FISHERIES OCEANOGRAPHY

FISHERIES OCEANOGRAPHY *Fish. Oceanogr.* 24:2, 135–149, 2015

Larval population structure of *Engraulis encrasicolus* in the Strait of Sicily as revealed by morphometric and genetic analysis

ANGELA CUTTITTA,[1,*] BERNARDO PATTI,[1] TERESA MAGGIO,[2] ENZA MARIA QUINCI,[1] ANNA MARIA PAPPALARDO,[3] VENERA FERRITO,[3] VITO DE PINTO,[3] MARCO TORRI,[1] FRANCESCA FALCO,[1] ALDO NICOSIA,[1] MARIANNA MUSCO,[1] GRAZIA MARIA ARMERI,[1] FRANCESCO PLACENTI,[1] GIORGIO TRANCHIDA,[1] ROBERTA MIFSUD,[4] ANGELO BONANNO[1] AND SALVATORE MAZZOLA[1]

[1]*Istituto per l'Ambiente Marino Costiero, UOS Capo Granitola Consiglio Nazionale delle Ricerche, via del mare, 3 – 91021, Campobello di Mazara, Trapani, Italy*
[2]*ISPRA sts, Via Puglisi Salvatore 9 - 90143, Palermo, Italy*
[3]*Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, Università di Catania, Corso Italia, 57 - 95129, Catania, Italy*
[4]*Department of Fisheries and Aquaculture, MSDEC Għammieri, Ngiered Road, Marsa, MRS 3303, Malta*

ABSTRACT

In order to investigate the larval population structure, specimens of *Engraulis encrasicolus* larvae from five different locations in the Strait of Sicily were analyzed by means of otolith readings, morphometric and genetic techniques. The distribution of age in day, identified by means of the otolith readings and associated with the oceanographic parameters, was useful to identify possible spawning areas and transport dynamics. The presence of more than one spawning area suggested the possibility that two or more sub-populations may co-exist in the study area. The morphometric characteristics were more adequate than genetic parameters to discriminate the different larval groups. The most relevant variables for the separation were the mouth length (ML) and the body diameter (BD). The population structure by means of genetic data reported the presence of two phylogroups co-occurring among samples in each sampling locations specimens. The pattern of genetic divergence among anchovy larvae in the Strait of Sicily was congruent with previous studies conducted on adult populations present in other Mediterranean areas with different molecular markers. The habitat-specific nature of the morphological variation and the lack of corresponding genetic variation among larvae from the different locations suggested that the observed differences in morphology could be linked to environmental parameters. The body form differences among different larvae samples could reflect the nutritional status of larvae. In fact, these differences were found among anchovy larvae collected in areas with different oxygen and fluorescence, which is an index of primary productivity and is linked to the availability of food for anchovy larvae.

Key words: *Engraulis encrasicolus*, morphometric and genetic analysis, population structure, Strait of Sicily

INTRODUCTION

Identification of fish population structure is a general issue for both ecological and evolutionary contexts as well as for conservation and management of species. Information about space-temporal configuration of distinct groups of individuals of the same species supports the study of population connectivity and, therefore, helps the recognition of population structure. In recent years, it was demonstrated that detailed and valid knowledge of population connectivity is crucial for population structure analysis of marine organisms, and in the case of financially important species it is an important requirement for spatial management of fisheries (Armsworth, 2002; Fogarty *et al.*, 2003; Botsford and Hastings, 2006). Traditionally in the case of fishery species, as reported by Cadrin *et al.* (2004), the stock identification model assumes that the population is well mixed throughout the management area and the spatial management units are defined on the basis of genetic structure, differences in morphological and demographic characteristics, fishing patterns, and/or movement and dispersal patterns of individual species. In the particular case of species of small pelagic fish, their populations tend to be distributed in wide geographic areas, where oceanographic features directly

---

*Correspondence. e-mail: angela.cuttitta@iamc.cnr.it

Received 11 July 2014
Revised version accepted 6 January 2015

doi:10.1111/fog.12098

influence the population size; the interplay between oceanographic features and fishery exploitation results is very complex and causes large interannual and interdecadal variations of abundance as well as fishing collapse (Bakun and Parrish, 1982, 1991).

One important and well-studied small pelagic fish in the Mediterranean Sea is the European anchovy, *Engraulis encrasicolus*; it is widely distributed in the Eastern Atlantic and in the Mediterranean Sea (Whitehead *et al.*, 1988; Palomera and Rubies, 1996). As with other short pelagic species, the European anchovy is very sensitive to its physical environment (Guisande *et al.*, 2004; Basilone *et al.*, 2006); environmental features were demonstrated to be strictly linked to early life stage survival as well as to recruitment success/failure (Cingolani *et al.*, 1996). Vasconcellos and Castello (1996) hypothesized that early life stage survival is linked to trophic availability, advection and absence of predation; in this regard Bakun and Parrish (1991) suggested that water column stability, horizontal transport, water temperature, advection and the dispersion phenomenon directly affect the larval survival. In addition, recruitment success of anchovy depends on nutrient enrichment, water turbulence, egg and larvae retention in the nursery area (Bakun and Parrish, 1991; Dulčić, 1995; Borja *et al.*, 1998).

The European anchovy (*E. encrasicolus*) represents an important fishery resource for the Mediterranean sea, where it is caught by purse seiners and also by midwater pair trawlers (Patti *et al.*, 2010). In some areas, it is subject to an intense fishing pressure (Morello and Arneri, 2009) and the population size showed fluctuations and collapses probably because of overfishing coupled with low recruitment level owing to adverse oceanographic conditions (Dulčić, 1995; Santojanni *et al.*, 2003).

The population structure of the European anchovy has been widely studied in the Mediterranean sea by means of growth parameter (Levi *et al.*, 1994), morphological/morphometric variations (Shevchenko, 1980; Tudela, 1999; Caneco *et al.*, 2004; Traina *et al.*, 2011) and acoustic data (Patti *et al.*, 2004; Bonanno *et al.*, 2005; D'Elia *et al.*, 2009); the major role of environmental conditions on anchovy population structure has been demonstrated (DeVries and Frie, 1996). Growth is affected by temperature (Pauly, 1980), whereas fish production, which is partly determined by growth, is mainly controlled by primary production (Iverson, 1990).

The population structure of the European anchovy was also investigated by means of different molecular markers; in the Atlantic Ocean and the Mediterranean basin, most of the authors supported the presence of two co-occurring phylads resolved with different class of molecular markers (mtDNA, microsatellites, SNP and nuclear intron) and with different phylad proportions (Grant, 1985; Magoulas *et al.*, 1996, 2006; Borsa *et al.*, 2004; Zarraonaindia *et al.*, 2012).

Unfortunately until now no paper has taken in consideration population structure in the Strait of Sicily, but even despite this, it can be considered a key zone in the basin for population structure. This area is characterized by a very complex sea circulation that can strongly influence the spawning strategy and the recruitment success of many fish species (Fortibuoni *et al.*, 2010; Garofalo *et al.*, 2010) and, in particular, of the European anchovy (Cuttitta *et al.*, 2006). Garcìa Lafuente *et al.* (2002) and Cuttitta *et al.* (2003) investigated the relationship between the physical processes mentioned by Agostini and Bakun (2002), such as enrichment, concentration and retention present in the Strait of Sicily, and larval population, which yield favorable conditions for the survival of anchovy larvae in the Strait of Sicily. Cuttitta *et al.* (2003) and more recently Basilone *et al.* (2013) observed the presence of more than one spawning area in the northern part of the Strait of Sicily, suggesting the possibility that two or more sub-populations may exist in the study area. Moreover, although Garcìa Lafuente *et al.* (2002) identified the Strait of Sicily as one of the main spawning areas of anchovies, larval population size showed interannual fluctuations that can affect the biomass of the adult stock (Patti *et al.*, 2004).

According to previous studies it seems that mesoscale processes influence the early life stage directly through favourable environmental conditions for larval survival and mixing of demes after spawning and so, indirectly, the population structure of adults and the presence of different units.

The aim of the present study was to investigate the population connectivity of *E. encrasicolus* by studying the early life stage using an integrated approach, comprising morphometric variation, growth and mtDNA sequence analysis in the Strait of Sicily. The use of genetic analysis may account for the hypothetical presence of different unit and the morphometric parameters and the larval otolith readings can be a good tool for the evaluation of larval survival and thus the subsequent recruitment in relation to environmental variability (Ré, 1986; Diaz *et al.*, 2009).

MATERIALS AND METHODS

Study area

The Strait of Sicily plays a crucial role in determining the water mass exchanges and related properties between western and eastern Mediterranean. The highly irregular bottom topography of the Sicilian Channel, in the form of a submarine ridge characterized by shallow banks along the Tunisian (Galite and Skerki banks) and Sicilian (Adventure Bank and Malta Channel area) coasts, limits the water flow particularly at deeper levels (Gasparini *et al.*, 2005; Sorgente *et al.*, 2011). An anti-estuarine circulation is mainly characterized by a two-layer system: a surface layer composed of Atlantic Water (AW) flowing eastward, essentially dominated by mesoscale processes, and an intermediate layer composed of Levantine Intermediate Water (LIW) and transitional Eastern Mediterranean Deep Water (tEMDW) flowing in the opposite direction. Two main veins of the AW are known to flow in the Strait of Sicily: the Atlantic Tunisian Current (ATC – Sammari *et al.*, 1999) in the south along the African coast and the Atlantic Ionian Stream (AIS – Robinson *et al.*, 1999) in the north along the Sicilian coast. The core of Atlantic water mass below the surface layer is identified by the salinity minimum (Robinson *et al.*, 1999). In the AIS, an important spatial variability exists in terms of shape, position and strength of permanent or quasi-permanent sub-basin gyres and their unstable lobes, meanders patterns, bifurcation structures and strength of permanent jets, transient eddies and filaments (Robinson *et al.*, 1999; Sorgente *et al.*, 2011). AIS and ATC are considered as quasi-permanent or permanent features of the area (Béranger *et al.*, 2004; Sorgente *et al.*, 2011).

Survey and Sampling

Chemical and physical data and ichtyoplankton samples used in the present study were collected during an oceanographic survey on board the *O/V Urania*, carried out from 25 June to 14 July 2010 in the Strait of Sicily. The sampling scheme was based on a station grid of 4 × 4 nautical miles in sea zones closer over the continental shelf, whereas a grid of 12 × 12 nautical miles was adopted for the off-shore areas (Fig. 1).

Temperature, salinity, dissolved oxygen and fluorescence data were acquired by means of a conductivity/temperature/depth (CTD) probe (Sea-Bird Inc mod. 9/11 plus) for each meter of the water column at each sampling station. The collected downcast data were quality checked and processed according to the Mediterranean and Ocean Data Base instructions (Brankart, 1994), using the Seasoft-Win32 software.

At each station, ichthyoplankton samples were collected by means of a Bongo40 net, which is composed of two coupled nets with the inlet mouth diameter of 40 cm and mesh size of 200 μm. The plankton oblique tows were carried out to a depth of 100 m, wherever possible. The zooplanktonic samples were stored in formaldeid at 4% buffered with borax (for morphometric analysis) and 70% ethanol (for otholits reading and genetic analysis). The ichtyoplanktonic species were observed by stereomicroscope for the taxonomic analysis.

A total of 3114 eggs (Fig. 1a) and 3036 larval (Fig. 1b) individuals from various locations in the survey area were collected. The entire study area was divided into six areas: three areas within the bathymetry of 200 m (Area 1 off the western coast until Sciacca; Area 2 off the central coast until Pozzallo; and Area 3 in the eastern part of the study area off Capo Passero), two areas at a bottom depth over 200 m (Area 4 in the Ionian waters and Area 5 in the deeper areas of the Strait of Sicily) and Malta islands waters. When the number of larvae was very low for the analysis, the areas were grouped together or not considered.

Morphometric analysis

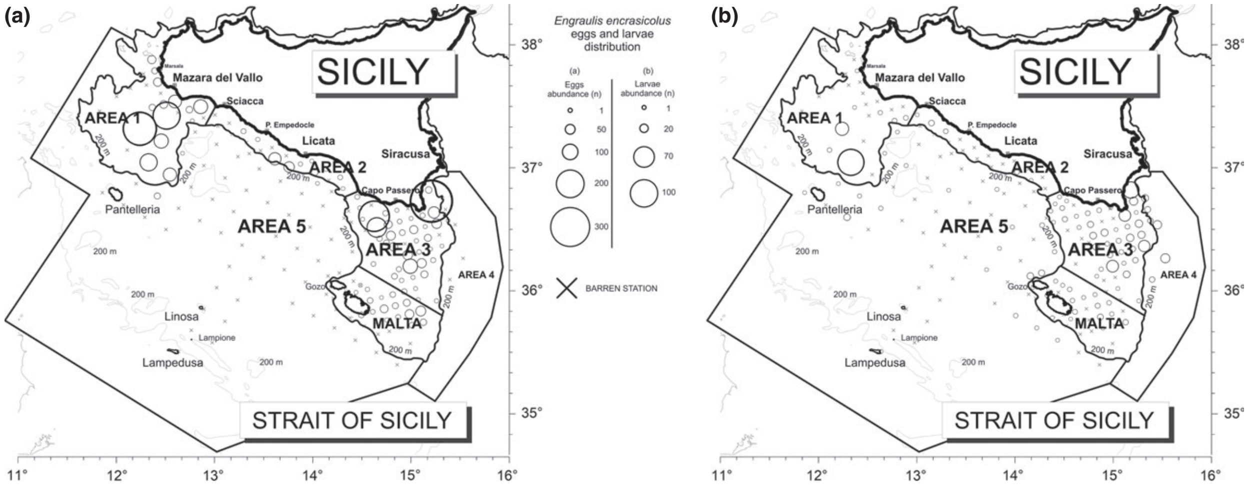
In order to characterize the anchovy larvae from the dimensional point of view and to assess morphological differences among individuals from different areas, we used multivariate morphometric analysis (Blackith and Reyment, 1971). In accordance with Erdoğan *et al.* (2009), the larvae were individually placed in the binocular stereo microscope and photographed. Morphometric parameters were acquired by software Image Pro Plus 6.0 (Image Cybernetics, RoperIndustries, SilverSpring, MD, USA; image analysis and processing): total length (TL), standard length (SL), body diameter (BD), head length (HL), eye diameter (ED) and mouth length (ML) (Diaz *et al.*, 2009).

In order to obtain a set of measures, which are better correlated among them and to increase the accuracy of the study, it was also considered appropriate to add another morphometric measure characteristic of the species, the anal length (AL: distance from the extreme front end of the upper jaw of the digestive tract).

These measures were normalized using the procedure presented by Thorpe (1975) and generalized by Lleonart *et al.* (2000), to remove the allometric effect of the TL on the other parameters: it completely removes all the information related to size, scaling all individuals to the mean size and adjusting their shape to that they would have in the new size.

The univariate Permutational Multivariate Analysis of Variance (PERMANOVA) was used on standardized morphometric variables to test if the differences observed among the various areas were

Figure 1. Eggs (a) and larval (b) distribution in the study area. The circle dimension is proportional to the density. The cross indicates the absence of eggs in the haul.



significant. All PERMANOVA analyzes used 999 permutations. When differences were detected, additional pairwise tests were conducted using permutational *t*-tests with 999 permutations as in the main test.

Multivariate discrimination was examined in order to verify if the morphometric variables can discriminate among areas. Levene's test revealed homoscedasticity in multivariate variance and covariance matrices, therefore, a quadratic discriminant analysis was performed rather than the simpler linear discriminant analysis. A backwards stepwise approach was adopted to eliminate the variables that contributed least to the prediction of group membership. The resultant discriminant functions were used to classify larvae into groups and then to compare them with the selected geographic areas. The expected error rates of the classification functions were estimated using cross-validation by the leaving-one-out procedure (Fung, 1996).

All statistical analyzes were carried out using the statistical software R 3.1.0.

Daily growth analysis

A total amount of 123 larval otoliths (sagittae) were analyzed in order to identify age class structure of anchovy larvae from band width, corresponding to daily growth. The larvae were immersed into a drop of water on a glass slid, and microneedles extracted the otoliths before the cutting of the otic capsules. Afterwards, they were located on a glass slide and fixed with fast drying transparent resin (Entellanr). Each pair of sagittae was observed under the optical microscope in order to count the increments, starting from the core outwards, representing the larval age expressed in days (Ré, 1986).

Genetic analyzes DNA extraction, PCR amplification and sequencing mtDNA control region

Genomic DNA was extracted from larval fish tissue using the DNeasy tissue kit (Qiagen, Milan, Italy), according to the manufacturer's instructions. PCR primers (ENG_FW: 5′ TTCTAAAGTTAAACTAC CCTCT; ENG_REV1: 5′-TTAAGTGAACGCTCG GCATGG-3′) specific to European anchovies were designed from the species' complete mtDNA sequence to amplify a 608-bp segment of the mitochondrial control region between the Pro-tRNA and the conserved sequence block D (CSBD). Polymerase chain reactions were carried out in 50 μl. Each reaction contained 0.5 mM of each primer, 0.2 mM deoxynucleoside triphosphate (dNTPs), 1.5 mM $MgCl_2$, 1× PCR buffer, 1 U of Taq polymerase (Invitrogen, Milan, Italy) and 50–100 ng of genomic DNA. The PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by denaturation at 94°C for 45 s), annealing at 55°C (45 s) and the extension at 72°C (1 min) repeated for 35 cycles and by a final extension at 72°C for 5 min. Negative controls were included in all PCR runs to ascertain that no cross-contamination took place. Double-stranded products were checked in agarose gel electrophoresis and purified with the Qiaquick PCR purification kit (Qiagen, Venlo, Netherlands) and subsequently sequenced in the forward and reverse direction using an ABI 137Prism 3100 automated sequencer (Applied Biosystems, Thermo

Scientific, Waltham, USA). All sequences have been carefully checked and deposited in Genbank.

Sequence data analysis

Sequences were aligned using ClustalX (Thompson *et al.*, 1997) with default settings and, thereafter, the sequence alignment was visually rechecked. Genetic variability was estimated using two parameters: nucleotide diversity (π) described as the average number of nucleotide differences per site between two sequences (Nei, 1987) and haplotype diversity (h). Substitution saturation within sequences was tested employing the entropy-based index of substitution saturation approach of Xia *et al.* (2003) calculating the Iss and Issc values. The phylogenetic reconstruction was inferred by using the Maximum Likelihood method based on the Tamura–Nei model (Tamura and Nei, 1993). The robustness of internal branches of distance was estimated by bootstrapping (Felsenstein, 1985) with 1000 replicates.

The relationships between mtDNA control region haplotypes were described with a Minimum Spanning Network (MSN) as implemented in Arlequin 3.0 (Excoffier *et al.*, 2005). In addition, population differentiation was assessed by a hierarchical analysis of molecular variance (AMOVA), using Arlequin 3.0 for the clustering of samples according to their geographic location. This analysis allowed us to verify the partitioning of genetic variation among and within populations, this reported the correspondent fixation indices: Φ_{ST} (the average within-populations within a group).

Principal coordinates analysis (PCoA) was conducted by GenAlEx 6.1 (Peakall and Smouse, 2006) as a way of visualizing the patterns of the genetic relationship contained in a genetic distance matrix as calculated for haploid data. Furthermore, larvae sequences from the Strait of Sicily were compared with adults sequences from the Mediterranean sea deposited in GenBank by Viñas *et al.* (2014) (Accession Number from HQ215598 to HQ215748 and JQ595010 to JQ595270). Phylogenetic relationships among larvae and adult sequences in the Mediterranean sea were inferred using the Maximum Likelihood method based on the Tamura–Nei model (Tamura and Nei, 1993) with MEGA software. The robustness of internal branches of distance was estimated by bootstrapping (Felsenstein, 1985) with 1000 replicates.

RESULTS

Surface water hydrology

The use of the θS diagram allowed us to discriminate the AW from the Ionian Water IW in the Strait of Sicily (Fig. 2a). A clear signal of the AW was recorded in the west stations of the study area between 12–14°E and 36.5–37.5°N, whereas the IW was present over the 15°E (Fig. 2a). A water layer with intermediate characteristics between the AW and IW was present between 14 and 15°E. The AW were characterized by a range of temperature, salinity and density of 15–19°C, 37.05–37.8, 27–28.5 kg m^{-3}, respectively, whereas the IW were slightly warmer and saltier (Fig. 2a). The core of the AIS path (Robinson *et al.*, 1999) was defined by connecting the points of minimum salinity at each transect perpendicular to the coast, which allowed us to define the thermohaline properties of the particular AW vein (Fig. 2a).

The interpretation of the density interfaces (not shown) in the study period showed the presence of a cyclonic structure that affected the surface layer positioned between 12–13°E and at about 37°N. Such an anti-cyclonic structure probably adjoined the AW off the Sicilian coast, constraining the entry of AIS along the coast. On the contrary, the presence of anticyclonic circulation located in the eastern part of the Sicilian coast (14–15°E; 36°N) supported the spread of the AIS vein off the southern Sicilian coast.

The horizontal salinity patterns (Fig. 2b) showed the presence of a thermohaline front to the east of the Strait of Sicily (15°E), considered as a sort of physical barrier to the surface circulation dynamics as reported by Garcìa Lafuente *et al.* (2005).

Moreover, the horizontal temperature and fluorescence patterns (Fig. 2b) indicated the presence of upwelling localized off the Sicilian coast (37.5°E; 12.5°N), as well as previously reported for instance by Garcìa Lafuente *et al.* (2005), Basilone *et al.* (2013) and Bonanno *et al.* (2013). Specifically, the upwelling area was characterized by lower values of temperature and higher values of salinity, oxygen and fluorescence than the surrounding areas (Fig. 2b).

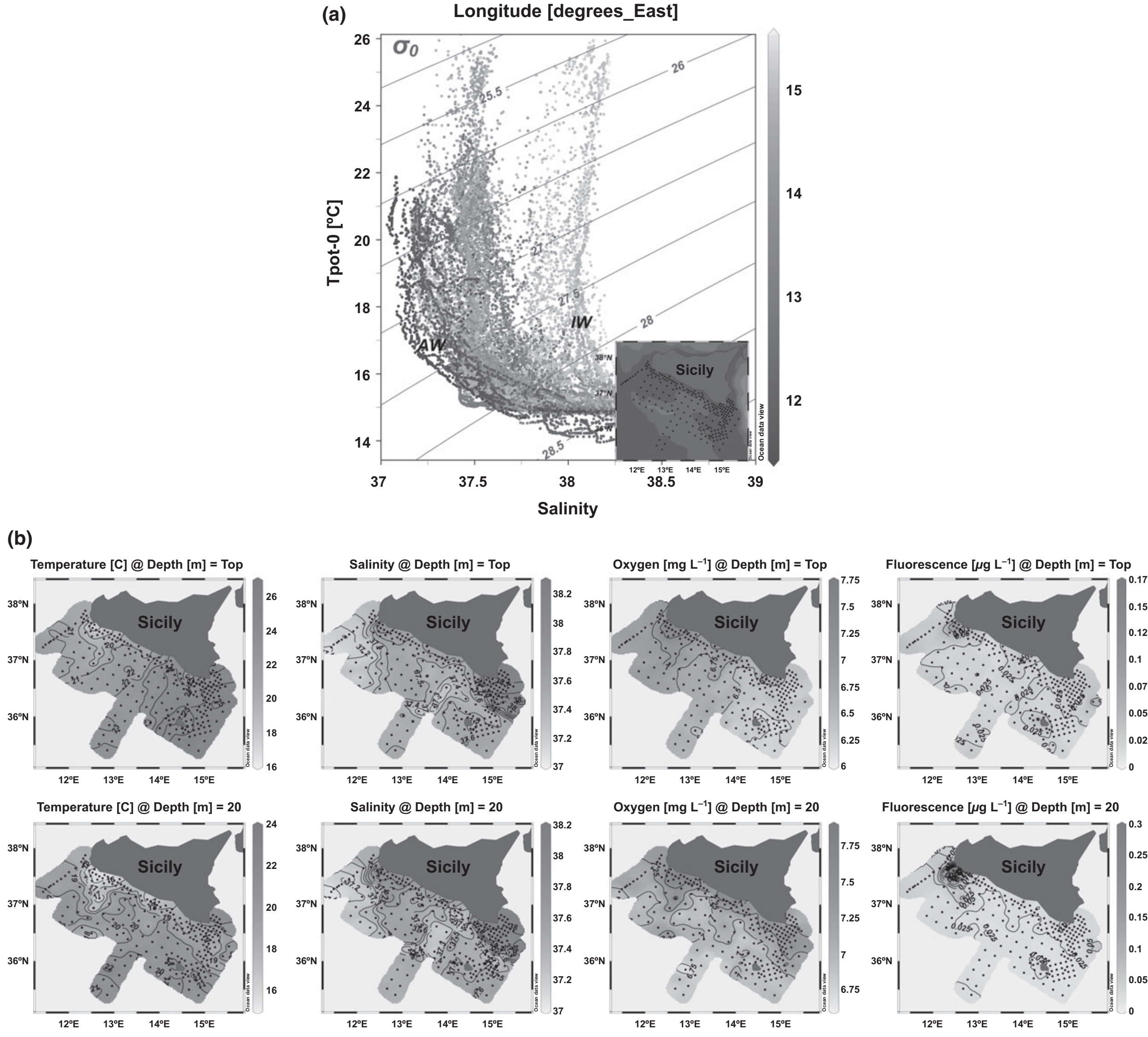
Morphometry

The morphometric parameters were measured for 43 Maltese and 435 Sicilian larval specimens (50 in area 1, 5 in area 2, 309 in area 3, 49 in area 4 and 22 in area 5).

The box plots provided information about the differences among the distributions of the normalized morphometric parameters in the different areas (Fig. 3).

PERMANOVA showed that there were significant differences in larval morphometry among the different areas ($F = 6.2218$; P-value < 0.001). Particularly, the univariate analyzes tested that the larvae significantly differed in terms of ML ($F = 20.52$; P-value < 0.0101),

Figure 2. (a) *θ*S diagram of the conductivity/temperature/depth (CTD) casts acquired in the surface water of the Strait of Sicily. (b) Isosurfaces of environmental parameters at the top and 20 meters of depth.



BD ($F = 9.5287$; P-value < 0.001) and ED ($F = 2.7117$; P-value $= 0.03$).

The pairwise comparisons explained that the values of ML distinguished three groups of larvae (areas 1–3; areas 4 and 5; area Malta). Areas 1 and 2 differed from area 3 in terms of BD, as well as area 4 from area 5. The BD values of larvae from Malta were significantly different from areas 4 and 5. Only areas 3 and 4 differed in terms of ED (Table 1).

The stepwise quadratic discriminant analysis revealed that the variables that mostly significantly contributed to the multivariate discrimination of the different areas were ML and then BD. The total correct classification rate estimated from the cross-validation procedure was 0.433. The correct classification rates ranged from about 0.42 to about 0.54 (Table 2). The classification of larvae from area 3 was the most confused.

The larvae from areas 1 and 2 were discriminated by lower values of ML and BD. The larvae from area 3 had no distinguishing characteristics compared with the other areas. The larvae from area 4 showed higher values of both variables. The larvae from area 5 had higher ML values and lower BD. The larvae from Malta were mainly discriminated by higher values of ML (Fig. 4).

Figure 3. Box plots of normalized morphometric parameters among different areas. The bottom and top of the boxes are the first and third quartiles. The band inside the box is the median. The bottom and top of the whiskers are minimum and maximum.

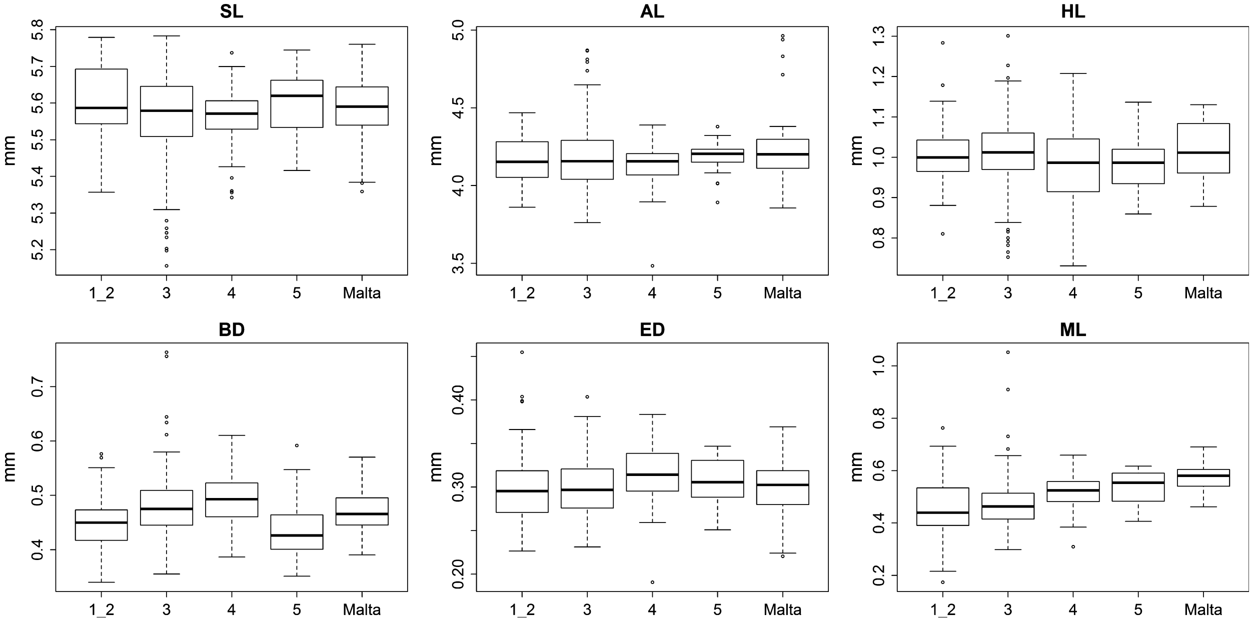


Table 1. *P*-value of the pair-wise tests using permutational *t*-tests of normalized mouth length (ML), body diameter (BD) and eye diameter (ED) among areas. Bold text indicates that the difference is significant (*P*-value < 0.05).

	Area	1–2	3	4	5	Malta
ML	1–2	1				
	3	0.784	1			
	4	**0.012**	**0.002**	1		
	5	**0.012**	**0.002**	0.463	1	
	Malta	**0.002**	**0.002**	**0.002**	**0.012**	1
BD	1–2	1				
	3	**0.002**	1			
	4	**0.002**	0.071	1		
	5	0.283	**0.002**	**0.002**	1	
	Malta	0.075	0.221	**0.010**	**0.008**	1
ED	1–2	1				
	3	0.910	1			
	4	0.213	**0.020**	1		
	5	0.763	0.595	0.595	1	
	Malta	0.910	0.910	0.085	0.595	1

Otholits

The distribution of age in day seems to be different among areas. The 20 larvae from area 1 and 13 larvae from area 2 exclusively belonged to the lower age classes (2 and 3 days). In contrast, in zones 4 and 5 only larvae older than 4 days were found. Area 3 and Malta showed a wider range of age classes (Fig. 5).

MtDNA

A 608-bp portion of the mitochondrial control region was sequenced in 74 individuals of anchovy from the Strait of Sicily (areas 1–3 and 5) and from the Maltese coast. The base composition showed an A-T bias (A+T content = 0.66), as usual for this mitochondrial region. A total of 51 nucleotide sites were found variable, of which 46 positions were parsimony informative. Saturation analysis showed that the mitochondrial control region data set bears little substitution saturation (the observed Iss values were significantly lower than Iss.c); thus, our data could be appropriate for our purposes. The analysis of all investigated sequences led to the identification of a total of 35 distinct mtDNA haplotypes with a frequency ranging from 0.027 to 0.216. The highest frequency haplotype is shared among three Sicilian sites. Notably, all other haplotypes resulted in either a unique or shared between two specimens of the same sampling sites.

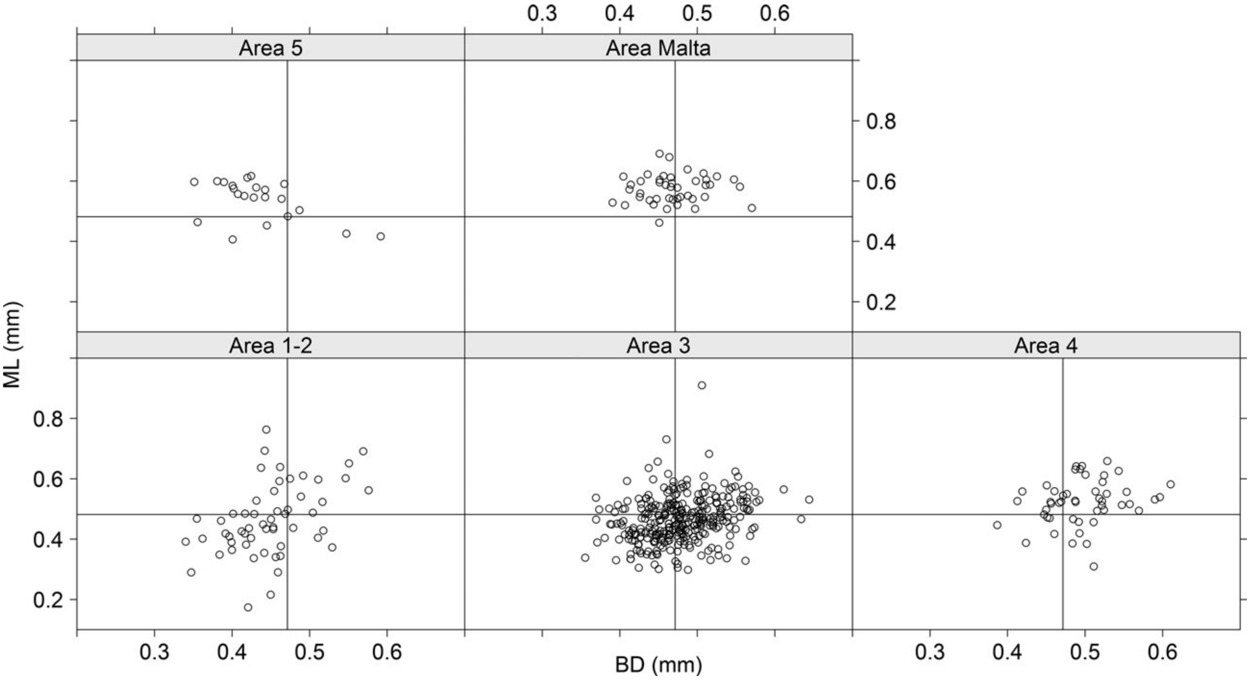
The genetic variation within each population was described by the haplotype diversity (h) and nucleotide diversity (π) indices. Overall values for (h) and (π) were 0.944 and 0.025 respectively; considering the single populations the haplotypes diversity ranged from 0.860 (area 3) to 0.962 (area 5), meanwhile nucleotide diversity ranged from 0.017 (Malta) to 0.024 (area 5) (Table 3).

The phylogenetic tree constructed with the Maximum Likelihood method (Fig. 6) revealed the

Table 2. Classification matrix by Quadratic Discriminant Analysis applied on normalized morphometric parameters of larvae from all areas.

	Number of larvae classified into estimated groups						
	Area	1–2	3	4	5	Malta	% correctly classified
Number of larvae classified into observed areas	1–2	24	10	8	6	7	0.436
	3	62	130	59	26	32	0.421
	4	7	9	22	3	8	0.449
	5	3	2	1	12	4	0.545
	Malta	6	2	4	12	19	0.442

Figure 4. Scatterplots of the normalized body diameter (BD) values versus normalized mouth length (ML) values for each origin area. Horizontal and vertical axes correspond to the ML and BD average, respectively.



presence of two well-defined clades A and B including both specimens from Sicilian areas and Maltese waters.

In order to investigate where the genetic variation was mainly distributed, among or within a population, in a hierarchical analysis of molecular variance (AMOVA) clustering samples according to their geographic location was conducted and reported a low but significant structure among locations with a FST value of 0.218 ($P < 0.001$).

Pairwise Φ_{ST} values resulted significant in all the comparisons except between area 1–2 versus area 5, confirming the genetic heterogeneity of the analysed samples.

Most of the variance among anchovy larvae was distributed in the first and second axes (65.23%) of the PCoA and the graphical pattern of genetic relationships among populations showed the presence of two different groups; in each group specimens from the three Sicilian areas and from the Maltese coast were present (Fig. 7).

The Minimum Spanning Network of the haplotypes clearly separated the two phylogroups (Fig. 8), each separated by the other from 17 mutational steps; the first comprised the most common haplotype shared among areas 1, 2, area 3 and area 5 and other shared haplotypes and the second one groups shared and unique haplotypes from all the sampling areas.

Figure 5. Histogram of the frequency distribution of the age classes of larvae for each origin area.

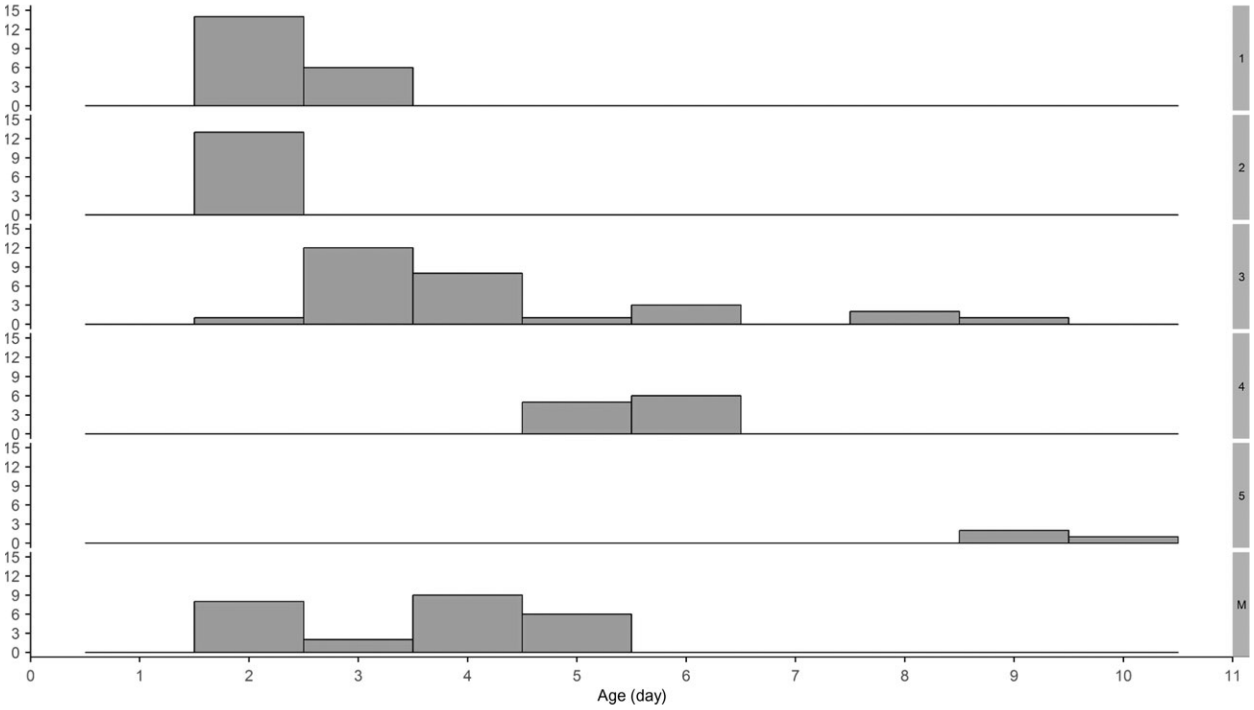


Table 3. Mitochondrial DNA variation summary or the five samples of E. encrasicolus; (N is number of specimens; H is number of haplotypes; h haplotype diversity; n nucleotide diversity).

Area	*N*	*H*	*h*	*π*
1–2	28	12	0.878	0.023
3	17	9	0.860	0.018
5	13	10	0.962	0.024
Malta	16	10	0.925	0.017
Tot	74	35	0.944	0.025

Sequence alignment of larvae specimens was integrated with mtDNA control region sequences from Viñas *et al.* (2014). The alignment of 400 bp from adults and larvae revealed 105 variable sites, 61 of which were informative. From the alignment, 250 haplotypes were present distributed in two well-defined groups as was evident from the Neighbour Joining tree used to infer phylogenetic relationships among larvae and adults. The two well-defined clades grouped specimens from all Mediterranean locations as in the case of the NJ tree based on larvae sequences.

DISCUSSION

The present study represents the first description of a larval population structure of European anchovy in the Strait of Sicily by means of otolith readings, morphometric and genetic techniques.

Several previous studies (Bembo *et al.*, 1996; Işmen, 2001; Magoulas *et al.*, 2006; Pollar *et al.*, 2007; Diaz *et al.*, 2009; Viñas *et al.*, 2014) have demonstrated that these methods represent a useful tool for studying the population structure of small pelagic species such as *Engraulis encrasicolus*.

In particular, the otoliths reading is a powerful parameter for the age in days determination of the larvae, useful to identify possible spawning areas and, associated with the mesoscale phenomena, their transport dynamics. The presence of eggs and younger larvae in the western part of the Sicilian coast could be interpreted as an indicator of the spawning grounds for anchovies. These results confirm previous findings reported in Garcìa Lafuente *et al.* (2002), Cuttitta *et al.* (2003, 2006) and Basilone *et al.* (2013).

The hydrological analyzes in the study area show that the distribution of anchovies in early life stages could highly depend on surface water dynamics (Garcìa Lafuente *et al.*, 2002; Cuttitta *et al.*, 2003; Sabatés *et al.*, 2007), which is mainly controlled by the Atlantic–Ionian Stream (AIS) (Robinson *et al.*, 1999). In fact, the absence of larvae of the higher age classes in the western part of the study area off Mazara

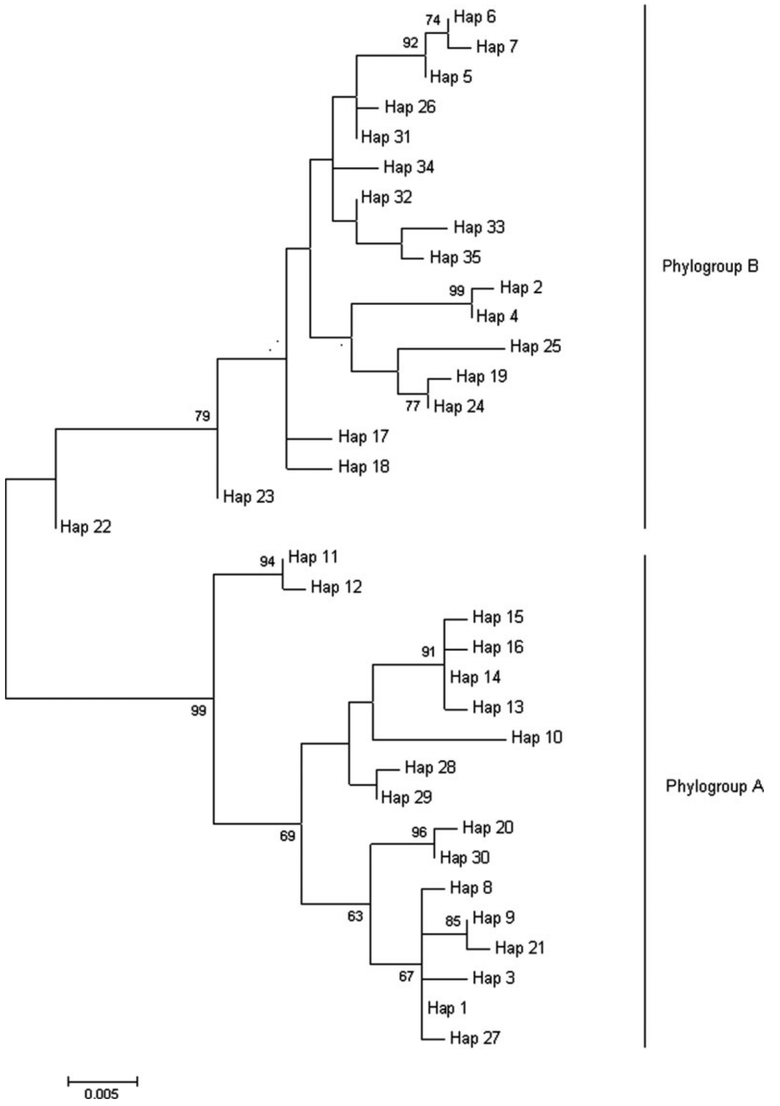


Figure 6. Maximum Likelihood tree based on the Tamura–Nei model [1]. The tree with the highest log likelihood (–1507.6763) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Bootstrap values lower than 50% are not reported.

suggests that the spawning product was transported into different areas.

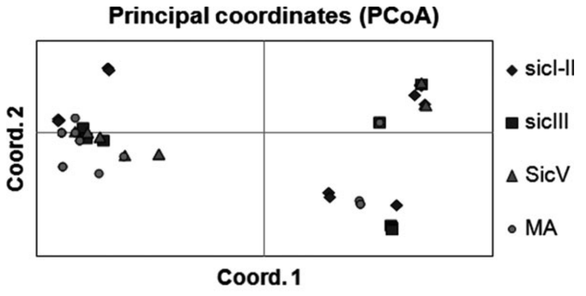
Presumably, the main branch of the AIS carried a large number of larvae towards the south-east end. The cyclonic structure, close to the coastline between Mazara and Sciacca, as well as the coastal upwelling, probably determined the offshore larval transport of a small number of larvae, which escaped the main branch of the AIS.

Moreover, the presence of larvae under Sciacca could be explained by transport from the area off Mazara, owing to the drift by currents parallel to the coast, highlighted by the colder and slightly less salty inshore waters.

The presence of eggs and young larvae also in the eastern area (off Capo Passero) and in the Maltese waters confirms these were also spawning areas for anchovies. Older larvae were found in these areas as well, probably owing to the presence of a thermohaline front that represented a sort of physical barrier to the dynamics of surface circulation and gave them characteristics that favored retention processes. Therefore, it is verified that during the summer of 2010 the anchovy-spawning habitat in the Strait of Sicily was primarily inshore and so it could be speculated that it was related to the bathymetry. Therefore, the observed distribution pattern confirms that anchovy mainly spawns over a continental shelf area characterized by waters with low salinity and high primary and secondary production (Garcìa and Palomera, 1996; Regner, 1996; Somarakis *et al.*, 2004; Somarakis and Nikolioudakis, 2007).

The presence of more than one spawning area suggests the possibility that two or more sub-populations

Figure 7. Principal coordinate analysis (PCoA) plots obtained from the Nei distance matrix. In the figure the percentage of variance explained by the first three axes is also reported.



Axis	1	2	3
%	53.88	11.35	6.46

may co-exist in the study area. In this regard, the morphometric studies provide useful results for identifying marine fish stocks and describing their spatial distributions.

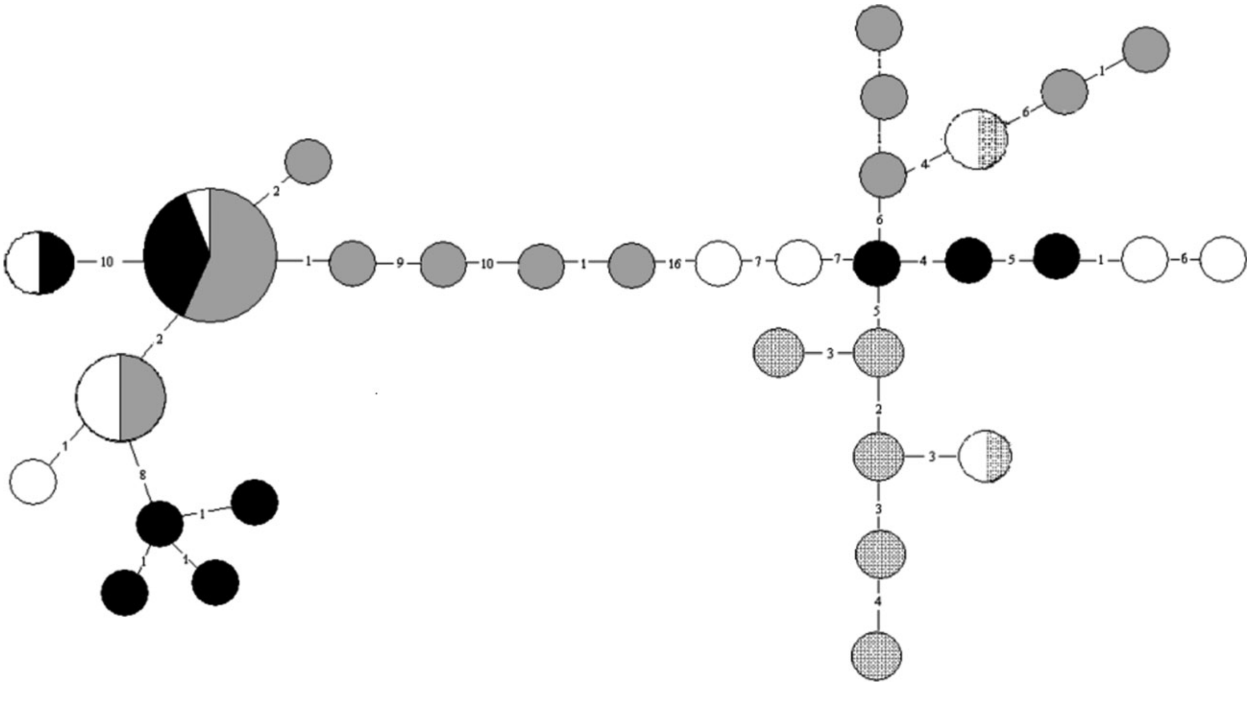
A discrete degree of morphometric discrimination among anchovy larvae samples is also noted, except for the area off Capo Passero. In fact, the larvae found in this area are not clearly discriminated from the morphometric parameters, and this could be as a result of their provenience from different areas of the Strait of Sicily.

The most useful parameters to discriminate the different larval groups are the length of the mouth and the body diameter. Statistical tests indicate differences mainly between samples taken in an area off Mazara and the offshore areas in the eastern part, characterized by the absence of mixing by surface currents.

The area off Capo Passero and the western offshore areas were characterized by larvae with similar morphometrics to western coastal larvae, confirming the transport of these larvae by means of the surface currents. The Maltese larvae seem to have had distinguishing features from all other areas.

In spite of the distribution of morphometrical variation among *E. encrasicolus* larvae from the Strait of Sicily, the population structure by means of genetic data reports the presence of two phylogroups co-occurring among samples; that is, in each sampling locations specimens belonging to the two philogroups were present. This conclusion of differentiation among locations comes from a different kind of analyzes such as hierarchical AMOVA in which the fixation index results are significantly different from zero and the pairwise Φ_{ST} values that result spread different from zero. Analysis of differentiation based on distance

Figure 8. Minimum Spanning Network of control region haplotypes detected for *Engraulis encrasicolus* samples. The area of each circle is proportional to the number of individuals exhibiting that haplotype. In each line connecting two haplotypes is reported in the number of mutational steps. Gray: Area 1–2; black: Area 3; white: Area 4; dotted: Malta.



(PCoA and Minimum Spanning Network) also corroborates the results showing the presence of two mixed groups in which all the specimens from the Strait of Sicily were widespread.

Furthermore, comparative analyzes of the mtDNA control region of larvae from the Strait of Sicily and adults from the Mediterranean waters (Alboran sea, Gulf of Lyons, Tyrrhenian sea, Adriatic sea and Aegean sea; Viñas *et al.*, 2014) indicate that the two larvae phylogroups correspond to the two phylogroups of the adults.

The pattern of genetic divergence among *E. encrasicolus* larvae in the Strait of Sicily is congruent with a previous study conducted on adult populations present in other Mediterranean areas with different molecular markers (allozymes Tudela, 1999; Ivanova and Dobrovolov, 2006; Sanz *et al.*, 2008; intron, Kristoffersen and Magoulas, 2008; RFLP, Magoulas *et al.*, 1996, 2006; SNP and mtDNA, Viñas *et al.*, 2014; Zarraonaindia *et al.*, 2013; Intron and microsatellites, Borrell *et al.*, 2012). The origin of genetic divergence between the two phylogroups is hypothesized to be the result of past geographical isolations brought about by climate cycles during the late Pliocene and throughout the Pleistocene. It seems that owing to the presence of physical barriers two phylogroups have evolved in isolated populations in the past and successively they have had a secondary mixing that caused their present coexistence in the Mediterranean (Magoulas *et al.*, 2006).

The strong concordance between population structure conducted both on adults and on larvae and based on nuclear markers as well as on mitochondrial markers heavily suggests that the observed structure accurately reflects the population structure of the species in the Mediterranean sea. A genetic population structure in the marine environment is usually explained by two main issues acting solely or in combination, namely the effect of oceanographic features such as currents, water fronts, physical barriers and features relating to life history, such as larval dispersal or adult displacement ability, etc. Commonly, pelagic fish species (and especially the ones with pelagic eggs and larvae) and highly migratory species are expected to show genetic homogeneity because of the absence of geographical barriers to gene flow in the marine environment and the dispersal capabilities of the species; furthermore in those cases where hydrographical conditions such as ocean fronts, upwelling areas and high levels of productivity can facilitate high levels of contemporary gene flow among populations such as in the Strait of Sicily, a homogeneous genetic pattern is expected. Although in the case of European anchovy the genetic imprint of historical events is still evident and supported by many kinds of molecular markers. Moreover, even though some hydrographical features (shorelines, local upwelling and ocean gyres, etc.) can sometimes explain part of the genetic variability among specimens, they cannot account for the divergence between the two major clades.

The co-occurrence of two distinct groups in the Mediterranean basin was demonstrated in other pelagic fish species, such as swordfish, *Xiphias gladius* (Alvarado Bremer *et al.*, 2005; Pappalardo *et al.*, 2011) and the Atlantic bonito, *Sarda sarda* (Viñas *et al.*, 2004). In this species two highly divergent clades were also found co-existing in the Mediterranean, one of which displayed a star-like phylogeny and the other a more complex network. To explain this pattern these authors have also considered allopatric isolation during the Pleistocene, population bottleneck followed by a sudden expansion that gave origin to the clade with a star-like phylogeny, and later secondary contact of the two previously isolated populations.

The habitat-specific nature of the morphological variation and the lack of corresponding genetic variation among larvae from the different locations suggests that the observed differences in morphology were environmentally induced.

The environmental conditions, especially in early life history, may have had a great influence in determining morphological variation (Winans, 1984). The larvae from the western area showed slender bodies compared to larvae from eastern areas and the larvae closer to coast have a smaller mouth then the larvae in the offshore areas. The larvae found in the Maltese waters were mainly characterized by a bigger mouth compared to the Sicilian larvae.

The body shape differences among different larvae samples could reflect the nutritional status of larvae (Ehrlich *et al.*, 1976; Theilacker, 1978; Powell and Chester, 1985; Frank and McRuer, 1989; Bombace, 1992). The length of the mouth and the body diameter are morphometric characters that change in response to starvation. In fact, this difference seems to be related to pattern of oxygen and fluorescence, which is an index of primary productivity and is linked to availability of food for anchovy larvae (Olivar *et al.*, 2001).

The integrated approach has been demonstrated to be a powerful tool for the analysis of population differentiation and so in the resolution of population structure (Begg and Waldman, 1999). This is primarily because genetic techniques are robust tools for identifying reproductive isolation between groups meanwhile morphological (phenotypic) markers are more useful for the detection of short-term variation induced by environmental conditions. Moreover the study of population structure through the analysis of early life stage, in this case the larvae, is innovative and decisive because population genetics of marine planktonic larvae or newborn are spatially and temporally much more dynamic than expected, given that their adult populations are large and apparently well connected by larval dispersal due to differential survival of genotypes after settlement (Koehn *et al.*, 1980; Hedgecock, 1986).

ACKNOWLEDGEMENTS

This study was supported by the Project MAGO funded by Italian Ministry MIPAAF and the FAO Project MedSudMed 'Assessment and Monitoring of the Fishery Resources and the Ecosystems in the Straits of Sicily', funded by the Italian Ministry MIPAAF.

REFERENCES

Agostini, V.N. and Bakun, A. (2002) 'Ocean triads' in the Mediterranean Sea: physical mechanisms potentially structuring reproductive habitat suitability (with example application to European anchovy (*Engraulis encrasicolus*). *Fish Oceanogr.* **11:**129–142.

Alvarado Bremer, J.R., Mejuto, J., Gómez-Márquez, J. *et al.* (2005) Hierarchical analyses of genetic variation of samples from breeding grounds and feeding grounds confirm genetic partitioning northwest Atlantic and South Atlantic population of swordfish (Xiphias gladius L.). *J. Exp. Mar. Biol. Ecol.* **327:**167–182.

Armsworth, P.R. (2002) Recruitment limitation, population regulation, and larval connectivity in reef fish metapopulations. *Ecology* **83:**1092–1104.

Bakun, A. and Parrish, R.H. (1982) Turbulence, transport, and fish in California and Perù currents. *CalCOFI Rep.* **23:**99–112.

Bakun, A. and Parrish, R.H. (1991) Comparative studies of coastal pelagic fish reproductive habitats: the anchovy (*Engraulis anchoita*) of the southwestern Atlantic. *ICES J. Mar. Sci.* **48:**342–361.

Basilone, G., Guisande, C., Patti, B. *et al.* (2006) Effect of habitat conditions on reproduction of the European anchovy (*Engraulis encrasicolus*) in the Strait of Sicily. *Fish Oceanogr.* **15:**271–280.

Basilone, G., Bonanno, A., Patti, B. *et al.* (2013) Spawning site selection by European anchovy (*Engraulis encrasicolus*) in relation to oceanographic conditions in the Strait of Sicily. *Fish Oceanogr.* **22:**309–323.

Begg, G.A. and Waldman, J.R. (1999) An holistic approach to fish stock identification. *Fish. Res.* **43:**35–44.

Bembo, D.G., Carvalho, G.R., Cingolani, N., Arneri, E., Giannetti, G. and Pitcher, T.J. (1996) Allozymic and morphometric evidence for two stocks of the European anchovy *Engraulis encrasicolus* in the Adriatic waters. *Mar. Biol.* **126:**529–538.

Béranger, K., Mortier, L., Gasparini, G.P., Gervasio, L., Astraldi, M. and Crépon, M. (2004) The dynamics of the Sicily Strait: a comprehensive study from observations and models. *Deep Sea Res. Part II Top. Stud. Oceanogr.* **51:**411–440.

Blackith, R.E. and Reyment, R.A. (1971) Multi-variate Morphometrics. New York: Academic Press, p. 412.

Bombace, G. (1992) Fisheries of the Adriatic Sea. In: G. Colombo, I. Ferrari, V.U. Ceccherelli and R. Rossi (eds.). Marine Eutrophication and Population Dynamics: 25th European Marine Biology Symposium. ed.: Olsen and Olsen, Fredensborg, pp. 379–389.

Bonanno, A., Patti, B., Goncharov, S. *et al.* (2005) Fluctuation of sardine and anchovy abundance in the Strait of Sicily investigated by acoustic surveys. *MedSudMed Tech. Doc.* **5:**39–47.

Bonanno, A., Zgozi, S., Cuttitta, A. *et al.* (2013) Influence of environmental variability on anchovy early life stages (*Engraulis encrasicolus*) in two different areas of the Central Mediterranean Sea. *Hydrobiologia* **701:**273–287.

Borja, A., Uriarte, A., Egaña, J., Motos, L. and Valencia, V. (1998) Relationships between anchovy (*Engraulis encrasicolus*) recruitment and environment in the Bay of Biscay (1967–1996). *Fish Oceanogr.* **7:**375–380.

Borrell, Y.J., Jorge, A., Piñera, J.A., Prado, S. and Blanco, G. (2012) Mitochondrial DNA and microsatellite genetic differentiation in the European anchovy (*Engraulis encrasicolus*). *ICES J. Mar. Sci.* **69:**1357–1371.

Borsa, P., Colleta, A. and Duranda, J.D. (2004) Nuclear-DNA markers confirm the presence of two anchovy species in the Mediterranean. *C. R. Biol.* **327:**1113–1123.

Botsford, L.W. and Hastings, A. (2006) Conservation Dynamics of Marine Metapopulations with Dispersing Larvae. In: Marine Metapopulations. J.P. Kritzer and P.F. Sale (eds) Amsterdam: Elsevier Academic Press, pp. 411–429.

Brankart, J.M. (1994) *The MODB Local Quality Control.* Technical Report, University of Liège, Liège, 5 pp.

Cadrin, S.X., Boutillier, J.A. and Idoine, J.S. (2004) A hierarchical approach to determining reference points for Pandalid shrimp. *Can. J. Fish Aquat. Sci.* **61:**1373–1391.

Caneco, B., Silva, A. and Morais, A. (2004) Morphometric variation among anchovy (Engraulis encrasicholus L.) populations from the Bay of Biscay and Iberian waters. *ICES J. Mar. Sci.* **60:**1352–1360.

Cingolani, N., Giannetti, G. and Arneri, E. (1996) Anchovy fisheries in the Adriatic Sea. *Sci. Mar.* **60:**269–277.

Cuttitta, A., Carini, V., Patti, B. *et al.* (2003) Anchovy egg and larval distribution in relation to biological and physical oceanography in the Strait of Sicily. *Hydrobiologia* **503:**117–120.

Cuttitta, A., Maneiro, I., Bonanno, A. *et al.* (2006) Fattori che influiscono sulla distribuzione della biomassa di zooplancton nello Stretto di Sicilia. *Biol. Mar. Medit.* **13:**954–958.

D'Elia, M., Patti, B., Sulli, A. *et al.* (2009) Distribution and spatial structure of pelagic fish schools in relation to the nature of the seabed in the Sicily Straits (Central Mediterranean). *Mar. Ecol. Evol. Persp.* **30:**151–160.

DeVries, D.R. and Frie, R.V. (1996) Determination of age and growth. In: Fisheries Techniques, 2nd edn. B.R. Murphy & D.W. Willis (eds), Bethesda: American Fisheries Society, pp. 483–512.

Diaz, M.V., Pájaro, M. and Sánchez, R.P. (2009) Employment of morphometric variables to assess nutritional condition of Argentine anchovy larvae (*Engraulis anchoita*). *Rev. Biol. Mar. Oceanog.* **44:**539–549.

Dulčić, J. (1995) Spawning of the anchovy, *Engraulis encrasicolus* (L.), in the northern Adriatic Sea in 1989, the year of intensive blooms. *Annales* **7:**51–54.

Ehrlich, K.F., Blaxter, J.H.S. and Pemberton, R. (1976) Morphological and histological changes during the growth and starvation of herring and plaice larvae. *Mar. Biol.* **35:**105–118.

Erdoğan, Z., Turan, C. and Koç, H.T. (2009) Morphologic and Allozyme Analyses of European anchovy (*Engraulis encrasicolus* (L. 1758)) in the Black, Marmara and Aegean Seas. *Acta Adriat.* **50:**77–90.

Excoffier, L., Laval, G. and Schneider, S. (2005) An integrated software package for population genetics data analysis. *Evol. Bioinform. Online* **1:**47–50.

Felsenstein, J. (1985) Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution* **39:**783–791.

Fogarty, N.M., Safari, E., Taylor, P.J. and Murray, W. (2003) Genetic parameters for meat quality and carcass traits and their correlation with wool traits in Australian Merino sheep. *Aust. J. Agric. Res.* **54:**715–722.

Fortibuoni, T., Bahri, T., Camilleri, M., Garofalo, G., Gristina, M. and Fiorentino, F. (2010) Nursery and spawning areas of deep-water rose shrimp, Parapenaeus longirostris (Decapoda: Penaeidae), in the Strait of Sicily (Central Mediterranean Sea). *J. Crustacean Biol.* **30:**167–174.

Frank, K.T. and McRuer, J.K. (1989) Nutritional status of field-collected haddock (Melanogrammus aeglefinus) larvae from Southwestern Nova Scotia: an assessment based on morphometric and vertical distribution data. *Can. J. Fish Aquat. Sci.* **46:**125–133.

Fung, W.K. (1996) Diagnosing influential observations in quadratic discriminant analysis. *Biometrics* **52:**1235–1241.

Garcìa Lafuente, J., Garcìa, A., Mazzola, S. *et al.* (2002) Hydrographic phenomena influencing early life stages of the Sicilian Channel anchovy. *Fish Oceanogr.* **11:**31–44.

Garcìa Lafuente, J., Vargas, J.M., Criado, F., Garcia, A., Delgado, J. and Mazzola, S. (2005) Assessing the variability of hydrographic processes influencing the life cycle of the Sicilian Channel anchovy, *Engraulis encrasicolus*, by satellite imagery. *Fish Oceanogr.* **14:**32–46.

Garcìa, A. and Palomera, I. (1996) Anchovy early life history and its relation to its surrounding environment in the Western Mediterranean basin. *Sci. Mar.* **60:**155–166.

Garofalo, G., Ceriola, L., Gristina, M., Fiorentino, F. and Pace, R. (2010) Nurseries, spawning grounds and recruitment of *Octopus vulgaris* in the Strait of Sicily, central Mediterranean Sea. *ICES J. Mar. Sci.* **67:**1363–1371.

Gasparini, G.P., Ortona, A., Budillon, G., Astraldi, M. and Sansone, E. (2005) The effect of the Eastern Mediterranean Transient on the hydrographic characteristics in the Strait of Sicily and in the Tyrrhenian Sea. *Deep Sea Res. Part II Top. Stud. Oceanogr.* **52:**915–935.

Grant, W.S. (1985) Biochemical genetic stock structure of the southern African anchovy, *Engraulis capensis* Gilchrist. *J. Fish Biol.* **27:**23–29.

Guisande, C., Vergara, A.R., Riveiro, I. and Cabanas, J.M. (2004) Climate change and abundance of the Atlantic-Iberian sardine (*Sardina pilchardus*). *Fish Oceanogr.* **13:**91–101.

Hedgecock, D. (1986) Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? *Bull. Mar. Sci.* **39:**550–564.

Işmen, A. (2001) Use of a discriminant function for the morphometric and meristic separation of whiting stocks, *Merlangius merlangus euxinus*, along the Turkish Black Sea Coast. *Turk J. Zool.* **25:**297–304.

Ivanova, P.P. and Dobrovolov, I.S. (2006) Population – genetic structure on European anchovy (*Engraulis encrasicolus*, Linnaeus, 1758) (Osteichthyes: Engraulide) from Mediterranean Basin and Atlantic Ocean. *Acta Adriat.* **47:**13–22.

Iverson, R.L. (1990) Control of marine fish production. *Limnol. Oceanogr.* **35:**1593–1604.

Koehn, R.K., Newell, R.I.E. and Immermann, F. (1980) Maintenance of an aminopeptidase allele frequency cline by natural-selection. *Proc. Natl Acad. Sci. USA* **77:**5385–5389.

Kristoffersen, J.B. and Magoulas, A. (2008) Population structure of anchovy *Engraulis encrasicolus* L. in the Mediterranean Sea inferred from multiple methods. *Fish. Res.* **91:**187–195.

Levi, D., Andreoli, M.G., Arneri, E., Giannetti, G. and Rizzo, P. (1994) Otolith reading as a tool for stock identification. *Fish. Res.* **20:**97–107.

Lleonart, J., Salat, J. and Torres, G.J. (2000) Removing allometric effects of body size in morphological analysis. *J. Theor. Biol.* **205:**85–93.

Magoulas, A., Tsimenides, N. and Zouros, E. (1996) Mitochondrial DNA phylogeny and the reconstruction of the population history of a species: the case of the European Anchovy (*Engraulis encrasicolus*). *Mol. Biol. Evol.* **13:**178–190.

Magoulas, A., Castilhob, R., Caetanob, S., Marcatoc, S. and Patarnelloc, T. (2006) Mitochondrial DNA reveals a mosaic pattern of phylogeographical structure in Atlantic and Mediterranean populations of anchovy (*Engraulis encrasicolus*). *Mol. Phylogenet. Evol.* **39:**734–746.

Morello, E.B. and Arneri, E. (2009) Anchovy and sardine in the Adriatic Sea – an ecological review. *Oceanogr. Mar. Biol. Annu. Rev.* **47:**209–256.

Nei, M. (1987) Molecular Evolutionary Genetics. New York: Columbia University Press, pp. 287–326.

Olivar, M.P., Salat, J. and Palomera, I. (2001) A comparative study of spatial distribution patterns of the early stages of anchovy and pilchard in the NW Mediterranean Sea. *Mar. Ecol. Prog. Ser.* **217:**111–120.

Palomera, I. and Rubies, P. (1996) The European anchovy and its environment. *Sci. Mar.* **60:**1–277.

Pappalardo, A.M., Guarino, F., Reina, S., Messina, A. and De Pinto, V. (2011) Geographically widespread swordfish barcode stock identification: a case study of its application. *PLoS ONE* **6:**e25516.

Patti, B., Bonanno, A., Basilone, G. *et al.* (2004) Interannual fluctuations in acoustic biomass estimates and in landings of small pelagic fish populations in relation to hydrology in the Strait of Sicily. *Chem. Ecol.* **20:**365–375.

Patti, B., Quinci, E.M., Bonanno, A., Basilone, G. and Mazzola, S. (2010) Anchovy, *Engraulis encrasicolus*, GSA 16 (South of Sicily), presented at General Fisheries Commission for the Mediterranean, Sub-Committee On Stock Assessment (SCSA). Report of the SCSA Working Group on Stock Assessment of small Pelagic Species – Campobello di Mazara, 1–6 November 2010.

Pauly, D. (1980) On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. *J. Cons. Int. Explor. Mer.* **39:**175–192.

Peakall, R. and Smouse, P.E. (2006) Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6:**288–295.

Pollar, M., Jaroensutasinee, M. and Jaroensutasinee, K. (2007) Morphometric analysis of *Tor tambroides* by stepwise discriminant and neural network analysis. *World Acad. Sci. Eng. Technol.* **9:**111–115.

Powell, A.B. and Chester, A.J. (1985) Morphometric indices of nutritional condition and sensitivity to starvation of spot larvae. *Trans. Am. Fish. Soc.* **114:**338–347.

Ré, P. (1986) Otolith microstructure and detection of life history events in sardine and anchovy larvae. *Ciénc. Biol. Ecol. Syst.* **6:**9–17.

Regner, S. (1996) Effects of environmental changes on early stages and reproduction of anchovy in the Adriatic Sea. *Sci. Mar.* **60:**167–177.

Robinson, A.R., Sellschopp, J., Warn-Varnas, A. *et al.* (1999) The Atlantic ionian stream. *J. Mar. Syst.* **20:**113–128.

Sabatés, A., Olivar, M.P., Salat, J., Palomera, I. and Alemany, F. (2007) Physical and biological processes controlling the distribution of fish larvae in the NW Mediterranean. *Prog. Oceanogr.* **74:**355–376.

Sammari, C., Millot, C., Taupier-Letage, I., Stefani, A. and Brahim, M. (1999) Hydrological characteristics in the Tunisia–Sardinia–Sicily area during spring 1995. *Deep Sea Res. Part I Oceanogr. Res. Pap.* **46:**1671–1703.

Santojanni, A., Arneri, E., Barry, C. *et al.* (2003) Trends of anchovy (*Engraulis encrasicolus*, L.) biomass in the northern and central Adriatic Sea. *Sci. Mar.* **67:**327–340.

Sanz, N., García-Marín, J.L., Viñas, J., Roldán, M. and Pla, C. (2008) Spawning groups of European anchovy: population structure and management implications. *ICES J. Mar. Sci.* **65:**1635–1644.

Shevchenko, N.F. (1980) Geographical changing of anchovy *Engraulis encrasicolus* (L.) in the Mediterranean basin (in Russian). *Icthyol. J.* **20:**20–30.

Somarakis, S. and Nikolioudakis, N. (2007) Oceanographic habitat, growth and mortality of larval anchovy (*Engraulis encrasicolus*) in the northern Aegean Sea (eastern Mediterranean). *Mar. Biol.* **152:**1143–1158.

Somarakis, S., Palomera, I., Garcia, A. *et al.* (2004) Daily egg production of anchovy in European waters. *ICES J. Mar. Sci.* **61:**944–958.

Sorgente, R., Olita, A., Oddo, P., Fazioli, L. and Ribotti, A. (2011) Numerical simulation and decomposition of kinetic energy. *Ocean Sci.* **7:**503–519.

Tamura, K. and Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **10:**512–526.

Theilacker, G.H. (1978) Effect of starvation on the histological and morphological characteristics of jack mackerel, *Trachurus symmetricus*, larvae. *Fish. Bull.* **76:**403–414.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25:**4876–4882.

Thorpe, R.S. (1975) Quantitative handling of characters useful in snake systematics with particular reference to intraspecific variation in the Ringed Snake Natrix natrix (L.). *Biol. J. Linn. Soc.* **7:**27–43.

Traina, A., Basilone, G., Saborido-Rey, F. *et al.* (2011) Assessing population structure of European Anchovy (*Engraulis encrasicolus*) in the Central Mediterranean by means of traditional morphometry. *Adv. Oceanogr. Limnol.* **2:**141–153.

Tudela, S. (1999) Morphological variability in a Mediterranean, genetically homogeneous population of the European anchovy, *Engraulis encrasicolus*. *Fish. Res.* **42:**229–243.

Vasconcellos, M.C. and Castello, J.P. (1996) Distribucion de larvas de anchoita (*Engraulis anchoita*) y el ambiente oceanografico en el sur de *Brasil. Atlantica.* **18:**77–100.

Viñas, J.R., Alvarado Bremer, J. and Pla, C. (2004) Phylogeography of Atlantic bonito (*Sarda sarda*) in the northern Mediterranean: the combined effects of historical vicariance, population expansion, secondary invasion and isolation by distance. *Mol. Phylogenet. Evol.* **33:**32–42.

Viñas, J., Sanz, N., Peñarrubia, L. *et al.* (2014) Genetic population structure of European anchovy in the Mediterranean Sea and the Northeast Atlantic Ocean using sequence analysis of the mitochondrial DNA control region. *ICES J. Mar. Sci.* **71:**391–397.

Whitehead, P.J.P., Nelson, G.J. and Wongratana, T. (1988) FAO species catalogue. Vol 7 Clupeoid fishes of the world (suborder Clupeioidei). An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads, anchovies and wolf-herrings. Part 1 – Chirocentridae, Clupeidae and Pristigasterdae. *FAO Fish Synop.* **125:**305–579.

Winans, G.A. (1984) Multivariate morphmetric variability in Pacific salmon: technical demonstration. *Can. J. Fish Aquat. Sci.* **41:**1150–1159.

Xia, Y., Yang, P., Sun, Y. *et al.* (2003) One-dimensional nanostructures: synthesis, characterization, and applications. *Adv. Mater.* **15:**353–389.

Zarraonaindia, I., Iriondo, M., Albaina, A. *et al.* (2012) Multiple SNP markers reveal fine-scale population and deep phylogeographic structure in European anchovy (*Engraulis encrasicolus* L.). *PLoS ONE* **7:**e42201.

Zarraonaindia, I., Smith, D.P. and Gilbert, J.A. (2013) Beyond the genome: community-level analysis of the microbial world. *Biol. Philos.* **28:**261–282.