Stock discrimination of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: integrating the results from different stock identification approaches.

P. Abaunza, A. Murta, S. Mattiucci, R. Cimmaruta, G. Nascetti, A. Magoulas, A. Sanjuan, S. Comesaña, K. MacKenzie, J. Molloy, A.T. Santos, S. Iversen, G. Dahle, L. Gordo, C. Stransky, C. Zimmermman, M.T. Santamaria, P. Ramos, R. Quinta, A.L. Pinto, A. Ruggi, N. Campbell.

The goal of horse mackerel stock discrimination was tackled by integrating both established and innovative approaches such as genetic markers (allozymes, mtDNA, microsatellite DNA and SSCP), morphometry and parasites, tagging experiments and life history traits (growth, reproduction and distribution), within the EU-funded HOMSIR project. The sampling covered almost the whole distribution range of horse mackerel through 20 sampling localities. 200 fish per sampling site were collected taking in consideration the spawning season and temporal variation (two years of sampling). The results from genetic approaches (Multilocus Allozyme Electrophoresis, mtDNA and microsatellite DNA) showed that there was only slight genetic differentiation among sampling sites. Another genetic technique (SSCP), was successful in finding a genetic marker that demonstrated substructuring in horse mackerel populations. Several approaches (SSCP, morphometrics, parasites) support the separation between the Atlantic Ocean and the Mediterranean Sea in horse mackerel populations, although the most western Mediterranean area could also be connected with the Atlantic populations. In the Northeast Atlantic, various stocks can be distinguished: The west Atlantic coast of the Iberian Peninsula (southern stock); the west coasts of European countries from northern Spain to Norway (western stock) and the North Sea stock. This information implies the revision of the boundaries of the so-called southern and western stocks as currently defined. Various results suggested that adult horse mackerel could migrate through different areas/or stocks in the Northeast Atlantic. Horse mackerel from the Mauritanian coast is distinguished by its high production parameters (growth and batch fecundity). The population of horse mackerel in the Mediterranean Sea is sub-structured roughly in at least three main areas: western, central and eastern Mediterranean. In this contribution, we try to integrate the fundamental findings of differente approaches showing that the holistic approach is the appropriate way to identify stocks in horse mackerel.

Keywords: Stock identity, *Trachurus trachurus*, genetic markers, biological tags, biological parameters.

P. Abaunza: Instituto Español de Oceanografía, P.O. Box 240, 39080 Santander, Spain. [tel: +34 942291060, fax: + 34 942275072, e-mail: pablo.abaunza@st.ieo.es]. A. Murta, A.T. Santos, R. Quinta, P. Ramos, and A.L. Pinto: Instituto de Investigaçao das Pescas e do Mar, Av. Brasilia, 1400 Lisboa, Portugal. [tel: +351 21 3027120, fax: +351 21 3015948, e-mails: amurta@ipimar.pt, anateia@ipimar.pt, rquinta@ipimar.pt, pramos@ipimar.pt]. S. Mattiucci. Institute of Parasitology of the University of Rome "La Sapienza". Piazzale Aldo Moro nº 5, 00185 Rome, Italy.[tel: +39 0649914894, fax: +39 0649914644, e-mail: smattiucci@uniroma1.it]. R. Cimmaruta, G. Nascetti and A. Ruggi: Department of Environmental Sciences of the Tuscia University. Via S. Camillo de Lellis, I-01100 Viterbo, Italy [e-mails: roberta.cimmaruta@pescevolante.it, nascetti@unitus.it, alessandra.ruggi@virgilio.it]. A. Magoulas. Hellenic Centre for Marine Research, Institute of Marine Biology and Genetics, P.O. Box: 2214, GR 710 013, Iraklio, Greece [tel: +30 2810337853, fax: +30 2810337820, e-mail: magoulas@imbc.gr]. A. Sanjuan and S. Comesaña. Facultade de Ciencias do Mar e Bioloxía, Universidade de Vigo, Lagoas-Marcosende 36200 Vigo, Spain. [tel: +34 986812569, fax: +34 986812556, e-mails: asanjuan@uvigo.es, basti@uvigo.es], K. MacKenzie and N. Campbell. School of Biological Sciences (Zoology), The University of Aberdeen, Tillydrone Avenue, Aberdeen, AB24 2TZ, UK. [tel: +44 1224 314532, fax: +44 1224 272396, e-mails: k.mackenzie@abdn.ac.uk, neil.campbell@abdn.ac.uk]. J. Molloy, The Marine Institute, Parkmore Industrial Estate, Galway, Ireland [e-mail: john.molloy@marine.com]. S. Iversen and G. Dahle. Institute of Marine Research, P.O. Box: 1870 Nordnes, N-5817 Bergen, Norway. [tel: +47 55238407, fax: +47 55238687, e-mails: sveini@imr.no, geird@imr.no]. L. Gordo. Facultade de Ciencias de Lisboa (IO/DBA). Bloco C-2, Campo Grande, 1749-016 Lisboa, Portugal [tel: +351 21 75000000, fax: +351 217572141, e-mail: lsgordo@fc.ul.pt]. C. Zimmermann and C. Stransky. Bundesforschungsanstalt f. Fischerei. Institut fur Seefischerei, Palmaille 9, D-22767, Hamburg, Germany [tel: +49 4038905177, fax: +49 40 38905263, e-mails: czimmermann@ish.bfa-fisch.de, christoph.stransky@ish.bfa-fisch.de]. M.T. Santamaría. Instituto Español de Oceanografía, Carretera de San Andrés s/n, 38120, Santa Cruz de Tenerife, Spain [tel: +34 922 549439, fax: +34 922549554 e-mail: teresa.garcia@ca.ieo.es].

INTRODUCTION

The concept of stock is a slippery one and there is no universally applicable definition of the term "stock" (Carvalho and Hauser, 1994). In general, the various proposals answered to the predilections of the different collectives involved in the study and exploitation of living marine resources, from evolutionary and/or population genetics research to social and economic impacts of harvesting. It is therefore difficult to find a general definition and it should be recommended to include in each case the definition adopted. The term stock usually can be considered under two points of view: the genetic approach and the operational approach. (Tyler & Gallucci, 1980; Carvalho & Hauser, 1994). Genetic stock is defined as a reproductively isolated unit, which is genetically different from other stocks,

thus the degree of integrity is very high (Carvalho and Hauser, 1994). In the operational approach, stocks are defined as locally accessible fish resources in which fishing pressure has no effect on the abundance of fish in another contiguous resource and the values of productive parameters (growth, fecundity, mortality) are homogeneous (Gauldie, 1988, 1991). In essence, the stock concept describes the characteristics of the units assumed homogeneous for a particular management purpose (Begg and Waldman, 1999). Fish stocks are identified on the basis of differences in characteristics between stocks. Investigation of any single characteristic will not necessarily reveal stock differences even when "true" stock differences exist (Type I error). To overcome this difficulty, a holistic approach of fish stock identification, involving a broad spectrum of techniques, appears to be pertinent (Begg & Waldman, 1999). In the last few decades, the stock separation has been largely neglected partly because the techniques available were crude (Gulland, 1989). Nowadays, the thechniques for stock delineation are improving and applied elshewere (i.e. Pawson and Jennings, 1996; Begg and Waldman, 1999, and references therein). To assess the fishing pattern and the state of the living marine resource a comprehensive knowledge of the stock structure, thus the object to be modelled using the stock assessment methods, is required (Gulland, 1971; Pitcher and Hart, 1982; Hilborn and Walters, 1992).

The catches of *Trachurus trachurus* (Linnaeus, 1758) in the study area raised up over 500,000 t in the middle of 90's decreasing to around 250 000 tonnes in the period 2000-02 (FAO, 2004). These catches therefore represent an important source of income for local economies. There is a stock characteristic in Carangidae that make them very suitable for developing sustainable fisheries, in contrast with the clupeids. This is their natural pattern of variation characterized by the "steady situation", that means without significant variation in catches year after year (Caddy and Gulland, 1983; Bas, 1995). An explanation could be that their relatively long life span and high fecundity, increase the probability of the presence of various abundant cohorts in the population. However, this property could change if the pattern of fishing changes as can be observed in the historical development of the so called "western stock" in the Northeast Atlantic horse mackerel (ICES, 2003).

Until now, in the ICES area the stocks were defined mainly according to the eggs distribution, resulting in: the "western stock" (northeast continental shelf of Europe, from France to Norway); "North Sea stock" (North Sea area) and the "southern stock" (Atlantic waters of the Iberian Peninsula) (Figure 1). However, there is no any recognizable boundary in the distribution of eggs between the so called western and southern horse mackerel stocks (ICES, 1999a). Special attention has been focused on this current stock definition, recognizing the uncertainties in the distribution limits and the lack of biological

information to support such stock units (ICES, 1992, 1999b). The papers about the horse mackerel stock structure in the ICES area are very few and they cover only a small part of the stock distribution, or the information is so scarce that it is no possible to conclude the delineation of subpopulations. Using allozymes, Nefedov *et al*., 1978 analysed muscle esterease allotypes and found differences between the North Sea and the western of the British Isles, although their results seem to be rather tenuous (see Borges et al., 1993 for the discussion of the document). However, Borges et al. (1993) using plasma transferrin phenotypes did not find any difference in samples collected through the Northeast Atlantic area. Abaunza *et al* (1995) analysed the anisakid infestation levels for their use as biological tags and found significant differences between the Cantabrian Sea and Galician waters, two areas that are considered to belong to the southern stock. MacKenzie (2002) using the parasitological data from the EU funded project HOMSIR showed that there were significant differences in the mean abundances of *Anisakis sp*. and *Hysterothylacium aduncum* between the North Sea and west of the British Isles. The analysis of morphological variation was applied by Murta (2000) who found some similarities between the Portuguese and the North African Atlantic coasts, which cast doubts on the current boundaries of southern stock. In the Mediterranean area the information on stock structrure is very scarce (Lleonart and Maynou, 2003), although general fishery statistics already exist (Fiorentini et al., 1997). In the East-Central Atlantic, Maxim (1995) described the dynamics of the Saharo-Mauritanian stock (fig.1). The information on the basic biology of *T. trachurus* is much more abundant, and can be used in the anlaysis of life history traits: growth, reproduction and distribution, for the characterization of management units. Abaunza et al. (2003a) did a comprehensive review of horse mackerel growth and reproduction in which can be found references to most of the published work on the subject. Useful information on horse mackerel fishery distribution and migratory patterns can be found in Pawson, 1995, Iversen et al. (2002) and ICES (2003) for horse mackerel in the Northeast Atlantic and in Maxim (1995) for Northwestern African coasts.

To know the horse mackerel stock structure along its distribution area and to fill in the lack of knowledge on this subject, a project to the European Union was proposed in 1999. The EU-funded HOMSIR project (**A multidisciplinary approach using genetic markers and biological tags in horse mackerel (*Trachurus trachurus*) stock structure analysis**), was designed taking in mind the holistic approach philosophy, since it is the best way to obtain the information requirements for stock identification (Ihseen, 1981; Begg and Waldman, 1999). The project was conducted until June 2003 and its main results are briefly presented

here. The findings from the different methodologies are shown in an integrated way rather than to describe the results from each technique separately.

MATERIAL AND METHODS

The sampling of horse mackerel was conducted almost along its entire distribution area, in the Atlantic Ocean and Mediterranean Sea. To carry out this task, 21 sampling sites (see figure 2) were selected from which at least a total of 200 specimens/per sampling site were caught (100, each year in two years of sampling). In addition six specific areas were identified in case of having to carry out additional sampling in the development of growth and reproductive studies (fig. 2). The sampling sites were selected according to a priori information on current stock units, distribution area and biology knowledge. In general, the European Atlantic waters and the European coasts in the Mediterranean Sea were sampled with more intensity due to the interest in improving the knowledge in areas where there are active programmes for managing horse mackerel fisheries. The sampling was conducted during the spawning season in accordance with the information available about the spawning time in each area. The sampling was not exent of difficulties. In some areas, it was impossible to get a whole set with all the fish in spawning activity. When there were difficulties in catching fish, the obtention of specimens in any time was favoured over the restriction of catching only during the spawning season. This was the case in some of the Mediterranean areas. Another case is the northern North Sea where adult horse mackerel appear following the feeding migration when spawning season is over. Another difficulty was to obtain samples in the southern North Sea due to the seasonality of the operating fishing fleets in that area.

From each specimen we collected the following basic biological information: total fish length, tatal and gutted fish weight, sex, "on sight" or macroscopic determination of sexual maturity and the otoliths were removed for ageing and shape analysis. In addition two muscle pieces were removed from each specimen for posterior genetic analysis: a) one piece to store frozen in Eppendorf cryo-test tubes for posterior allozyme analysis; b) the other piece to kept in 95% etanol in Eppendorf test tubes for posterior DNA extraction.

It is not included here a detailed description of the methods used within each of the techniques applied because it would be too long. Instead of that, we are going to enumerate the techniques with few outlines. The reader who is interested in getting a complete description, can look for it in Abaunza et al (2003b).

The same specimen was utilized for each of the techniques used in the project. The techniques were:

Genetics: We applied the following techniques: multilocus allozyme electrophoresis (MAE) using 25 loci, microsatellite DNA (msDNA) for five microsatellite loci, mitochondrial DNA sequencing (mtDNA) on control region, ND4 locus and ND2 locus, single-strand conformation polymorphism (SSCP) on nuclear DNA (four pairs of primers).

Parasites: A complete authhopsy was applied to each speciment to get the parasitefauna, (metazoan and protozoan parasites) from each sampled fish. In the case of anisakids it was necessary to apply molecular techniques (i.e. MAE analysis) to identify species.

Morphometry: Horse mackerel specimens from each location were analysed to find body and otolith shape differences among areas or samples. Each fish in a sample was photographed and film pictures were digitised and the cartesian coordinates of the 11 landmarks selected were taken using and image analysis system. In otholith shape analyis only samples from the left body side were included to ensure that one sample represents one fish and only otoliths from fish measuring 20-35 cm length were selected to reduce area-length interactions. Otolith outlines were digitised using an image analysis system.

Tagging: It was explored the possibility of using artificial tags for migratory studies. Unfortunately, the observed mortality in tagged fish was so high, that the application of the method at larger scale was discarded. New methods for catching and handling fish without damage should be explored, since they were identified as the critical processes in the survivorship of tagged fish.

Life history traits: Growth, Reproduction and Distribution. All the specimens sampled were aged following the standard criteria stablished in the ICES area. The search for growth differences among areas we analysed the length distributions at age by non-parametric methods. The ovaries were removed and prepared for histological analysis to assign microcopically the maturity stage for the estimation of the maturity ogive. In addition, in some areas the batch fecundity was also estimated.

Finally all the data were integrated to assess the status and the structure of horse mackerel stocks. For a comprehensive squeme of the project sampling design see figure 3.

RESULTS AND DISCUSSION

In general, horse mackerel shows low levels of genetic differentiation
The results from Multilocus Allozyme Electrophoresis, mt DNA sequencing (on control region, ND2 locus and ND4 locus) and microsatellite DNA show that there is very poor genetic differentiation among sampling sites. Thus, the gene flow along the entire area of distribution is an important evolutionary force for horse mackerel species. This finding is quite common among marine fishes (Ward et al., 1994). However, sample homogeneity does not necessarily equate population homogeneity. For example, gene flow rates of 1% will give genetic homogeneity among samples and thus cannot be distinguised (Ward, 2000). In addition, it is also possible that the markers selected are not the appropriate ones to detect subdivisions in this species. Each genetic technique has also its own especific discriminatory power depending of the research objetive. For example, DeWoode and Avise (2000) reviewing several fish species found that within-population microsatellite variation was higher than that recorded in earlier allozyme surveys. In spite of the difficulties in determining the stock structure of marine fish by genetic techniques (due to the presence of few barriers to migrations between populations and the small amount of gene flow needed to maintain genetic homogeneity) one can find a genetic marker, which show population differentiation.

The Single Strand Conformation Polymorphism (SSCP) technique on nuclear DNA, was succesful in finding such a genetic marker.
It showed that there were significant differences between the horse mackerel from the Atlantic Ocean and the Mediterranean Sea and some substructuring within the two areas (figure 4).

Horse mackerel showed high genetic variability and a stable genetic structure over time.
The levels of genetic variability (polimorphism and heterozigosity) found by allozyme analysis, microstellite DNA and mitochondrial DNA control region sequencing was quite high. Even, it constituted a problem for data analysis (in mtDNA on control region and microsatellite DNA) since the available methods did not fit well for cases with such high variability. Genetic variability is necessary for adaptive evolutionary change (Carvalho, 1993) and could be used as an indicator of the state of exploited fish populations (Smith et al., 1991). The overall indication is that the biomass depletion suffered by the horse

mackerel did not impoverish its genetic resources. The relative lower variability levels found in the Norhteast Atlantic, could indicate an area of concern to avoid overexploitation.

A major separation of horse mackerel populations between the Atlantic Ocean and the Mediterranean Sea exists.

Several techniques agree in delineateing this major division among horse mackerel sampling sites. The SSCP genetic technique gave a very clear result (based on the polimorphic patterns of Primer D2, figure 4) supporting this differentiation. Parasites also reveal the existence of particular parasite-faunas in both areas, such as the different anisakid composition (figure 5) and the presence of the digenean *Bathycreadium elongatum*, the copepod *Lernanthropus trachuri*, the monogenean *Paradiplectanotrema trachuri* and the myxosporean *Alatospora solomoni,* only in the Mediterranean samples. Body morphometrics (figure 6) and otolith shape analysis (figures 7 and 8) were shown as powerful methods in discriminate both areas as a whole: the Atlantic sites from the Mediterranean ones. In general, the first length-at-maturity is lower in the Mediterranean sites than in the Atlantic areas.

Although the horse mackerel from the Alboran Sea (the most western Mediterranean Sea) and from the Atlantic Ocean (near to Strait of Gibraltar) could be partially connected.

Several findings support this hypothesis. A monogenean species typically from the Atlantic Ocean is also found in horse mackerels from Alboran Sea. The significant presence of the nematode parasite *Anisakis simplex sensu stricto* (typically Atlantic species) in the Alboran Sea and the presence of the *Anisakis pegreffii* (typically Mediterranean species), in Iberian and African coasts, is also indicative for a possibility of mixing (figure 5). Body morphometrics and otolith shape analysis showed that Alboran Sea is an special area, almost and outlier, that not fit well with the rest of the Mediterranean areas and, indeed, it is closer to the proximate Atlantic areas than to the Mediterranean areas (figures 6, 7 and 8). The wide range observed in the length-at-age distributions of the Alboran Sea (area number 17) could indicate the existence of a mixing area between populations or individuals, that show different growth rates (figure 9).

The Mediterranean and Atlantic Regions are long-recognised marine zoogeographic provinces, as identified by a more traditional type of zoogeographic evidence (Briggs, 1974). However, recently, a varying intraspecific genetic differentiation between Mediterranean and Atlantic populations have been reported for different marine organisms, mainly fishes, molluscs and crustaceans (Sanjuan et al. 1996, Borsa et al. 1997, Pérez-Losada et al. 2002, and references therein). These Atlantic-Mediterranean genetic

differences seems to be associated with the Strait of Gibraltar and the presence of a well defined hydrographic boundary of surface waters in the Alboran Sea between Almería (SE Spain) and Oran (Algeria), the Almería-Oran oceanographic front (Tintore *et al*, 1988).

Horse mackerel from the west Iberian Atlantic coast can be distinguished from the rest of the Atlantic areas.

SSCP results showed significant differences between the west Iberian Atlantic coast and the rest of the Atlantic areas (figure 4). The same result was obtained with the analysis of body morphometrics (figure 6) and partially with the otolith shape analysis (figures 7 and 8), identifying in the same cluster the areas located in the west coast of the Iberian Peninsula. The presence of *Anisakis simplex pegreffii* (a typical Mediterranean species) is decresing as latitude increase along the Atlantic Iberian coast (figure 5).

In the Atlantic Ocean, the northern boundary of the so called "southern stock" ought to be revised, and accordingly, the shouthern boundary of the so called "western stock".

The body morphometrics and the otolith shape analysis joined the northwest of the Iberian Peninsula (North Galicia) to the areas located more to the North in the Atlantic Ocean, Bay of Biscay and Celtic Sea (figures 6, 7 and 8). On the other hand, the genetic results from SSCP associates the northwest of Iberian Peninsula to the Portuguese sampling sites. These differences between the techniques suggest that North Galicia (area 07 in figure 2) may correspond to a transition area between two possible stock units. Therefore, it is proposed to move the actual boundary of the "Southern" and "Western" stocks from the Cape Breton Canyon (southeast of Bay of Biscay) to the northwest of Iberian Peninsula (Galician coasts) and specifically to the Cape Finisterre at 43º N latitude, which could be considered as a boundary for certain hydrographic features like the influence of North-Atlantic Central Water (Fraga et al., 1982).

The southern boundary of the so called "southern stock" is more uncertain.

The finding of parasites typically distributed in tropical areas such as the monogeneans *Heteraxinoides atlanticus* and *Cemocotyle trachuri* (Gaevskaya and Kovaleva, 1979) in Portuguese coasts suggest migrations of *T. trachurus* from West Africa into European waters. The similarities found in otolith shape analysis between the Portuguese coast and the very far southwards coast of Mauritania, open the question of the connexion between the Portuguese sampling sites and the Northwest African coasts as was also suggested by Murta (2000). Given that the only area sampled in the African coast (area 11) is very far southwards (coast of Mauritania), data from the Moroccan coast is needed to allow a

definitive delimitation of the southern boundary of this stock.

Parasites and body morphometrics indicated that horse mackerel in the North Sea could constitute a stock well differentiated from the rest of adjacent Atlantic areas.

This result is in accordance with the current definition of North Sea stock and Western stock in the Northeast Atlantic. The distinctive pattern of infection with the nematodes Anisakis spp. And Hysterothylacium aduncum, observed in samples from the North Sea (area 05) clearly distinguishes it from the nearest stations 01, 02, 03 and 06 located in the Northern North Sea, Celtic Sea and Bay of Biscay (figure 10). However, the three anomalous fish in sample 05 (North Sea) identified by infections more characteristic of fish from the putative "western stock" and the temporal distribution of commercial catches (ICES, 2003) also suggest a limited migration from the so called western stock into the North Sea stock, which could produce in the North Sea a mixed stock fisheries in some seasons.

Unfortunately, we have no enough information to look for possible migrations and/or mixing areas in southern North Sea and English Channel. In addition, the batch fecundity values reached by horse mackerel in the North Sea were much lower than those found in the adjacent Atlantic areas (Figure 11).

Horse mackerel in western european coasts, from the northwest of Spain to Norway, seems to be a unique stock.

This definition is very similar to the current so called "western stock", excepting that from the results of this project it is also included the north coast of the Iberian Peninsula. In other species such as hake (*Merluccius merluccius*) Lundy et al (1999) using microsatellite loci also joined the samples from the noth Iberian coast to those from the Celtic Sea.

Nor the SSCP results (figure 4) neither the parasite composition showed any contradiction with this definition. Anisakid species composition are homogeneus through this area. otolith shape analysis and body morphometrics include the sampling sites from this area in the same cluster showing a great similarity in their morphometric characters (figures. 6, 7 and 8).

However, the population structure in the western european coasts could be more complicate and more research is needed.

In the SSCP analysis the results showed a complicate picture in this area. Area 05 (North Sea), area 01 (Norwegian coast) and area 21 (South of Bay of Biscay) are in the same group. Parasites also indicate some temporal changes in parasite composition in the Bay of

Biscay and it is suggested to be an indicative of a mixing area. In body morphometrics area 03 (south of Ireland) is defined as outlier. It is dessirable in the future to get more information from the Cantabrian Sea (north of Spain), Bay of Biscay (central and northern areas) and English Channel to obtain a more clear picture of the horse mackerel population structure in the Northeast Atlantic.

Some of the results suggest the existence of migrations within the Atlantic Ocean.

The lack of genetic differentiation among sampling sites found by allozyme analysis, mtDNA and microsatellite DNA analysis could support this hipothesys. Parasite composition indicate that through the Northeast Atlantic some of the older and bigger specimens could migrate through the different areas, as it is indicated by the presence of tropical parasitic species (i.e. the monogenean *Cemocotyle trachuri*) in some specimens from the Norwegian waters. In the Northeast Atlantic, differences in lengths-at-age distributions between sampling areas are very clear (figure 9). These differences and those found in fecundity (figure 11) may be due to differential growth or reproduction rates between areas, or can be also originated by length-dependent migrations during the spawning or feeding seasons, in which larger fishes, or in better condition, tend to drift more than small fish (Dodson, 1997). Considering this last hypothesis, our data would suggest that the following migrations during the spawning season would take place: A) from the south of Ireland in the Celtic Sea to the North, along the west coast of Ireland, and to the Bay of Biscay; B) From the south of Portugal to the North of Portugal (figure 12). As in the case of the Alboran Sea (area 17) the great variability of growth data in area 03 (south of Ireland) and area 21 (south of Bay of Biscay) suggests that it may be a mixing area for different populations or for different migrating fish from the same stock (figure 9). The data of Batch fecundity is in accordance with the pattern of the growth findings, being the values in relation with latitude (figure 11). The analysis of international catches in the Northeast Atlantic, over the different years of the project, also suggests the existence of active migrations. During the feeding season (autumn-winter) fish seem to move from the Celtic Sea to Northern North Sea (ICES, 2003). This pattern also agree with the hypothesis of length-dependent migration since the results from growth analysis showed that the median of length at age distributions from Northern North Sea (area 01) is greater than in the other northeast Atlantic areas (figures 9 and 12).

Horse mackerel from the Mauritanian coasts is differentiated mainly by the high growth rates and high batch fecundity.

The highest median values of length at age distributions were reached in the Mauritanian coasts, area 11 (figure 9). The estimated batch fecundity was also very high (figure 11). The homogeneity in stock production parameters (such us growth and fecundity) is one of the factors that one stock should be met. From this point of view, and taking in consideration the large distance between the Mauritania and Iberian coasts, it seems coherent that this population could be assessed and managed apart from the other Atlantic areas located in the Iberian Peninsula (fig 12).

The population of Horse mackerel in the Mediterranea Sea is sub-structured in at least two or three main areas.

Almost all the approaches suggest this substructuring. SSCP distinguised two main groups in the Mediterranean: the Eastearn Mediterranean and the western Mediterranean (figure 4). This last group is closer to the Atlantic patterns. Parasitological analysis suggested the existence of three main groups which had characteristic parasitofauna: The western Mediterranean (Iberian coasts), the central Mediterranean (Italy-Sardinia) and the eastern Mediterranean (Greek waters). Body morphometrics results are also in accordance with the parasitological findings (figure 6). The differences in growth among areas (i.e.: high growth in area 13, Thyrrenian Sea, and 15, Ionean Sea) could indicate different growth rates or length-dependant migrations. The high growth variability in Ionean Sea could indicate an area of mixing populations or migrating fish with different growth rates (figures 9 and 12).

The Thyrrenian Sea, in the central Mediterranean, showed particular results

Interestingly, the genetic analysis of allozymes and microsatellite DNA found the Thyrrenian Sea as the more particular area. There are also some particularities in the parasitefauna from Thyrrenian Sea. Moreover, the highest mean lengths-at-age in the Mediterranean Sea were obtained in the Thyrrenian Sea.

The information about horse mackerel stock structure in the Mediterranean Sea will not be complete until new information on horse mackerel from Mediterranean African coasts be available.

Unfortunately, the Mediterranean coasts of Africa were not sampled. Although, initially these areas were contemplated, the logistic problems made impossible to carry out the sampling. The stock structure can not be assessed completely if the distrubution area is not cover completely. In the Mediterranean Sea significant areas like the Algerian coast, the Gulf of Sirte and the Israel-Lebanon shelf, should be sampled to get a complete picture of

the horse mackerel structure.

The holistic approach is the appropriate way to identify stocks in horse mackerel species
Several markers have proved to be succesful in delineate horse mackerel stocks. With the genetic technique SSCP on nuclear DNA we found an appropriate genetic marker. Several parasite species were very useful as biological tags (i.e. anisakid species) which allowed for example to identify clearly the North Sea population. Body morphometrics and otolith shape analysis were also successful in delineate stocks.
It is interesting to collect all the possible biological information, such us growth and reproduction, to consider the influence of these covariables in the rest of the approaches and to identify areas with homogeneus production parameters.

For a tagging programme, horse mackerel appear to be very sensitive to conditions and must be carefully handled immediately after tagging.
Although the results of tagging experiences within the project were extremely disappointing there are some important points to emerge. 1) it does not seem advisable to embark on a large scale tagging porgramme at the moment. 2) Further investigations are necessary on the best type of gear by which to catch horse mackerel. 3) Horse mackerel appear to be very sensitive to conditions and must be carefully handled immediately after tagging. Horse mackerel do not appear to be as tough or strong as reported and are similar to other fish in that they require to be handled very gently before and during tagging operations. It appears to be particularly important to be very gentle in order to prevent damage of the jaws. Fish appear very susceptible to infectiion and rquier clean water to survive. 4) Fish appear to retain tags satisfactorily and no fish appeared to die as a direct result of the tagging procedure.

The project has also provided other useful information regarding the biology of these species.
The knowledge of the horse mackerel parasitofauna has led to dicover probably various new species of parasites and new host and locality-area records for some of the parasites.
The project allowed the opportunity to make a review on growth and reproduction of horse mackerel, setting the basis for a better approach to these two areas of knowledgement. It has also demonstrated density dependent growth in horse mackerel. The data obtained from reproductive studies support the idea that horse mackerel is an indeterminate spawner, which has a direct impact in the use of the historical series of egg abundance for stock assessment purposes (ICES, 2003).

CONCLUDING REMARKS

Horse mackerel has a broad geographical range and for management purposes, it makes sense to divide its range into populations or stocks that are relatively independent of other stocks. The delineation of stocks is also useful ecologically because population dynamics may be understood in the context of local environmental change (Levington, 2001) and the basic questions of population regulation (population richness, geographical patterns, abundance of stocks, temporal fluctuations) can be undertaken (Sinclair, 1988).

The absence of significant genetic structure, as can be inferred from the results of allozymes analysis, mitochondrial DNA and microsatellite DNA, can not always be equated with true equilibrial panmixia. It may take a long time following subdivision for genetic markers to reveal genetic structure (Grosberg and Cunningham, 2001). The lack of obvious physical barriers to dispersal in the ocean led to consider that species with broadly dispersing larvae should exhibit little genetic structure across their range. In many marine fish, like large migratory fishes, this could be the case but we have to take in consideration that larval exchange rates may be overestimated. Cowen et al. (2000), using simple advection models, showed that larval retention near local populations exists and that may be of great importance in the maintenance of population structure and management of coastal marine resources.

Stock identification is a complex task, and as in any complex research, it can be attempted from different techniques and points of view that are complementary to each other. Despite of the diversity of approaches, all are based on a common principle: the signal from the among-stock variation must exceed the noise of whithin-stock variation (Waldman, 1999). The techniques of stock identification are costly and it is desirable to select the more useful approaches. In this sense, this project has allowed us to compare the different techniques and to select the more appropriate ones for future stock delineation studies in horse mackerel species.

The stock identity research can give the answer to two practical problems, related to each other in fishery management. One is just the identification of stocks and their boundaries and the other is the stock mixture analysis. In stock delineation, each technique is analyzed under its own methodology. The results obtained from all the techniques are then discussed, pointing up and explaining the similarities and the differences among the techniques to perceive the stock structure. On the other hand, in stock mixture analysis, the

researchers know which are the actual stocks (or at least some of them) and appropriate statistical tools can be applied to discriminate the stock composition of the catches in a certain area (Wood et al., 1989; Prager and Fabrizio, 1990; Waldman, et al., 1997; Pella and Masuda, 2001). It is interesting to say that Waldman et al. (1997) found that in mixed stock analysis, it is risky to compare estimates among approaches when they are divergent, and that it may be better to monitor stock composition with a single approach and to treat the results in relative terms.

The HOMSIR project was dealing with the first approach: the stock identity and the identification of their boundaries. The problem of mixed stock fisheries in horse mackerel has probably not the same relevance as in salmonids or other commercially important anadromous fishes. Anyway, HOMSIR results allow the possibility to develop further statistical procedures in mixed stock analysis. This will be explored in future publications (Fisheries Research special publication devoted to HOMSIR, that will be done in the next future).

According to Waldman (1999), HOMSIR results could be categorised into planned comparisons among divergent approaches (although if we limit the number of approaches, then one can also have the possibility of doing planned comparisons among similar approaches, i.e. among approaches in genetics). There are techniques that showed differences among areas meanwhile others did not. To analyze properly, one has to take into account the nature and the temporal scale of the techniques. For example, some of them have fundamentally an evolutionary meaning and then the time scale could be more important than the geographic one (this could be the case for the genetic techniques). However, when the differences are detected with whatever technique (using parasites as biological tags, morphometry, genetics, life history traits, etc.) those are real and one would have to explain why there are differences. Thus, only heterogeneity and not homogeneity can be demonstrated (Waldman, 1999).

We have considered that the correct approximation is first to get all the data and to look at them. In that sense, we have started with discussing and relating the different findings taking in consideration the time scale (microevolution-macroevolution), the geographical factors and the environmental information. Various multivariate statatistical tools were applied for the analysis of results within each approach (i.e., discriminant analysis, clusters, ANCOVA, dendograms…. etc). Simple correlation analysis could be taking in consideration when joining the data. The Kappa analysis (Waldman *et al*., 1997) could be useful to identify the sensibility of each of the technique to discriminate true stocks, although this technique is more suitable when there is a knowledge of the actual stock structure.

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TABLES

Table 1. HOMSIR sampling activity during 2000 and 2001. Specimens collected with an indication of the length and age ranges in each sampling area.

AREA	Nº OF SPECIMENS	LENGTH RANGE (cm)	AGE RANGE
01	201	20.8 – 40.0	3 – 23
02	382	25.4 – 41.0	5 – 29
03	351	18.5 – 38.7	2 – 19
04	0	-	-
05	218	18.5 – 39.0	2 – 25
06	198	16.1 – 28.2	2 – 14
07	262	25.6 – 33.0	3 – 19
08	259	15.2 – 33.0	1 – 19
09	161	19.0 – 27.7	1 – 7
10	212	16.2 – 31.5	1 – 7
11	188	20.0 – 39.5	2 – 19
12	200	14.4 – 22.9	1 – 3
13	193	25.0 – 41.3	3 – 18
14	197	09.6 – 32.0	1 – 19
15	219	11.5 – 43.1	1 – 19
16	167	13.9 – 37.7	1 – 25
17	225	06.1 – 39.5	0 – 16
18	100	17.1 – 37.8	4 – 19
19	199	17.5 – 41.0	2 – 20
20	195	08.5 – 29.8	0 – 5
21	292	19.6 – 40.2	2 – 21
TOTAL	**4419**	**06.1 – 43.1**	**0 - 29**

FIGURES

Figure 1. Horse mackerel (*Trachurus trachurus*): distribution of stocks as presently defined. Map source: GEBCO, 200 m depth contour drawn.
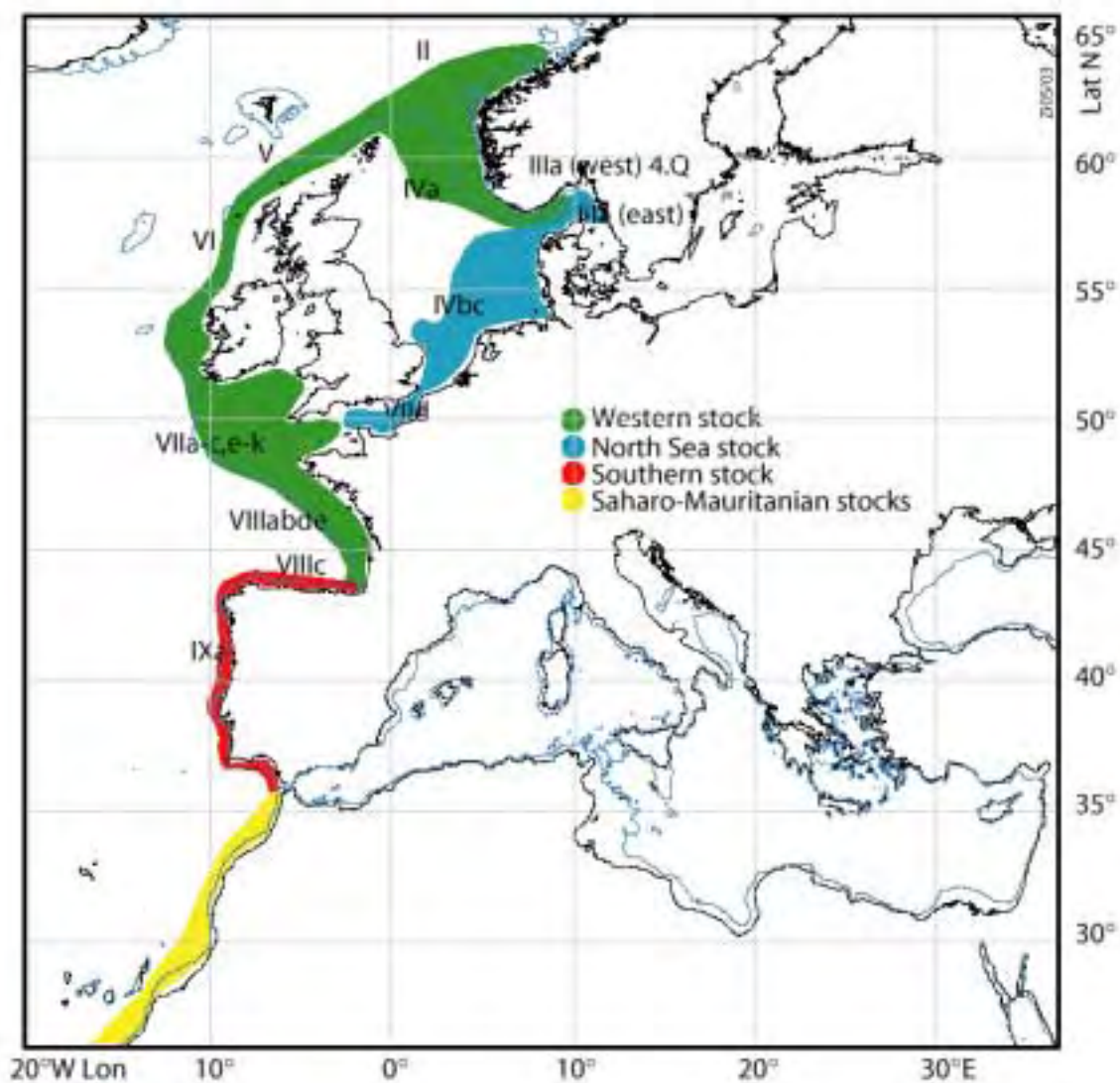
Figure 2. Realised sampling site positions for the EU-project HOMSIR in 2000 (circles) and 2001 (triangles). Map source: GEBCO, 200 m depth contour drawn.
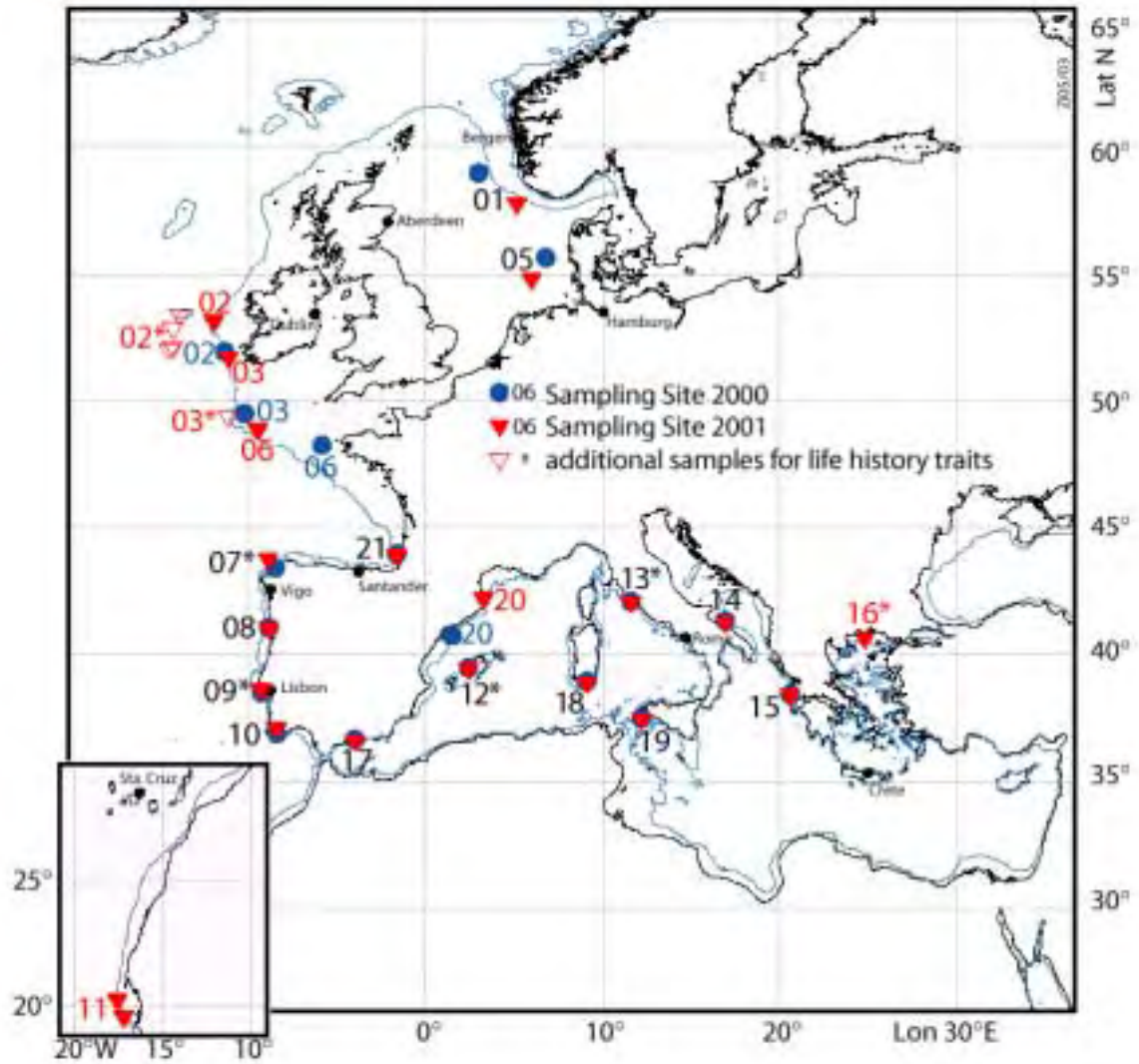
Figure 3. HOMSIR project: sampling details and flow of the material

Fig. 2: EU-Project HOMSIR - sampling scheme
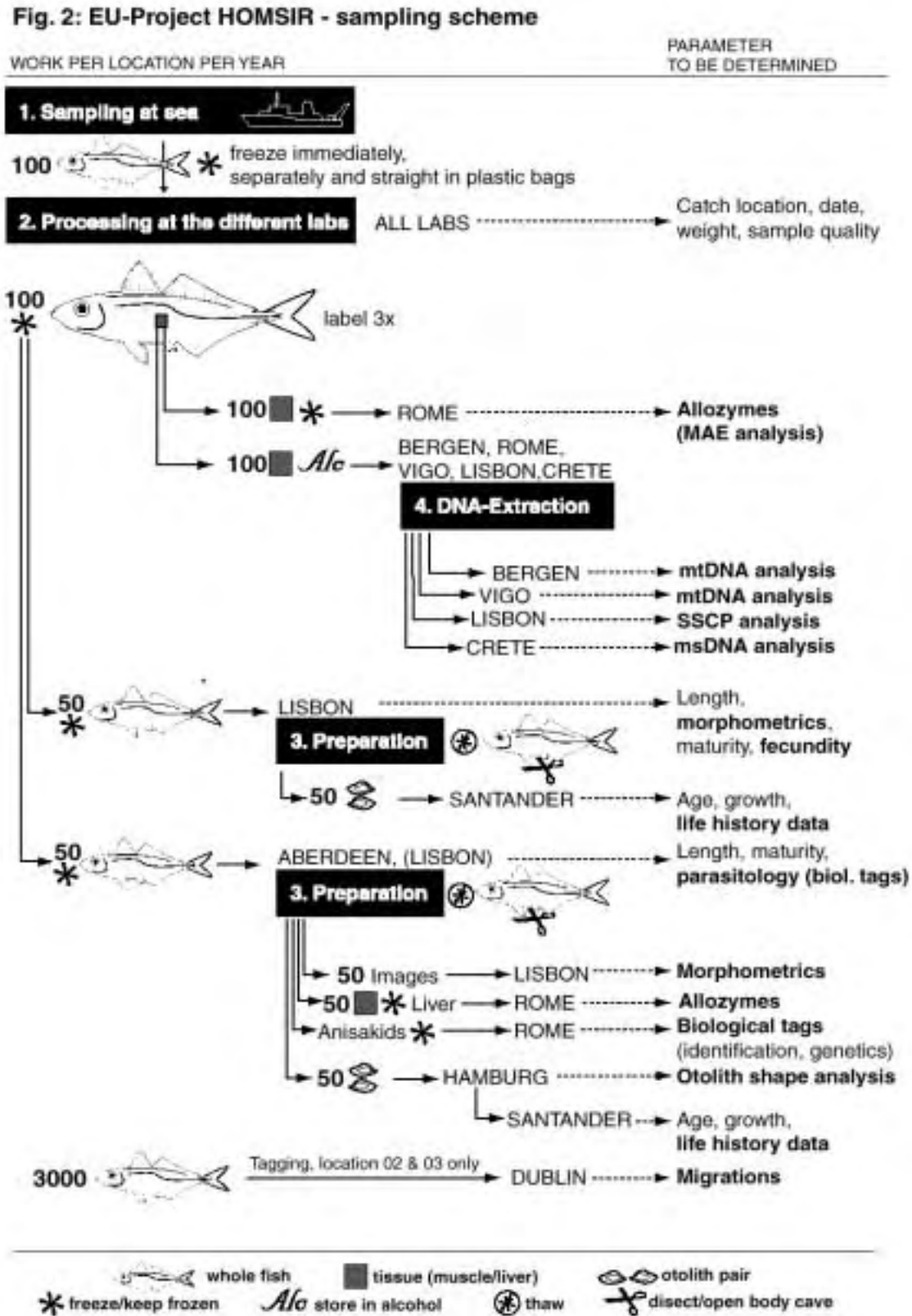
Figure 4. UPGMA dendogram constructed with Nei genetic distances from the samples captured in 2000 (upper figure) and 2001 (below).
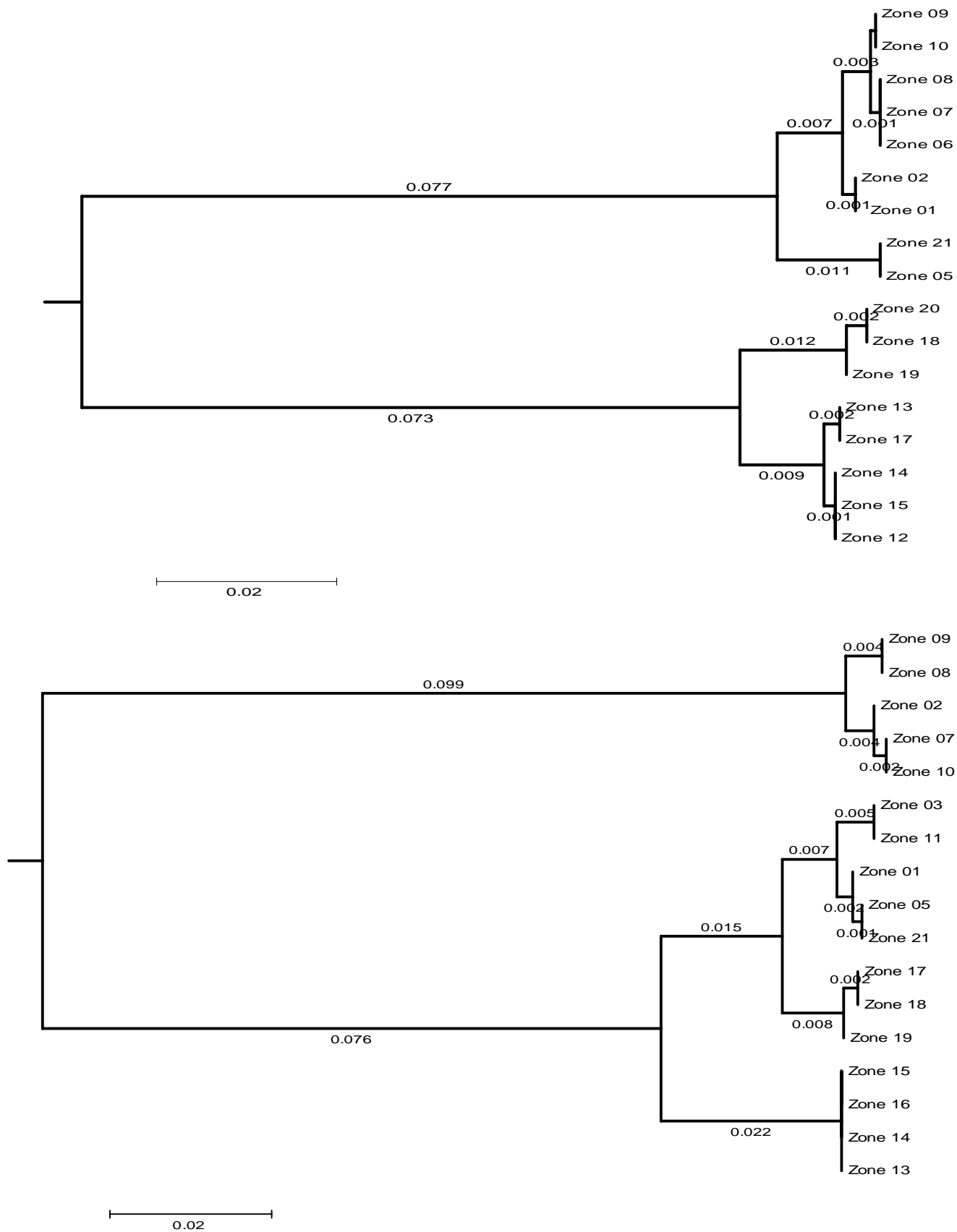
Figure 5. Relative proportions of Anisakis spp. in horse mackerel (*T. trachurus*) from different localities
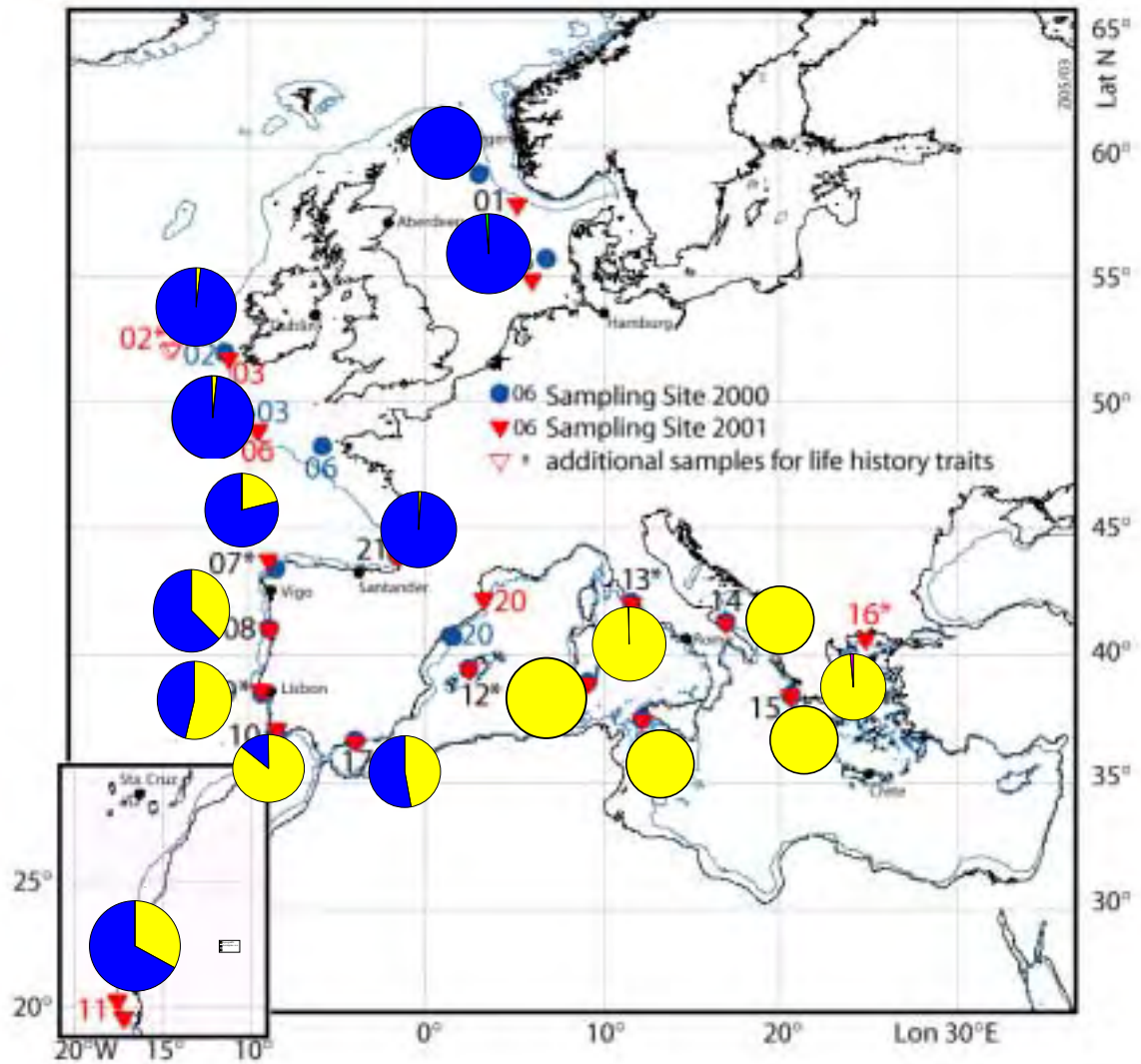
Figure 6. Horse mackerel (*T. trachurus*): dendogram of the distances between average configurations of each site in the Atlantic (upper) and in the Mediterranean (below).
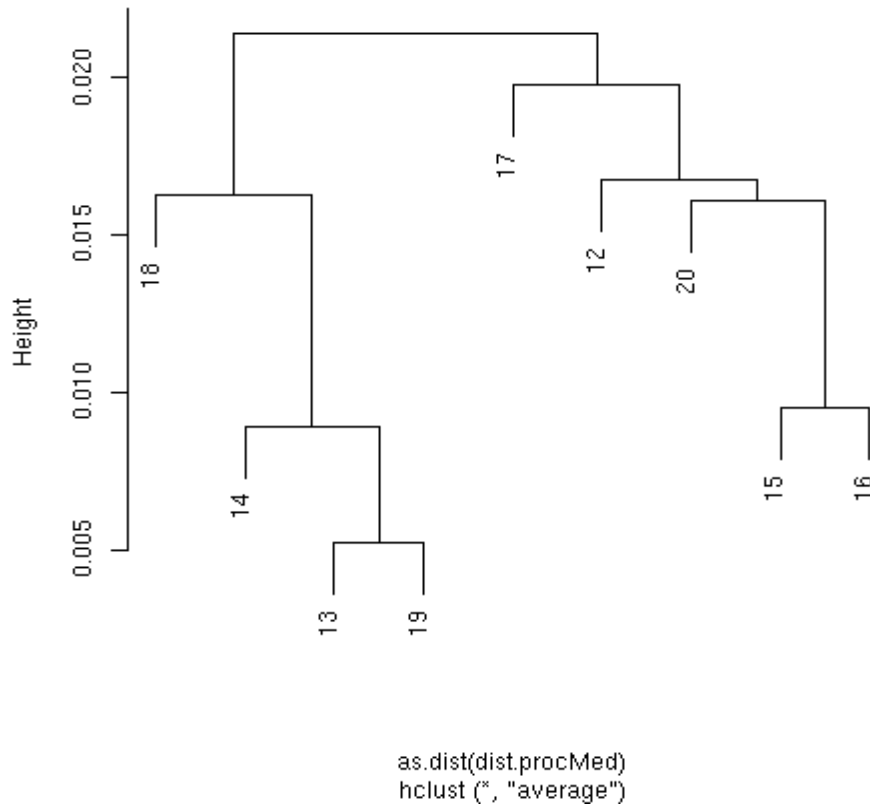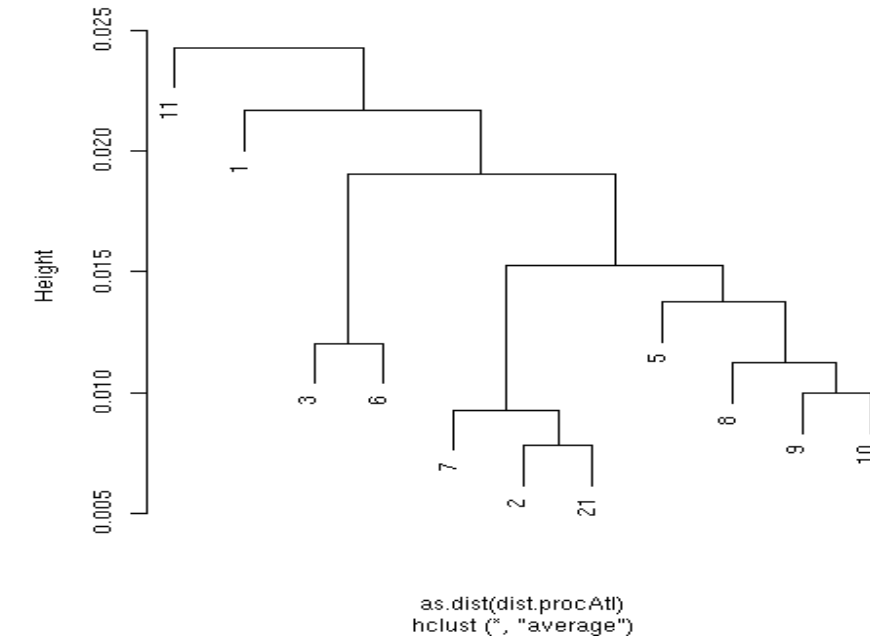
Figure 7. Hierarchical cluster dendrogram of the mean size-corrected Fourier descriptors in each sampling area, based on a Euclidean distances matrix, linked by unweighted pair-group average. Sampling years 2000 and 2001 were combined.
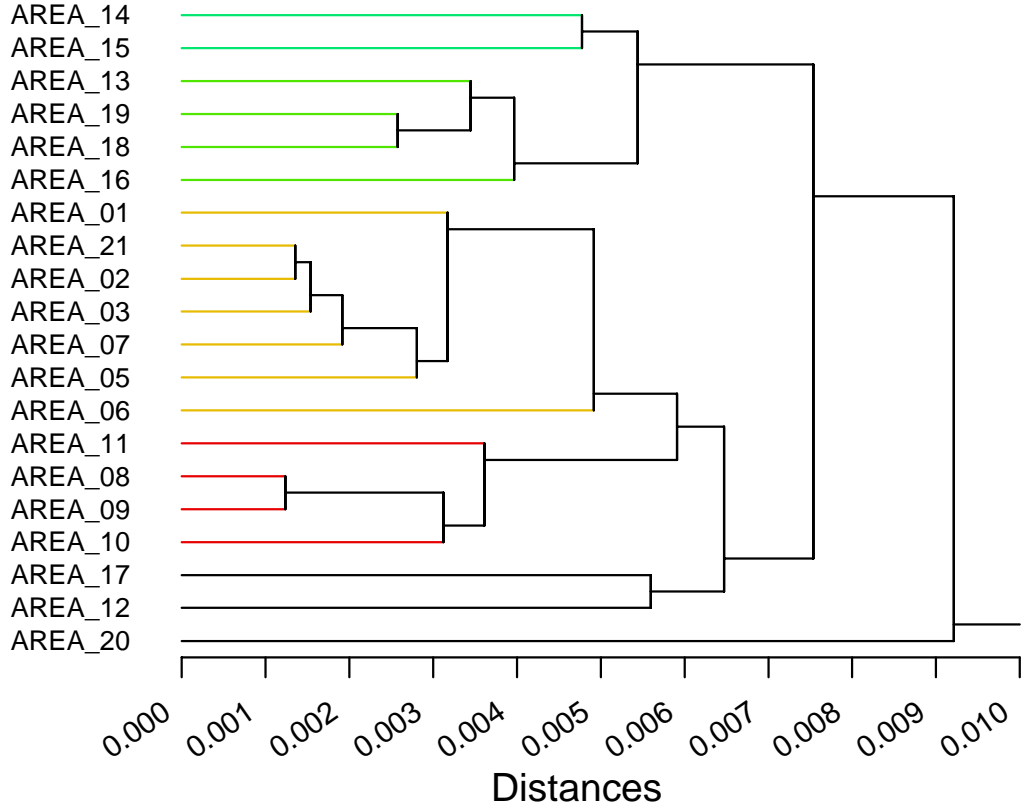
Figure 8. MDS ordination plot of the Euclidean distances between mean size-corrected Fourier descriptors in each sampling area (stress = 0.027). Sampling years 2000 and 2001 were combined.
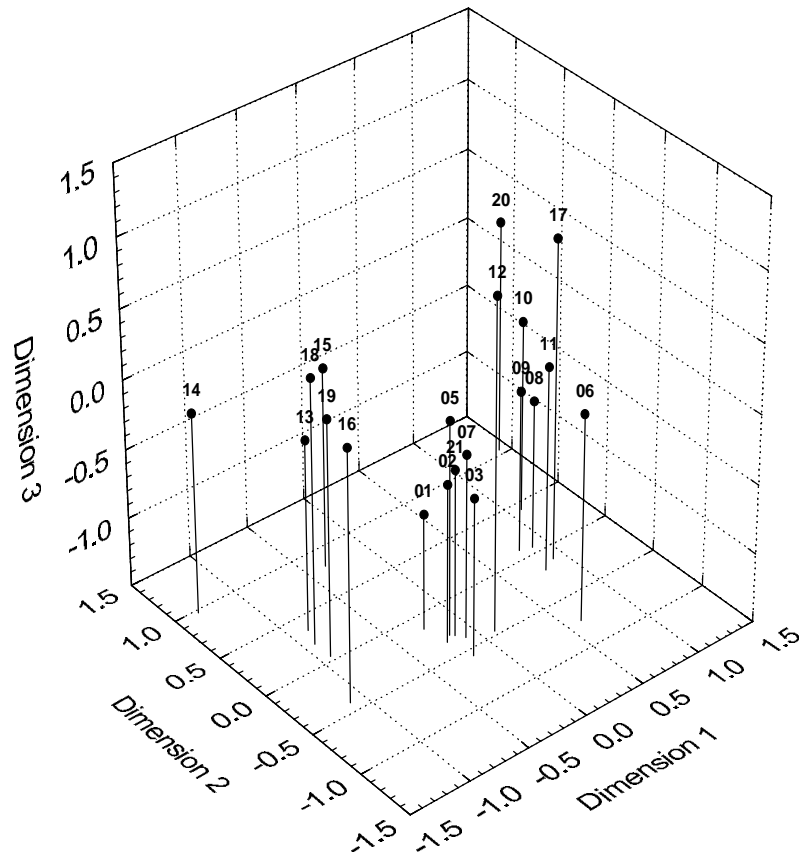
Figure 9. Boxplots of horse mackerel length distributions at age by area.
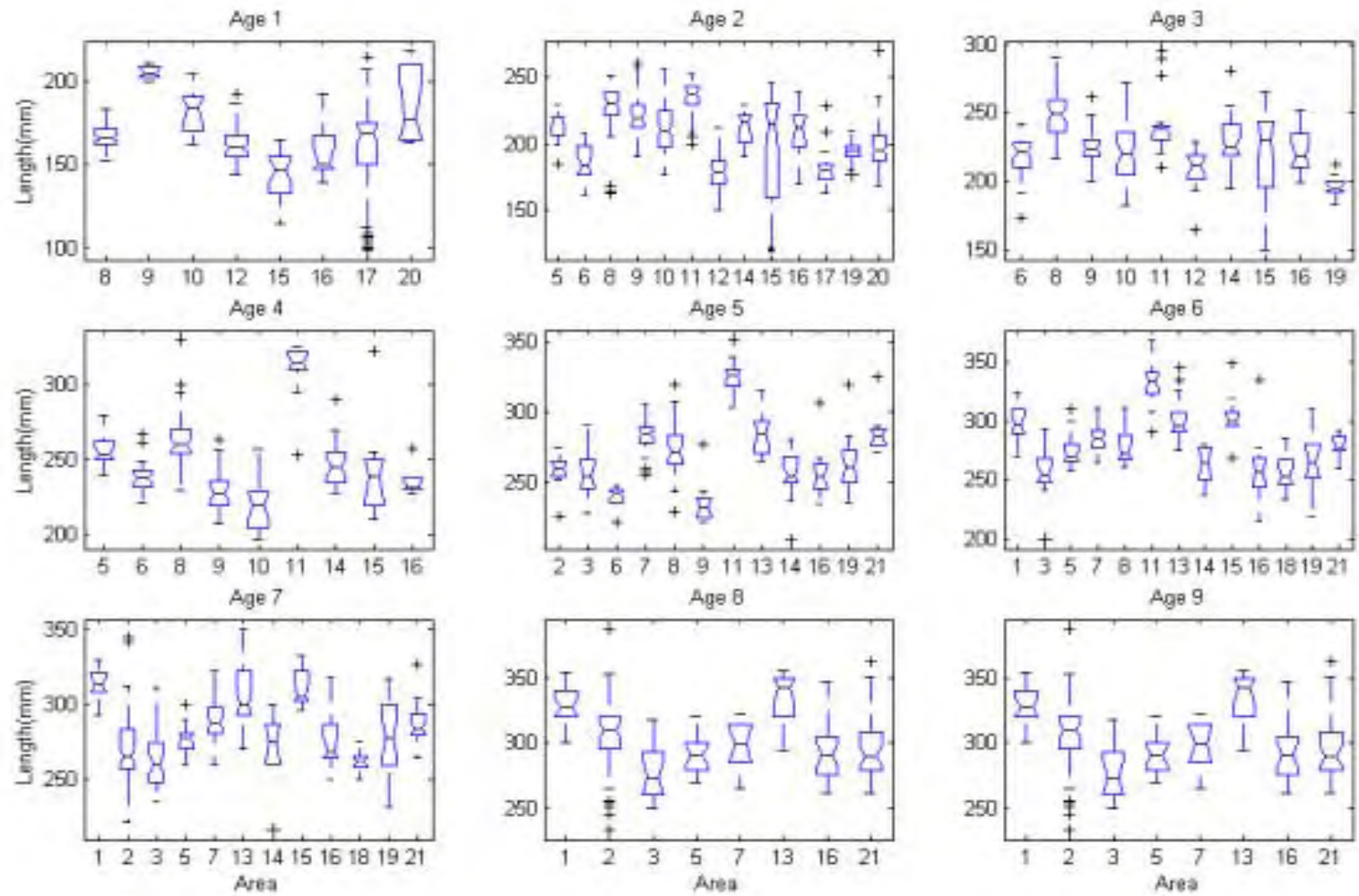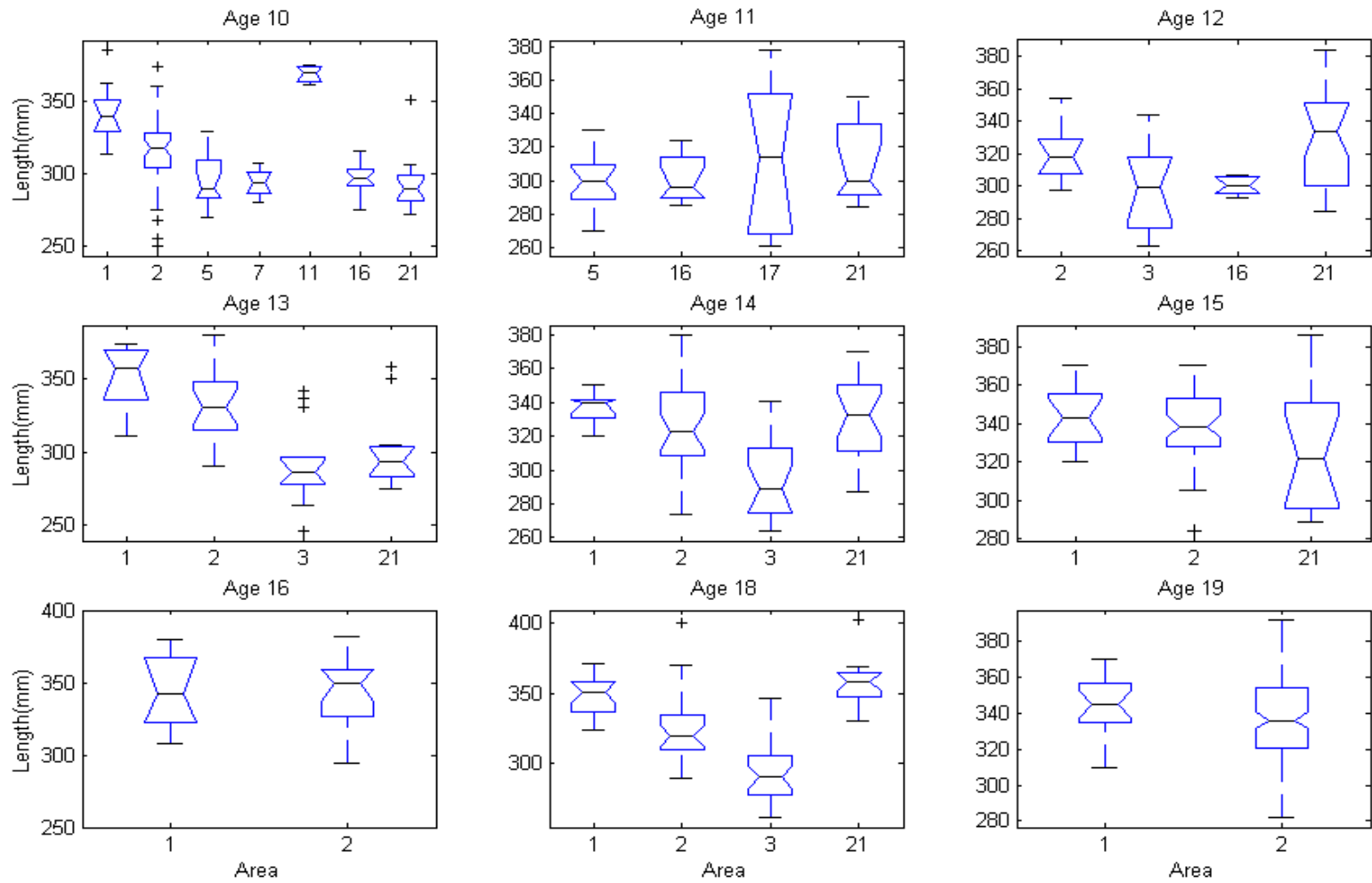
Figure 9. (Cont.)

Figure 10. Relative mean abundances of two nematode genera in horse mackerel aged 5-9 years from Atlantic samples taken in 2000.
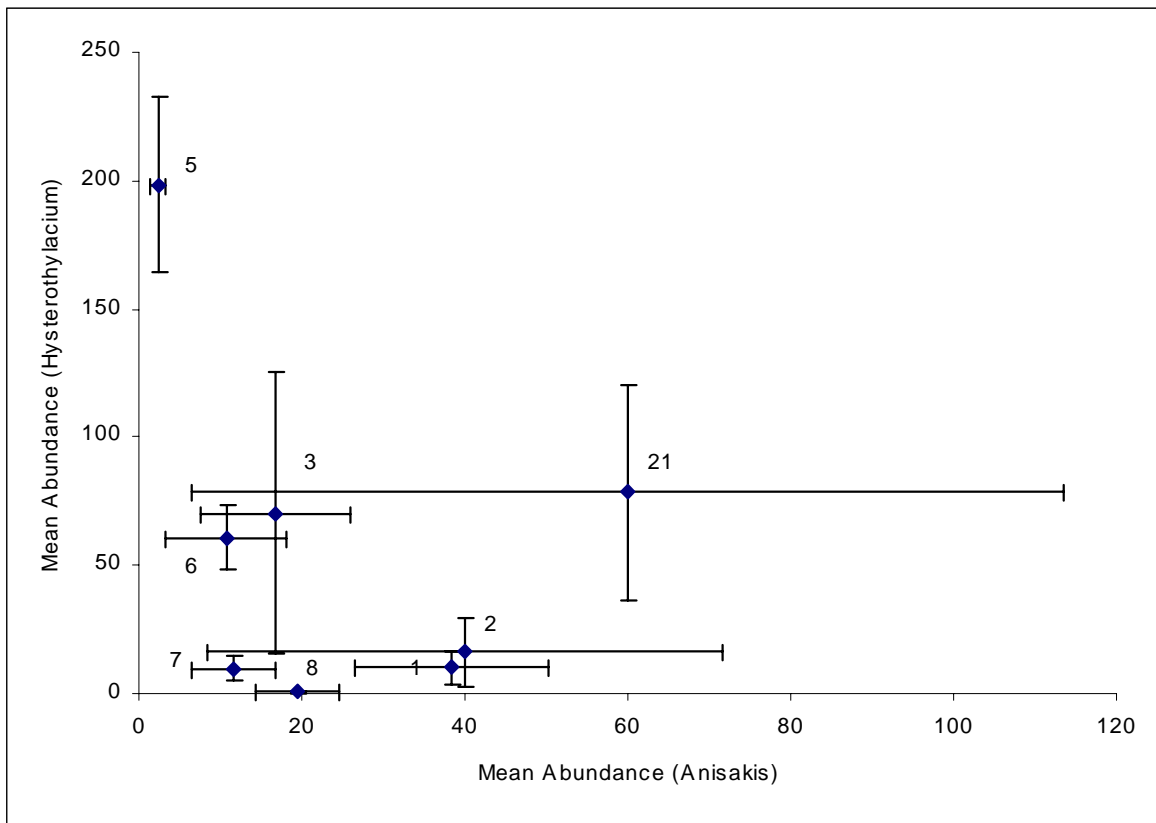
Figure 11. Covariance analysis between the variables "area" and "number of eggs released per gram of gutted weight", being "total length" as covariate.
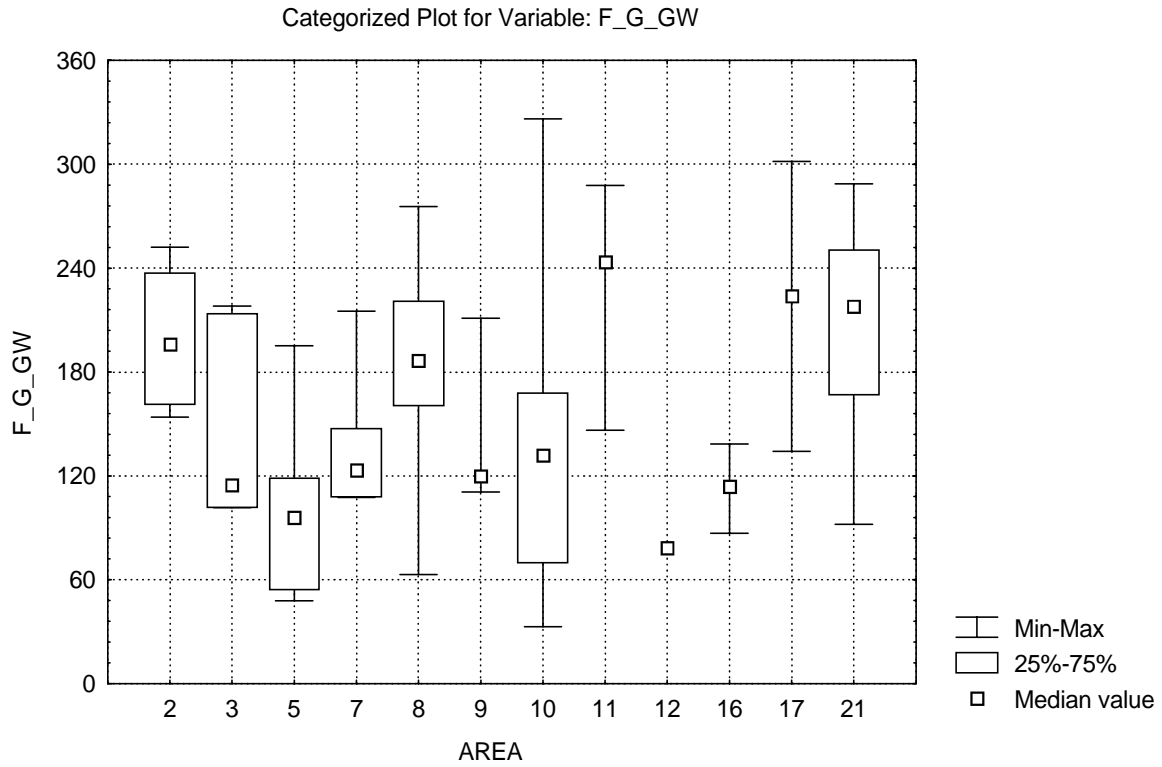

Categorized Plot for Variable: F_G_GW

Figure 12. Characterization of zones with respect to horse mackerel growth, with inclussion of some possible migration movements during spawning season inferred from growth and reproduction data and after spawning, inferred from grwth and fisheries distribution data. Blue row: feeding migration route. Red rows: movements during spawning season.