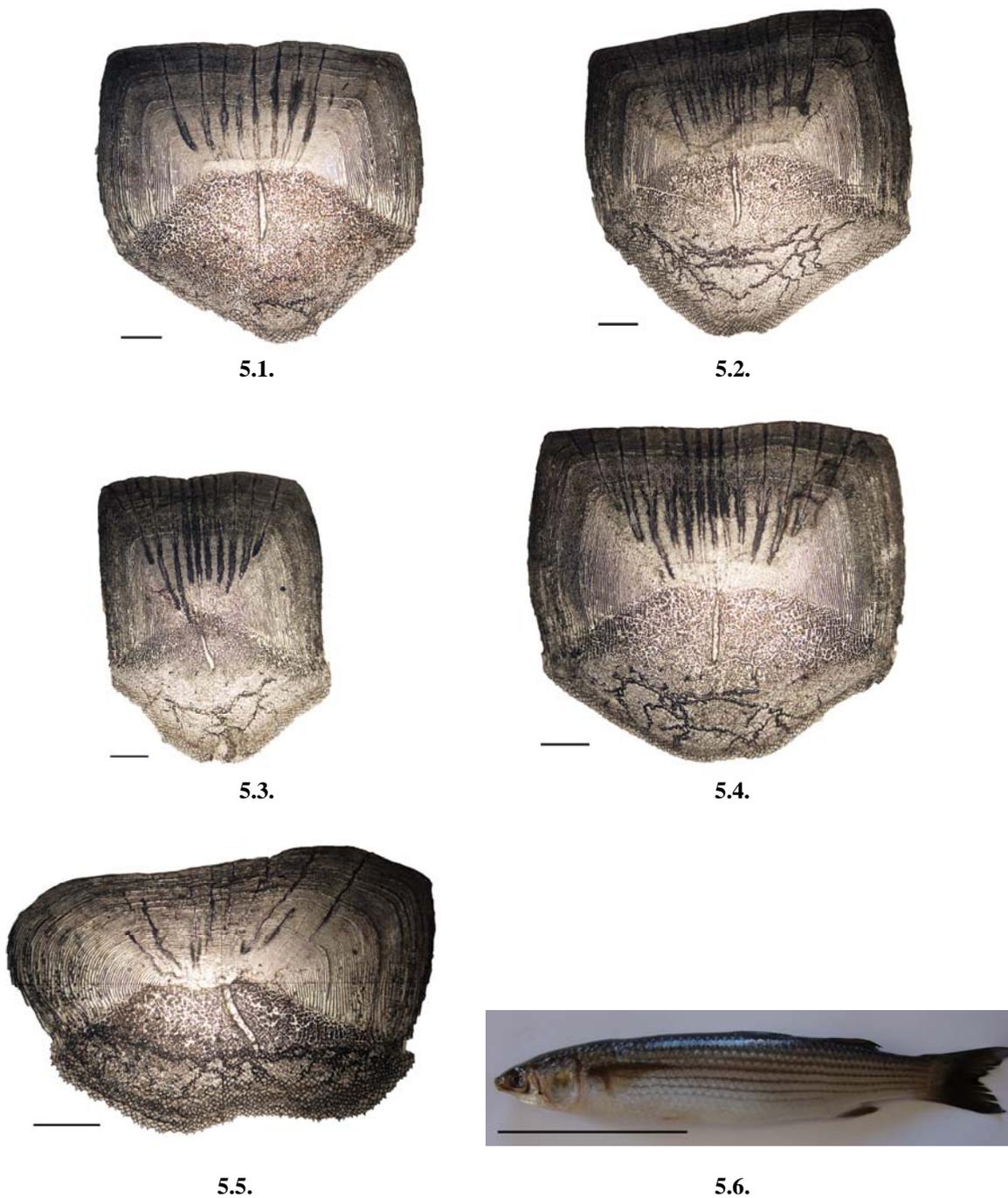
**Figure 3.6.** The species – Annular seabream (*Diplodus annularis*); scale bar = 10.0 cm.

**IV. Species:** *Engraulis encrasicolus* (Linnaeus, 1758) / European anchovy  
**Family:** Engraulidae  
**Genus:** Clupeiformes



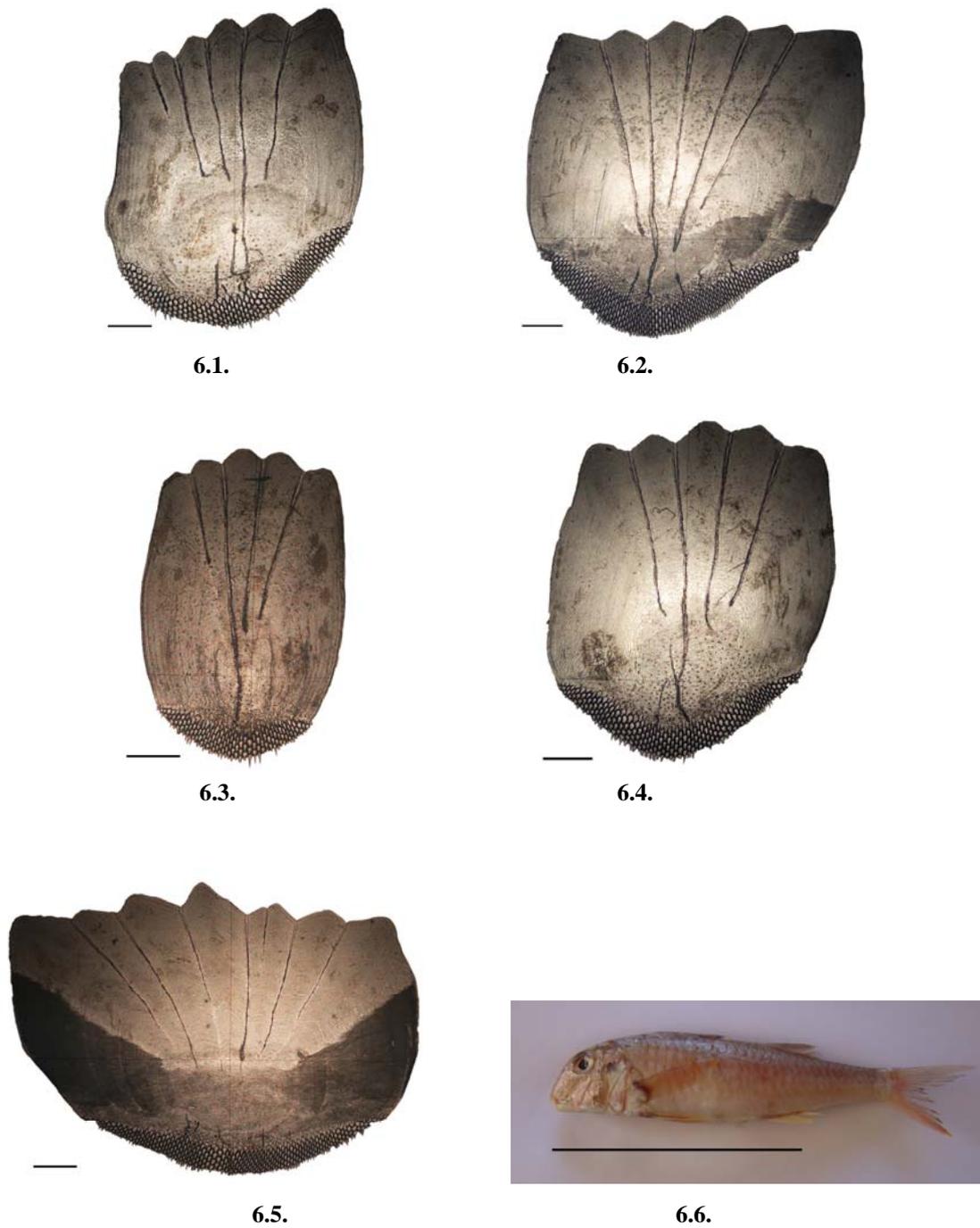
**Figure 4.1. - 4.5.** Scales of *Engraulis encrasicolus*; scale bar =1.0 mm.  
4.1. Scale derived from zone A (dorsal, anterior, above the lateral line); 4.2. Scale derived from zone B (ventral, below lateral line); 4.3. Scale derived from zone C (caudal); 4.4. Scale derived from zone D (dorsal, posterior, above lateral line); 4.5. Scale derived from zone E (caudal to operculum);  
**Figure 4.6.** The species – European anchovy (*Engraulis encrasicolus*); scale bar = 10.0 cm.

**V. Species:** *Liza ramado* (Risso, 1810) / Thinlip mullet  
**Family:** Mugilidae  
**Genus:** Mugiliformes



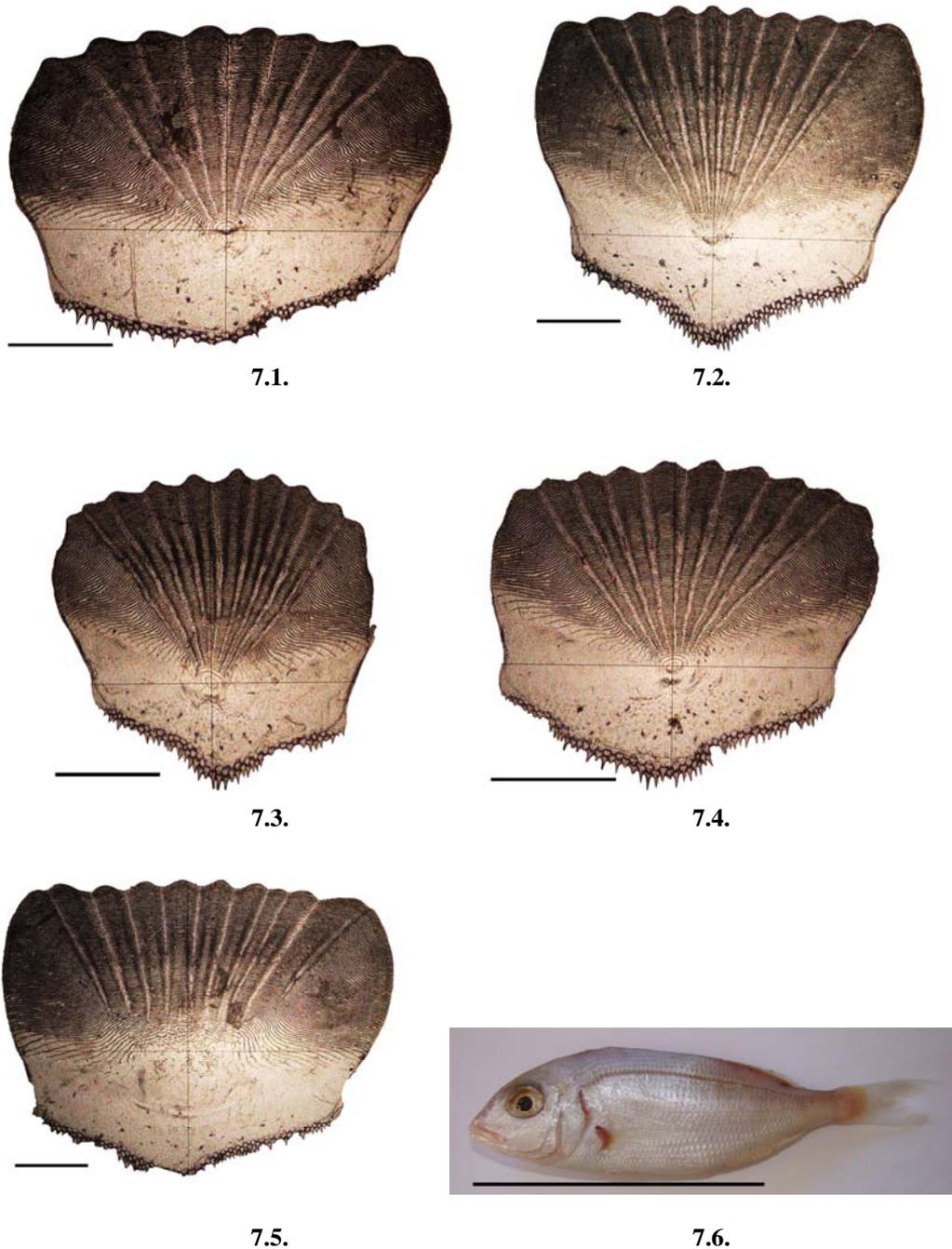
**Figure 5.1. - 5.5.** Scales of *Liza ramado*; scale bar =1.0 mm.  
5.1. Scale derived from zone A (dorsal, anterior, above the lateral line); 5.2. Scale derived from zone B (ventral, below lateral line); 5.3. Scale derived from zone C (caudal); 5.4. Scale derived from zone D (dorsal, posterior, above lateral line); 5.5. Scale derived from zone E (caudal to operculum);  
**Figure 5.6.** The species – Thinlip mullet (*Liza ramado*); scale bar = 10.0 cm.

**VI. Species:** *Mullus barbatus* (Linnaeus, 1758) / Red mullet  
**Family:** Mullidae  
**Genus:** Perciformes



**Figure 6.1. - 6.5.** Scales of *Mullus barbatus*; scale bar =1.0 mm.  
6.1. Scale derived from zone A (dorsal, anterior, above the lateral line); 6.2. Scale derived from zone B (ventral, below lateral line); 6.3. Scale derived from zone C (caudal); 6.4. Scale derived from zone D (dorsal, posterior, above lateral line); 6.5. Scale derived from zone E (caudal to operculum);  
**Figure 6.6.** The species – Red mullet (*Mullus barbatus*); scale bar = 10.0 cm.

**VII. Species:** *Pagrus pagrus* (Linnaeus, 1758) / Common seabream  
**Family:** Sparidae  
**Genus:** Perciformes

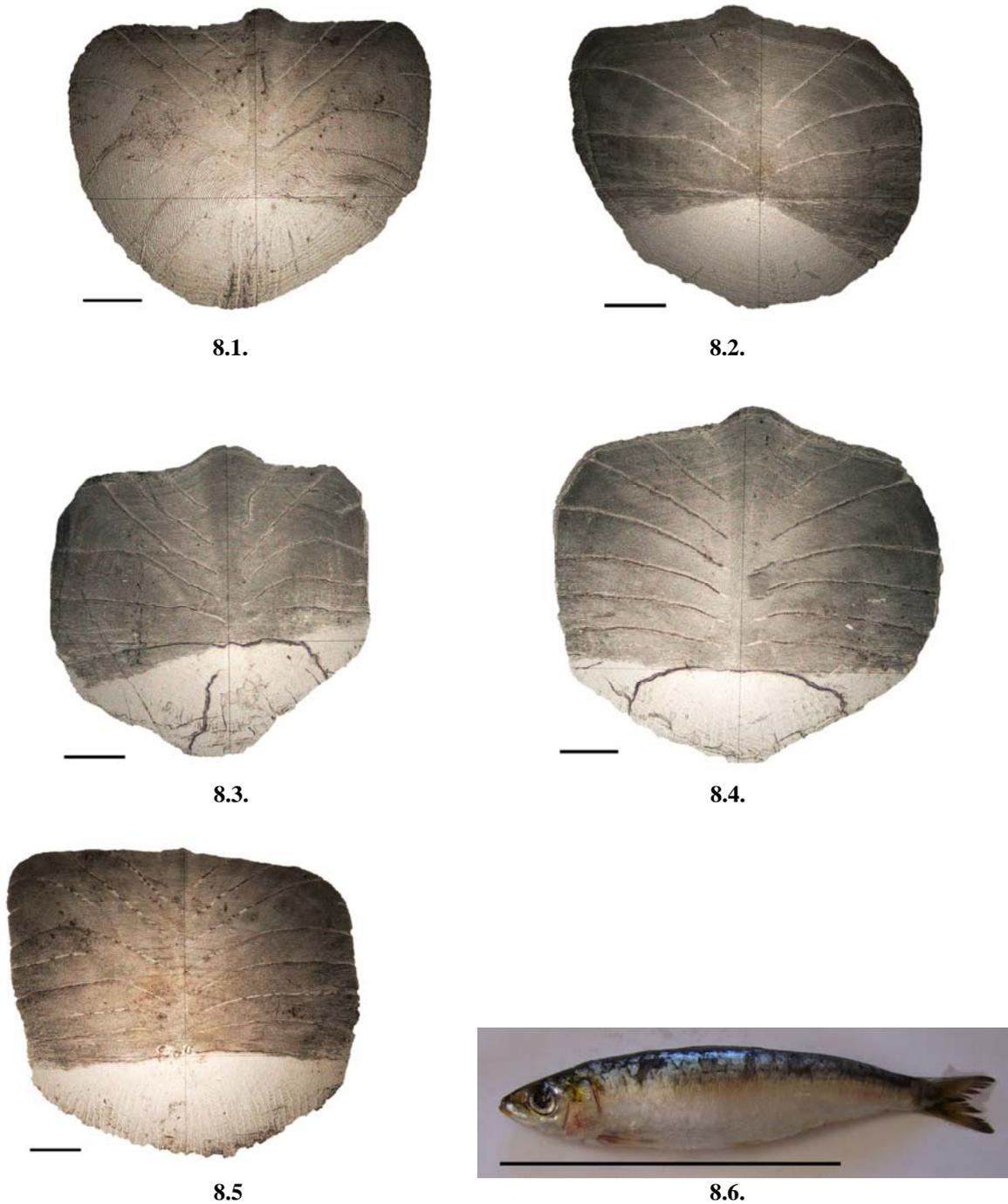


**Figure 7.1. - 7.5.** Scales of *Pagrus pagrus*; scale bar =1.0 mm.

7.1. Scale derived from zone A (dorsal, anterior, above the lateral line); 7.2. Scale derived from zone B (ventral, below lateral line); 7.3. Scale derived from zone C (caudal); 7.4. Scale derived from zone D (dorsal, posterior, above lateral line); 7.5. Scale derived from zone E (caudal to operculum);

**Figure 7.6.** The species – Common seabream (*Pagrus pagrus*); scale bar = 10.0 cm.

**VIII. Species:** *Sardina pilchardus* (Walbaum, 1792) / European pilchard  
**Family:** Clupeidae  
**Genus:** Clupeiformes

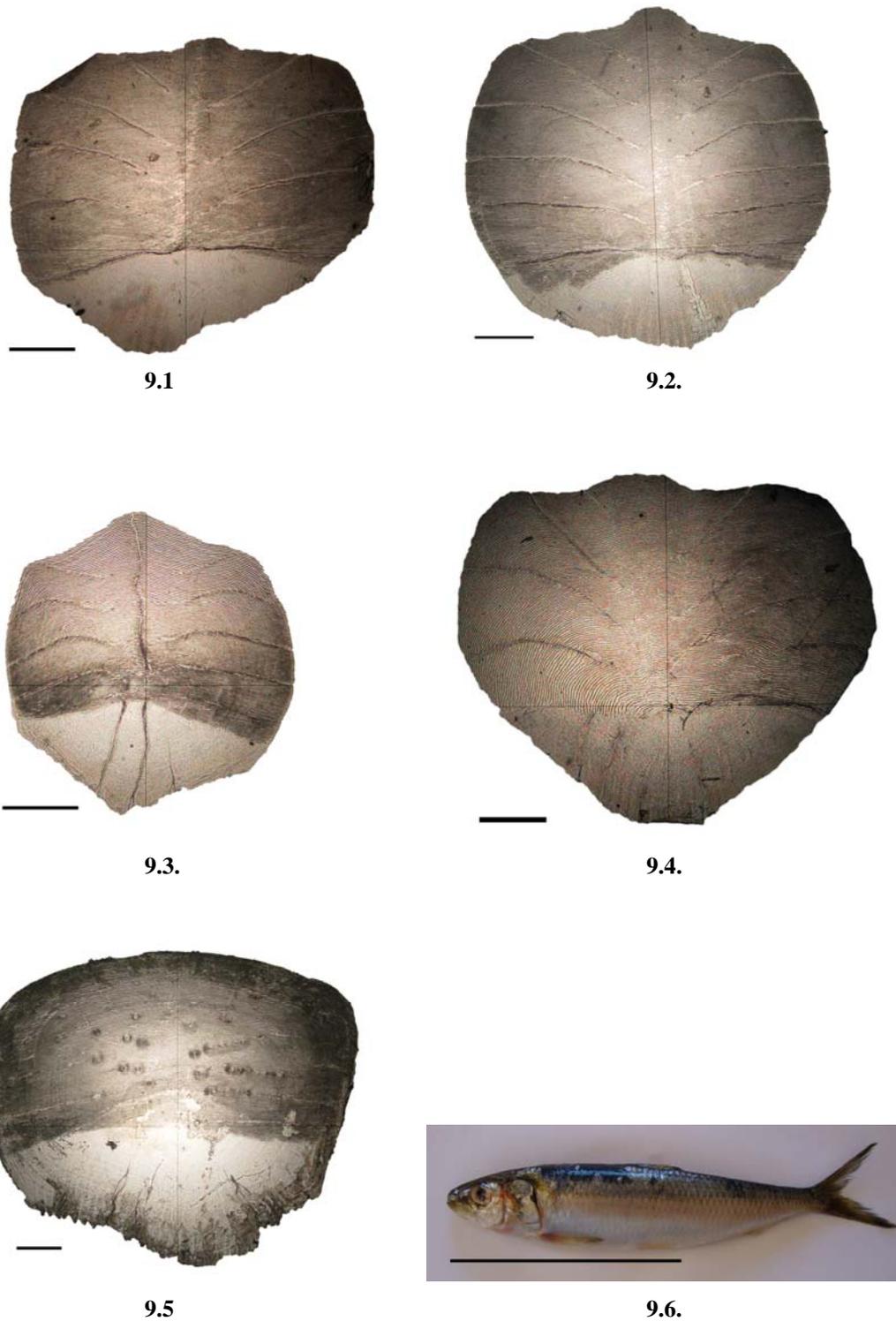


**Figure 8.1. - 8.5.** Scales of *Sardina pilchardus*; scale bar =1.0 mm.

8.1. Scale derived from zone A (dorsal, anterior, above the lateral line); 8.2. Scale derived from zone B (ventral, below lateral line); 8.3. Scale derived from zone C (caudal); 8.4. Scale derived from zone D (dorsal, posterior, above lateral line); 8.5. Scale derived from zone E (caudal to operculum);

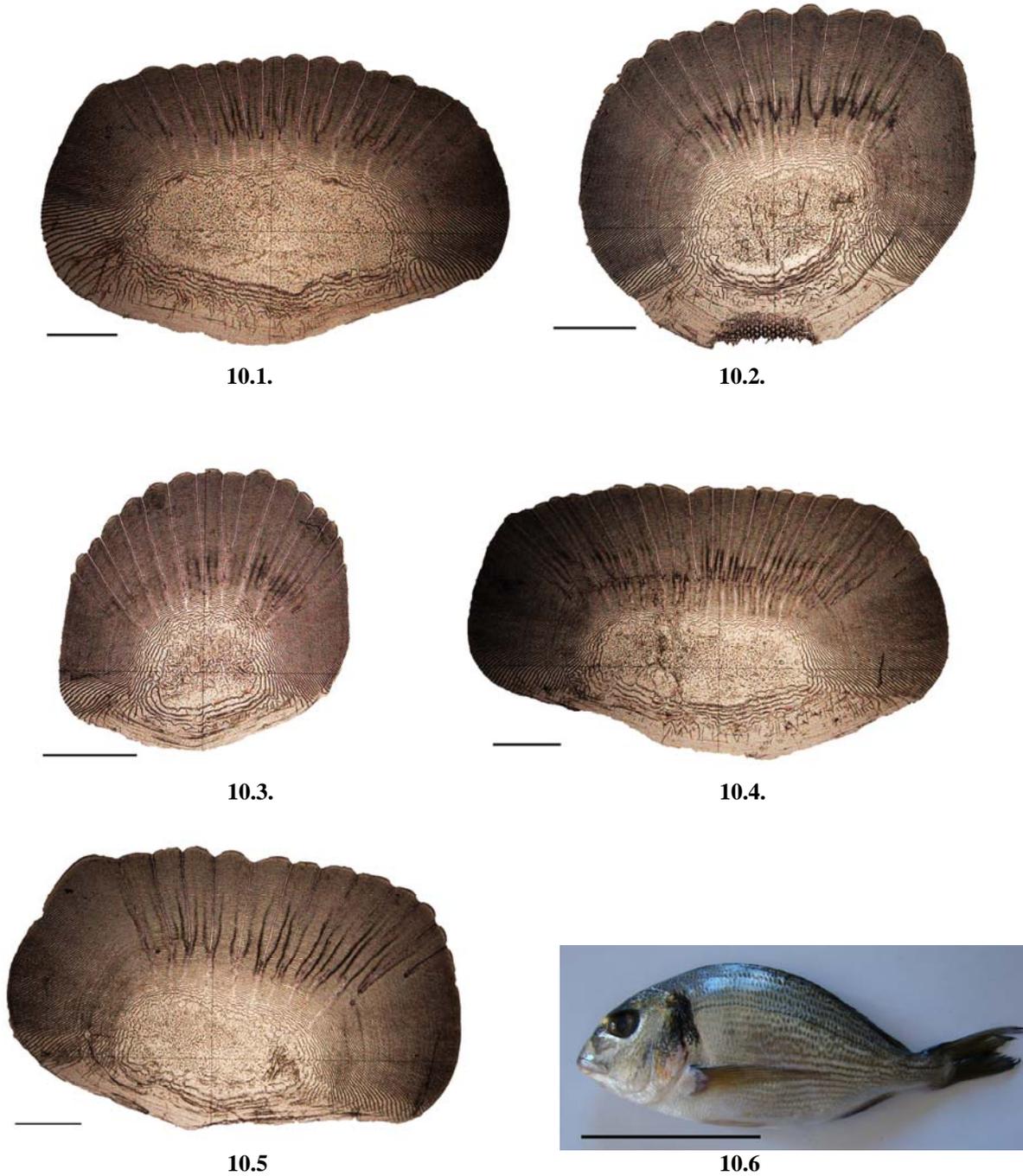
**Figure 8.6.** The species – European pilchard (*Sardina pilchardus*); scale bar = 10.0 cm.

**IX. Species:** *Sardinella aurita* (Valenciennes, 1847) / Round sardinella  
**Family:** Clupeidae  
**Genus:** Clupeiformes



**Figure 9.1. - 9.5.** Scales of *Sardinella aurita*; scale bar =1.0 mm.  
9.1. Scale derived from zone A (dorsal, anterior, above the lateral line); 9.2. Scale derived from zone B (ventral, below lateral line); 9.3. Scale derived from zone C (caudal); 9.4. Scale derived from zone D (dorsal, posterior, above lateral line); 9.5. Scale derived from zone E (caudal to operculum);  
**Figure 9.6.** The species – Round sardinella (*Sardinella aurita*); scale bar = 10.0 cm.

**X. Species:** *Sparus aurata* (Linnaeus, 1758) / Gilthead seabream  
**Family:** Sparidae  
**Genus:** Perciformes

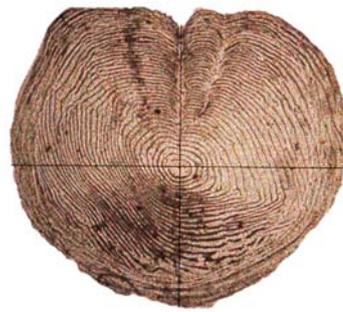


**Figure 10.1. - 10.5.** Scales of *Sparus aurata*; scale bar =1.0 mm.  
10.1. Scale derived from zone A (dorsal, anterior, above the lateral line); 10.2. Scale derived from zone B (ventral, below lateral line); 10.3. Scale derived from zone C (caudal); 10.4. Scale derived from zone D (dorsal, posterior, above lateral line); 10.5. Scale derived from zone E (caudal to operculum);  
**Figure 10.6.** The species – Gilthead seabream (*Sparus aurata*); scale bar = 10.0 cm.

**XI. Species:** *Trachurus trachurus* (Linnaeus, 1758) / European horse mackerel  
**Family:** Carangidae  
**Genus:** Perciformes



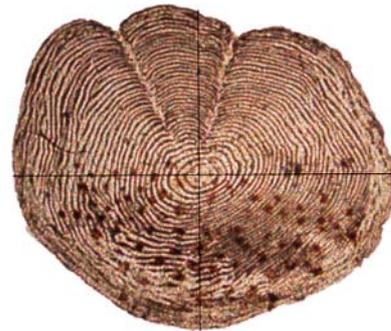
11.1.



11.2.



11.3.



11.4.



11.5.



11.6.

**Figure 11.1. - 11.5.** Scales of *Trachurus trachurus*; scale bar =1.0 mm.

11.1. Scale derived from zone A (dorsal, anterior, above the lateral line); 11.2. Scale derived from zone B (ventral, below lateral line); 11.3. Scale derived from zone C (caudal); 11.4. Scale derived from zone D (dorsal, posterior, above lateral line); 11.5. Scale derived from zone E (caudal to operculum);

**Figure 11.6.** The species – European horse mackerel (*Trachurus trachurus*); scale bar = 10.0 cm.