water

MDPI

Article

Inferring Population Structure from Early Life Stage: The Case of the European Anchovy in the Sicilian and Maltese Shelves

Angela Cuttitta [1], Bernardo Patti [2], Marianna Musco [1], Tiziana Masullo [1], Francesco Placenti [3], Enza Maria Quinci [3], Francesca Falco [4], Carmelo Daniele Bennici [1], Marilena Di Natale [1], Vito Pipitone [1], Matteo Cammarata [5], Isabel Maneiro [3], Stefania Russo [1,*] and Marco Torri [1]

1 Istituto di Studi sul Mediterraneo, Consiglio Nazionale delle Ricerche (ISMed CNR), SS di Palermo, Via Filippo Parlatore, 65, 90145 Palermo, Italy; angela.cuttitta@ismed.cnr.it (A.C.); marianna.musco@ismed.cnr.it (M.M.); tiziana.masullo@ismed.cnr.it (T.M.); carmelo.bennici@ismed.cnr.it (C.D.B.); marilena.dinatale@ismed.cnr.it (M.D.N.); vito.pipitone@ismed.cnr.it (V.P.); marco.torri@ismed.cnr.it (M.T.)
2 Istituto per lo Studio degli Impatti Antropici e Sostenibilità in Ambiente Marino, Consiglio Nazionale delle Ricerche (IAS CNR), SS di Palermo, Complesso Monumentale ex-Roosevelt, Lungomare Cristoforo Colombo 4521, Loc. Addaura, 90149 Palermo, Italy; bernardo.patti@ias.cnr.it
3 Istituto per lo Studio degli Impatti Antropici e Sostenibilità in Ambiente Marino, Consiglio Nazionale delle Ricerche (IAS CNR), SS di Capo Granitola, Via del Mare 3, Torretta Granitola, 91021 Campobello di Mazara, Italy; francesco.placenti@ias.cnr.it (F.P.); enza.quinci@ias.cnr.it (E.M.Q.); isabelmaneiro@gmail.com (I.M.)
4 Istituto per le Risorse Biologiche e le Biotecnologie Marine del Consiglio Nazionale delle Ricerche (IRBIM CNR), Via Luigi Vaccara, 61, 91026 Mazara del Vallo, Italy; francesca.falco@irbim.cnr.it
5 Università degli Studi di Palermo DISTEM Department, Via Archirafi, 22, 90123 Palermo, Italy; matteo.cammarata@unipa.it
* Correspondence: stefania.russo@ismed.cnr.it; Tel.: +39-0916810761

Abstract: The European anchovy is an important fishing resource in the Sicilian Channel that supports a high recruitment success variability. The presence of two spawning areas, the drifting of the larvae along the currents and the different oceanographic conditions within the region suggest the presence of different larvae subpopulations. Morphometric and biochemical approaches have been used to analyze the differences among larvae collected. The amino acid composition discriminates two larval groups closely related to the spawning regions: Adventure Bank and the shelf between the South of Sicily and Malta. In addition, there are morphometric and growth differences between recently hatched larvae in these two regions, reinforcing the hypothesis of two larval subpopulations and suggesting differences in the parental reproduction effort. Between the South of Sicily and Malta there are growth and biochemical composition differences since larvae from the Maltese coast present a higher protein content and a bigger growth rate than those from Sicily, pointing out that Malta is an area with a better nutritional condition environment. No differences in the growth rate have been observed between the Adventure Bank area and the Maltese shelf, therefore, a diverse nutritional condition cannot be suggested between these two areas despite the Maltese larvae having a higher protein content present.

Keywords: *Engraulis encrasicolus*; ichthyoplankton; amino acid; morphometry; population dynamics; biochemical composition; fisheries management; Sicilian Channel

check for updates

Citation: Cuttitta, A.; Patti, B.; Musco, M.; Masullo, T.; Placenti, F.; Quinci, E.M.; Falco, F.; Bennici, C.D.; Di Natale, M.; Pipitone, V.; et al. Inferring Population Structure from Early Life Stage: The Case of the European Anchovy in the Sicilian and Maltese Shelves. *Water* **2022**, *14*, 1427. https://doi.org/10.3390/w14091427

Academic Editor: José Luis Sánchez-Lizaso

Received: 31 March 2022
Accepted: 23 April 2022
Published: 29 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

The European anchovy (*Engraulis encrasicolus*) is one of the most important fishing resources in the Mediterranean Sea, making up about 50% of the total Mediterranean landings [1]. This means anchovy fisheries have an important economical role in local fishing communities, which are strongly affected by the observed annual variability in the volume of catches and the declining trends of stock abundance [2].

It is well known that in pelagic fish, production results mainly from fish growth and recruitment [3]. Like most pelagic species, the anchovy has a short life cycle, thus supporting a high recruitment variation [4,5] due to habitat constraints and their effects on the survival of early life stages [6–12] These conditions for fish production lead to the European anchovy being very susceptible to overexploitation as it was indicated by SGMED [2]. It has been pointed out that most Mediterranean stocks are fully exploited, particularly stocks in the Sicilian Channel that are considered to be in poor condition due to overexploitation [1]. Thus, as it has been previously shown, knowledge on recruitment success and body growth is necessary for an appropriate fisheries management of European anchovy in the Mediterranean Sea.

The Sicilian southern coast is a stable area with a weak current, produced by the impingement against the coast of the Atlantic–Ionian Stream (AIS) [13]. Although the current path fluctuates throughout the years, during the spawning period it is generally bound by one cyclonic eddy located in the northwestern region over the Adventure Bank, another one is situated offshore in the west of the Maltese Shelf and a third one is located in the Capo Passero region [13,14]. It is known that eddies contribute to larval retention as was indicated in Brazilian anchovies by Macedo-Soares et al. [15]. In agreement with the presence of eddies in the Strait of Sicily, Cuttitta et al. [16] suggested that the distribution pattern of anchovy eggs and larvae are linked to the AIS path and distance from the shoreline. Basilone et al. [17] confirmed the presence of two coastal recurrent spawning areas for anchovy, one next to the Adventure Bank in the area between Mazara del Vallo and Agrigento, and the second in the SE region, around the southernmost tip of the Sicilian coast, although it has to be pointed out that the variability of this current influences the distribution of anchovy eggs in the area. Even though the AIS can transport eggs and larvae towards the south-east end of the Sicilian coast (Capo Passero) [18–20], the presence of two different egg laying areas associated with stable oceanographic conditions suggests that two different larvae subpopulations could co-exist. In fact, Basilone et al. [17] indicated that there are both temperature and primary productivity differences between the two spawning areas, but whether such conditions increase egg production by the adults, survival and growth of the early life stages is still to be studied and modeled. In agreement with this hypothesis, Cuttitta et al. [3] observed different amino acid compositions of anchovy larvae among these areas in the Sicilian Channel. This spatial complexity of subpopulations within a stock may lead to the erosion of subpopulation units, with unknown consequences on stock viability [21].

Varieties of approaches have been used to discriminate fish populations, including anatomical [22–26], biochemical [3,27–30] and genetic tools [24,31–34]. Some studies have observed differences in stock discrimination using a different methodology [23,24,32,35], indicating that comparative studies based on the same samples are relevant and informative [36]. Nevertheless, it is confirmed that fish populations can be successfully discriminated using molecular tools [37] and European/Mediterranean anchovies present genetic divergence [33,38]. Genetic markers do not appear to be a good approach to study the presence of diverse larval subpopulations since the absence of a significant genetic structure does not indicate a real panmixia as it was pointed out by Abaunza et al. [31]. In contrast, the plasticity of fish allows them to respond adaptively to environmental changes by modifications in their physiology and behavior, which lead to changes in their morphology, reproduction or survival that mitigate the effects of environmental change [39]. This means morphological and biochemical tools are optimal to detect larval subpopulations adapted to different environmental conditions. Shape analysis has been widely used to discriminate fish populations/stocks and both morphometry and otolith shape are the traditional and most common measurements used to identify stocks although, to the best of our knowledge, it has been only used once to discriminate larval subpopulations [34]. Both approaches properly discriminate adult stocks, although body shape seems more discriminating in phenotypic groups [26].

Moving on to biochemical methods, these have already been used to distinguish larval populations [28,30,40]. Specifically, the amino acid composition (AAC) of marine fish has been successfully used to separate *Sardina pilchardus* larvae from diverse spawning areas [28]. Additionally, Cuttitta et al. [3] discriminated anchovy larvae from different areas in the Sicilian Channel using AAC. These authors firstly observed a different amino acid quantity and composition for anchovy larvae among different spawning grounds in the Strait of Sicily.

In examining the larval population of *Engraulis encrasicolus* under different environmental scenarios in the South coast of Sicily, the present study aims at giving further insight into the differences among the sub-populations of early life stages occupying regions characterized by different temperature and food conditions through the combined analysis of their morphology and biochemical content. The results are expected to provide new information on larval biology in relation to the spatial distribution, with potential application in support of the needs of fisheries management of this important fish resource.

2. Materials and Methods

2.1. Sampling Area

The ichthyoplankton sampling was carried out onboard the research vessel "Urania" in July 2009, during the peak spawning period of the anchovy in the study area [41]. Figure 1 shows the location of sampling stations and sub-areas. Six different areas were established: the five areas already described by Cuttitta et al. [3] and another one close to the Maltese shelf. Four areas (1, 2, 3 and Malta) are located over the continental shelf within the 200 m isobaths, representing the main spawning habitat for anchovies [17,42], whereas in areas 4 and 5 the bottom depth is greater than 200 m.

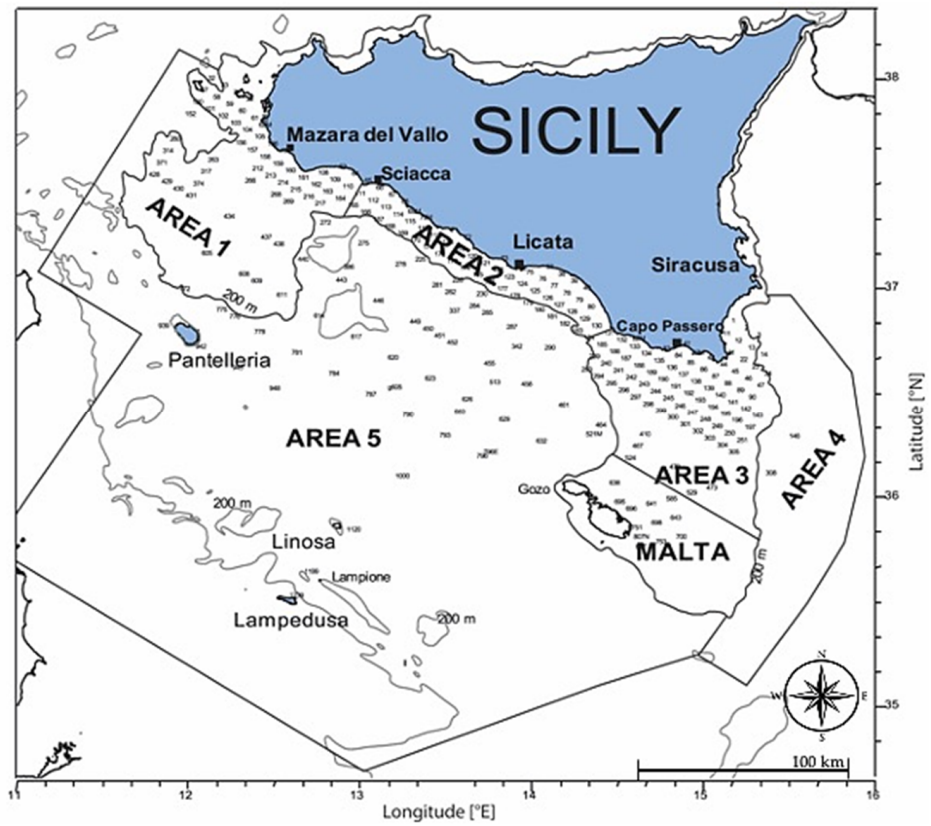

Figure 1. Sampling map. Map of the Sicilian Channel, showing the sampling stations and the six sampling areas (1, 2, 3 and Malta are within the 200 m isobaths, 4 and 5 at bottom depths >200 m).

2.2. Larval Sampling

Ichthyoplankton samples were collected using a Bongo 40 net with mesh size of 200 µm. The Bongo net was towed at 2 knots in oblique hauls. A total of 420 anchovy larvae were sorted and transferred to individual plastic vials containing 70% ethanol and conserved at 4 °C; however, to the aims of biochemical analyses, a sub-sample of 75 larvae were immediately fixed in liquid nitrogen and transferred to a −80 °C freezer on land.

2.3. Morphometric Analysis

Multivariate morphometry was used to describe the anchovy larvae from a dimensional point of view [43]. A total of 385 out of the sorted larvae were placed individually and photographed under a binocular stereo microscope. In order to obtain the morphometric characters, the images were processed using the Image Pro Plus 6.0 software (Media Cybernetics, Rockville, MD USA, Roper Industries, image analysis and processing). The parameters acquired were: standard length (SL), total length (TL), body diameter (BD), head length (HL), eye diameter (ED) [44] and anal length (AL), the latter being a typical criterion for identifying this larval species [34]. In order to analyze size-frequency distributions, 9 different size classes were stabilized according to Sturges' rule [45]. The smallest larva found in samples was 2.68 mm (TL) long and the biggest one 16.05 mm, therefore, 1.5 mm intervals were used, starting at 2.6 mm.

To remove the size component and allometric effects, all measurements were normalized to a standard body size, following the procedure by Lleonart et al. [46]. This method derives from the theoretical equations of allometric growth and removes information related to size, scaling all individuals to the same size (in this case, mean TL of collected material from all stations).

A random forest analysis [47,48] was subsequently applied in order to verify if the normalized morphometric parameters were able to discriminate among the different zones. The selection of the most important variables in the RF was made using a procedure that carries out variable elimination using the out-of-bag (OOB) error as a minimization criterion [49]. Using 999 iterations, we determined how often the variables were included in the final model [49]. The most commonly selected variables were used to develop a final random forest model [48], from which the confusion matrix and the accuracy were calculated.

2.4. Age Determination

The interpretation of otolith microstructure is the main tool for the study of age in the early life stages of fish due to the deposition of microscopic rings during growth. These rings are characterized by typical alternating opaque and hyaline layers that are deposited daily [50] as it was confirmed by Aldanondo et al. [51] for European anchovy. A total of 234 otoliths from the larvae also used for morphometric analysis were extracted under a binocular microscope and read using a light microscope, starting from the core outwards. Lab experiments confirm that the first increment ring lays down at the hatching [51], therefore, the number of daily increments (DI) corresponds to the larval age in days.

2.5. Growth Rate Estimation

Growth of the larvae population was described through the use of the Gompertz's growth equation, in order to relate size to age [52] in each one out of the six sub-regions in the study area (Figure 1). In addition, the individual growth rate (IGR, in mm·d^{-1}) was estimated from the time of hatching following the equation proposed by Takahashi and Watanabe [8] and modified by Costalago et al. [53]:

$$IGR = (SL - SL_0)/DI$$

where SL is the standard length of the larva, DI is the age of the larvae and SL_0 is the standard length at hatching, estimated at 2.5 mm in agreement with laboratory experiments

performed by Regner (1985) [54] and Rè (1999) [55]. A bootstrap sample (with replacement) was generated from the original data to estimate a 95% confidence interval of the growth parameter for each fitted Gompertz equation in order to assess whether there were significant differences in larval growth among the different zones.

2.6. Larval Amino Acid Composition

Larval amino acid composition was analyzed by high-performance liquid chromatography (HPLC) using a Shimadzu System, a Shimadzu RF-10-AXL fluorescence detector and a rp C18 column following the method described by Shimadzu with slight modifications (application 2008). The amino acid standard mix SIGMA AA-S-18 was used for the identification and quantification of amino acids. Ten amino acids were analyzed: aspartic acid, glutamic acid, glycine, serine, arginine, threonine, alanine, lysine, isoleucine and phenylalanine. Following Guisande et al. [56] and Brucet et al. [57], each amino acid was analyzed as a percentage of the total amino acid content.

To determine whether it was possible to discriminate among areas according to the amino acid composition of the larvae, a random forest analysis was applied on relative composition values (percentages) related to the total amino acid content. Using 999 interactions, a variable selection method based on minimization of the error rate was used to determine the most important amino acids for area discrimination [49]. The proximity matrix on selected variables was used to obtain multi-dimensional scaling (MDS) plots in two dimensions, which were useful in identifying groups and in comparing the observed areas.

2.7. Larval Organic Content

Each larva was transferred to an ultracentrifuge plastic tube with 1000 µL of bi-distilled water. Samples were homogenized using a pipette tip adapted to fit the shape of the vial. A volume of 200 µL of each homogenate was used for protein analysis, 400 µL for carbohydrate analysis and 75 µL for lipid analysis. The method described by Lowry et al. [58] and modified by Maxwell et al. [59] was used to analyze the protein content. Carbohydrate content was measured by the phenol-sulphuric acid method [60] and lipid content was determined using the sulphophosphovanillin method described by Zöllner and Kirsch [61]. Few larvae were available for these analyses in 2009, therefore, additional larval specimens from oceanographic cruises carried out in the same study area and with the same sampling protocol in summers 2005 and 2008 were also used for the determination of organic components. As biochemical compositions of larvae are dominated by ontogenic trends [62], leading to different types of best fitting models among studies [11,63], non-parametric generalized additive models (GAM) [64,65] were applied to relate the organic content (lipids, proteins and carbohydrates) and the size (TL) of the larvae. Two different models were applied to each element of larval organic content: the first model used size as an independent variable, the other one used the interaction between size and zone. The comparison between the two models was made to evaluate whether the interaction was more suitable to explain the variability of the organic content than the single effect of the size. The selection of the final model was based on the minimization of the AIC (Akaike's Information Criterion) [66].

3. Results

3.1. Oceanographic Conditions and Larval Density

Table 1 shows the average (± sd) values observed for temperature, salinity and fluorescence of the first 20 m depth of the water column by sampling area, including only the stations where anchovy larvae were found in planktonic samples.

Table 1. Environmental conditions. Mean (±sd) temperature, salinity and fluorescence within the first 20 m. Different superscript letters after temperature values indicate statistically significant difference between pairs of areas (permutational pairwise *t* test, *p*-value < 0.05).

	Temperature (°C)	Salinity (°/oo)	Fluorescence
Area1	18.80 (±2.74) [a]	37.69 (±0.16)	0.034 (±0.008)
Area2	19.20 (±0.88) [a]	37.64 (±0.12)	0.028 (±0.005)
Area3	21.89 (±1.67) [b]	37.64 (±0.23)	0.026 (±0.005)
Area4	22.26 (±0.67) [b]	38.17 (±0.25)	0.034 (±0.004)
Area5	22.58 (±1.45) [b]	37.52 (±0.14)	0.028 (±0.004)
Malta	24.49 (±0.31) [c]	37.60 (±0.02)	0.033 (±0.009)

Permutational analysis of variance (PERMANOVA; Anderson, 1998) indicated that there were statistically significant differences in the temperature among areas (F = 63.479; $p < 0.001$) (Table 1). Three different groups were detected in relation to temperature regime: a first group formed by areas 1 and 2, with the lowest average temperature; the Malta region, where the warmest waters were recorded; and an intermediate-temperature group, encompassing areas 3, 4 and 5.

The horizontal temperature pattern is shown in Figure 2. Four different upwelling processes were detected in the region during the survey period. Three of them were coastal phenomena located in areas 1 and 2 while the other reflected the upwelling regime associated to the cyclonic eddy on the Maltese shelf.

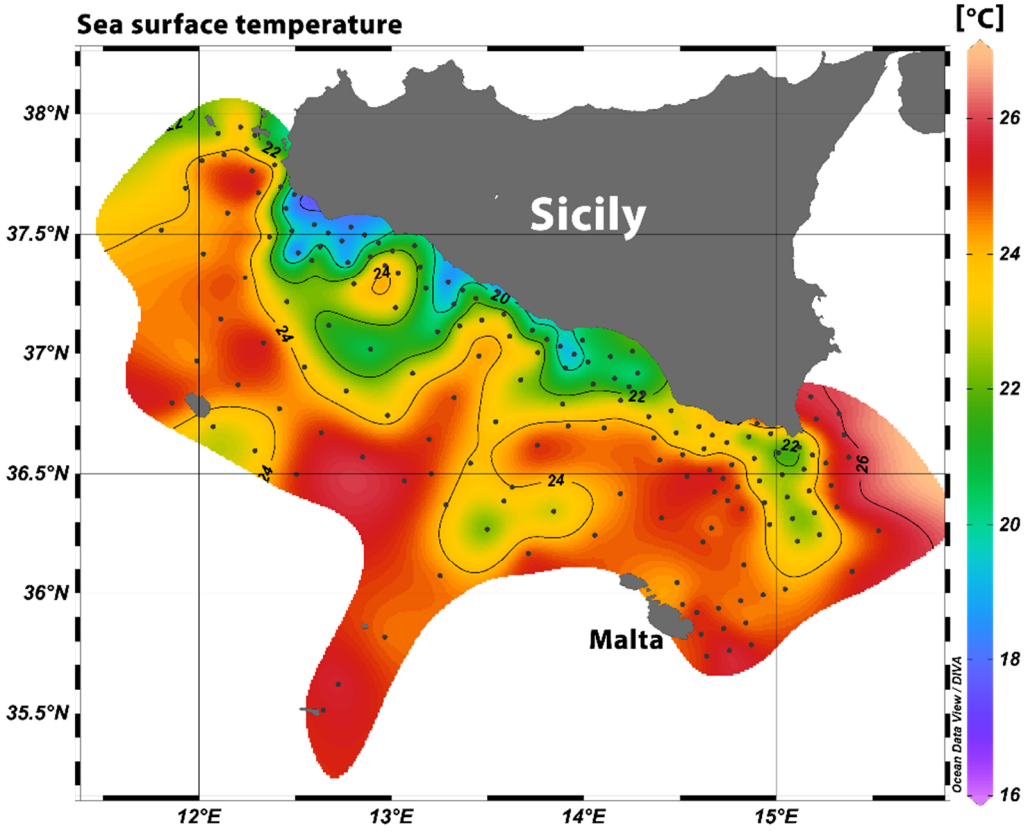

Figure 2. Map of the Sicilian Channel showing the surface temperature (units in °C) in the study area.

Higher larval densities were recorded in area 3, 5 and Malta, while few larvae were found in areas 1, 2 and 4 as it is shown in Figure 3.

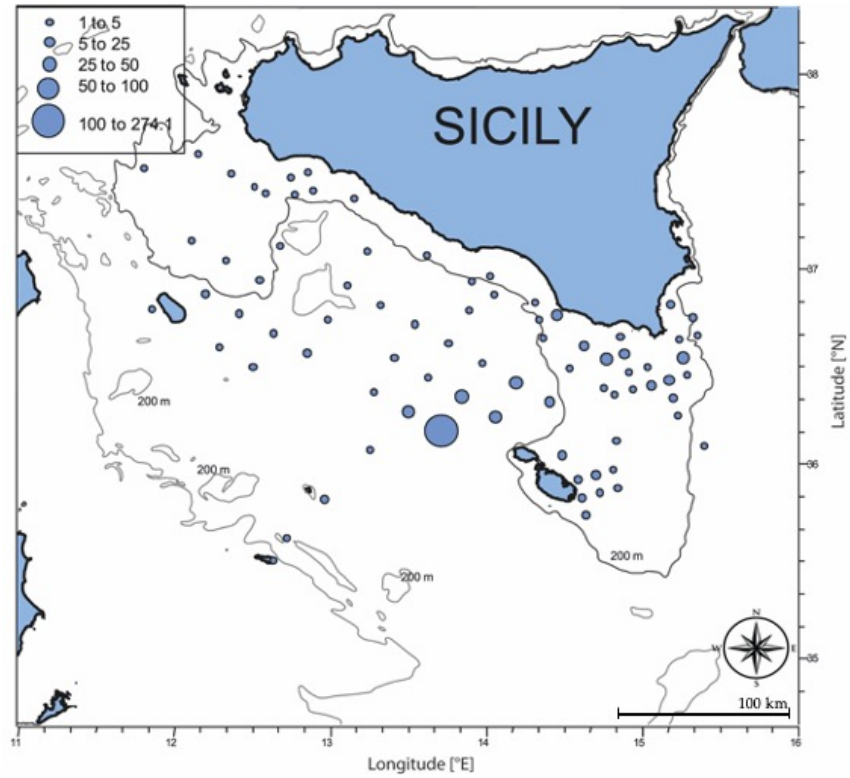

Figure 3. Abundance of anchovy larvae in the sampling stations of survey carried out in summer 2009. The size of filled circles is proportional to the number of larvae found in planktonic samples.

3.2. Morphometry

The total length (TL) distributions of the larvae collected in each sub-area are represented by box-plots in Figure 4A. PERMANOVA showed that there were statistically significant differences among the zones ($F = 65.861$; $p < 0.001$). In addition, pairwise tests indicated that the larval size was smaller in area 2 than in each one of the other areas (p-value < 0.05 in all cases), that the larval size in area 3 was significantly different compared to the other areas, except for area 1, and that there was a statistically higher larval size in Malta in relation to all areas, except for area 4 ($p < 0.05$).

Larval size distribution was also different among the areas, as shown in Figure 4B. In area 1 and area 2, the collected larval specimens were smaller than 8.6 mm, whereas in area 4, all larvae were greater than 10 mm. The normalized measurements, by area, of the morphometric parameters AL, HL, BD and ED (the mean TL value of all larvae, 7.95 mm, was used to obtain standardized values), are shown in Figure 5. Despite the size effect correction, there were statistically significant differences in all normalized morphometric parameters among the areas (PERMANOVA, $p < 0.05$ in all cases). Results of the pairwise tests are shown in Table 2. Two groups were distinguished in terms of AL (areas 3–5 with higher values and areas 2 and Malta with lower values); other groups were different in terms of BD (areas 2–5 with higher values and areas 3–4 and Malta with lower values) and in terms of ED (areas 2–5 with higher values and areas 3–4 with lower values). Areas 5 and Malta were statistically different in terms of HL only.

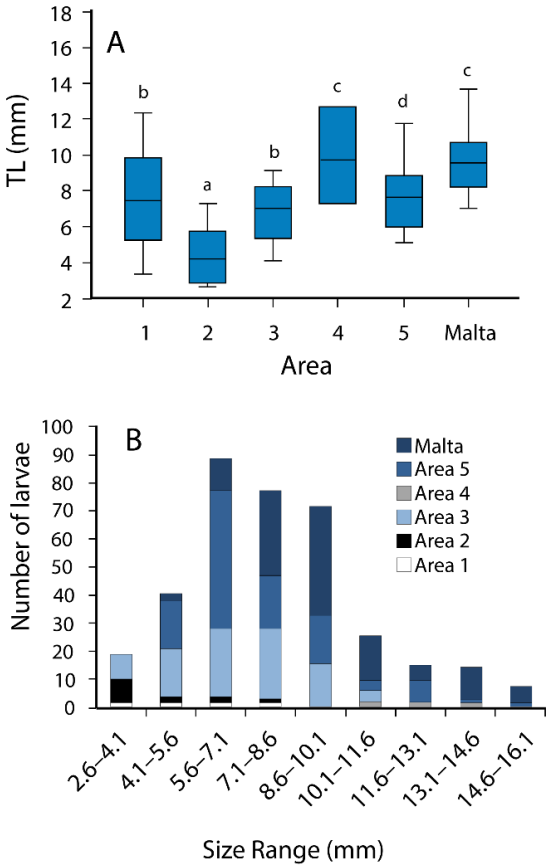

Figure 4. Mean total length (TL) and size distribution of sampled larvae. (**A**) Box plot of the TL among different areas. The bottom and top of the boxes are the first and third quartiles, while the whiskers indicate the 10 and 90 percentiles, and the band inside is the mean value. Lowercase letters (a–d) indicate statistically significant difference between the areas (pairwise tests, p-value < 0.05). (**B**) Histogram of size distribution of larval abundance (n) in the six considered areas.

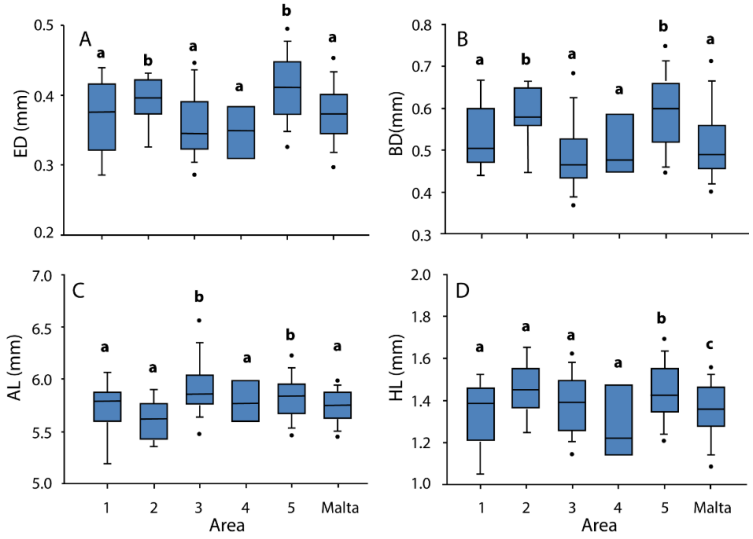

Figure 5. Box plots of the normalized morphometric parameters (eye diameter, ED (**A**); body diameter, BD (**B**); anal length, AL (**C**); head length, HL (**D**)), by area. The bottom and top of the boxes are the first and third quartiles, while the whiskers indicate the 10 and 90 percentiles, and the band inside is the mean value. Black points indicate outliers. Lowercase letters (a–c) indicate statistically significant difference between the areas (pairwise tests, p-value < 0.05).

Table 2. Results of permutational pairwise *t* test on normalized morphometric parameters between areas. "-" indicates non-significant differences, "?" indicates a *p*-value between 0.05 and 0.1, while "*" indicates *p*-value < 0.05.

	Zone2				**Zone3**				**Zone4**				**Zone5**				**Malta**			
	AL	**HL**	**BD**	**ED**	**AL**	**HL**	**BD**	**ED**	**AL**	**HL**	**BD**	**ED**	**AL**	**HL**	**BD**	**ED**	**AL**	**HL**	**BD**	**ED**
Zone1	-	-	-	-	**?**	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-
Zone2					*	-	*	*	-	-	*	*	*	-	-	-	**?**	**?**	*	-
Zone3									-	-	-	-	**?**	**?**	*	*	*	-	-	*
Zone4													-	-	*	*	-	-	-	*
Zone5																	*	*	*	*

Random forest analysis showed that 53.89% of samples were correctly classified by morphometry and confirms there were differences among the areas. The selection procedure involved the inclusion of ED, BD and AL (in order of importance) in the discrimination among areas excluding HL. Areas 1 and 4 were incorrectly classified at 100% and area 2 was highly confused, especially with areas 5 and Malta. Higher performance was found in areas 5 and Malta, where more than 60% of cases were correctly classified (Table 3).

Table 3. Morphometry confusion matrix. Classification applied on normalized morphometric parameters by random forest analysis. Bold numbers indicate the number of larvae correctly classified.

		Number of Larvae Classified into Estimated Groups						
	Area	***1***	***2***	***3***	***4***	***5***	***Malta***	***% Correctly Classified***
Number of larvae classified into observed areas	*1*	**0**	0	3	0	5	4	0
	2	0	**1**	1	0	6	5	7.69
	3	0	0	**48**	0	21	24	51.62
	4	0	0	1	**0**	1	3	0
	5	1	1	16	0	**72**	29	60.5
	Malta	0	1	18	0	26	**73**	61.86

3.3. Larval Growth

The relationship between TL and age in areas 3, 5 and Malta is shown in Figure 6. The estimated growth rates by area estimated by Gompertz's model were equal to 1.17 (Bayesian confidence interval: [1.04–1.29]) in area 3, 1.73 (Bayesian confidence interval: [0.70–2.76]) in area 5 and 1.61 (Bayesian confidence interval: [1.33–1.88]) in Malta. This means that, even though the growth rate value was lower in area 3 than in the other two areas, a statistically significant difference was only found with Malta, due to the wide confidence interval of estimated growth rate in area 5. In addition, statistically significant differences between area 3 and Malta ($p < 0.01$) were also found with a permutation *t* test on individual growth (IGR). The analysis of recently hatched larvae (age = 1 day) only from the two spawning regions suggested for the Sicilian Channel (the western one represented by areas 1, 2 and 5, and the eastern one represented by areas 3 and Malta), revealed a significant difference in daily growth (IGR) of the larvae between regions (Figure 7B, permutational *t* test = −4.089, $p < 0.05$), linked to the significant difference in total length of one day old individuals (Figure 7A, permutational *t* test = −3.953, $p < 0.05$).

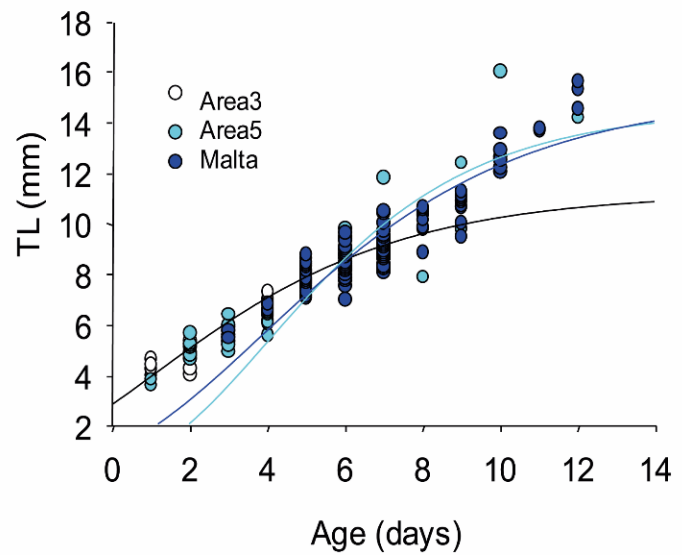

Figure 6. Relationship between total length (TL) and age of larvae from areas 3, 5 and Malta. Lines represent curves fitted by Gompertz's model by area.

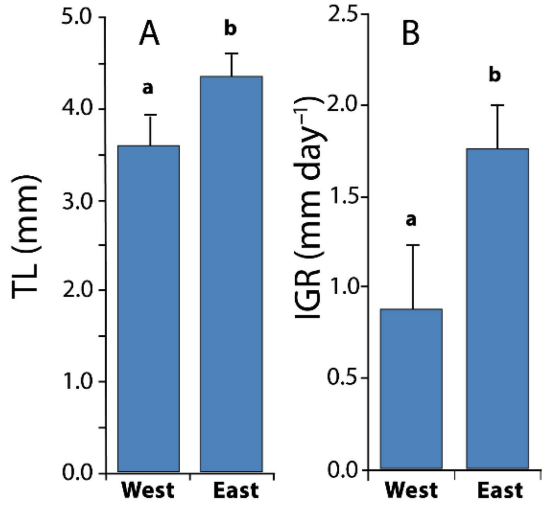

Figure 7. Size and growth of recently hatched larvae from the western and the eastern spawning areas. (**A**) Total length (TL). (**B**) Individual growth rate (IGR). Lowercase letters (a,b) indicate statistically significant difference between the spawning areas (permutational *t* test, *p*-value < 0.05).

3.4. Biochemical Composition of Larvae

No statistical differences were found in the amino acid fractions among areas (PERMANOVA, $p > 0.05$ in all cases). Nonetheless, random forest analysis based on AAC showed a moderate performance in classifying the samples by area (error rate = 36.25%). Figure 8 shows the score of the first two dimensions in the random forest analysis. Area 5 was completely differentiated from the other areas, which were all overlapping. The confusion matrix (Table 4) confirmed this result, showing that the larvae from area 5 were the most correctly classified (95.22%), followed by larvae from areas 3 and Malta (38.5% and 35.3%, respectively), that present a high rate classification confusion between them. Areas 2 and 4 were very confused and area 1 presented a high classification error percentage (83.33%, highly confused with the Malta area). The most important variables for the classification are in order of importance, glycine, alanine, threonine and lysine.

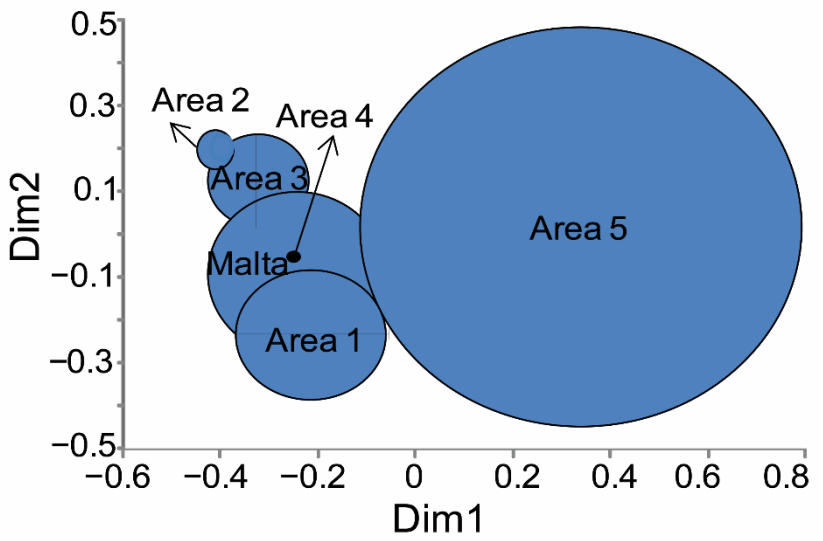

Figure 8. Amino acid random forest analysis. Plot of the mean ± sd of the first 2 dimensions' scores obtained with the random forest performed on the amino acid composition in the different studied areas.

Table 4. Confusion matrix resulting from random forest analysis on amino acid compositions. Bold numbers indicate correctly classified larvae.

		Number of Larvae Classified into Estimated Groups						
	Area	*1*	*2*	*3*	*4*	*5*	*Malta*	*% Correctly Classified*
Number of larvae classified into observed areas	*1*	**1**	1	0	0	1	3	16.67
	2	0	**0**	1	0	0	1	0
	3	0	1	**5**	0	1	6	38.46
	4	0	0	0	**0**	1	0	0
	5	0	0	0	0	**39**	2	95.12
	Malta	2	1	5	0	3	**6**	35.3

No differences in mean TL were found among the areas or years on the larval subsamples used for the evaluation of organic content (PERMANOVA, $p > 0.05$). Mean values of carbohydrates, lipids and proteins content as well as the ratio between lipids and proteins in the areas and years of study are shown in the descriptive box plots of Figure 9. Permutational *t* tests among the areas only pointed out statistically significant differences in carbohydrates between areas 1–2 and Malta, and in proteins between areas 1–2, area 5 and Malta. Carbohydrate content was statistically different between the years 2008 and 2009, as well as the protein content in the year 2009 compared to years 2005 and 2008. A 2-way PERMANOVA was performed on the ratio lipid–proteins using years and areas as factors showing that neither the area nor year had a significant effect on this ratio ($p > 0.05$). Following the AIC criterion, the best GAM models estimated for the three response variables (carbohydrates, lipids and proteins), always included as a statistically significant (p-value < 0.001) explicative variable, the size–area interaction, which explained 63.4% of the observed variability in the carbohydrate content, 53.1% in the lipid content and 72.4% in the protein content. The relationship between size and each organic component was significantly positive in the model (Figure 10). In fact, the contents remained almost constant up to 13 mm and grew faster for sizes greater than 13 mm. Moreover, the models indicated that the carbohydrate and protein contents increased faster with size in the Malta area compared to the other areas (higher coefficient), and that the increment had been different throughout the years.

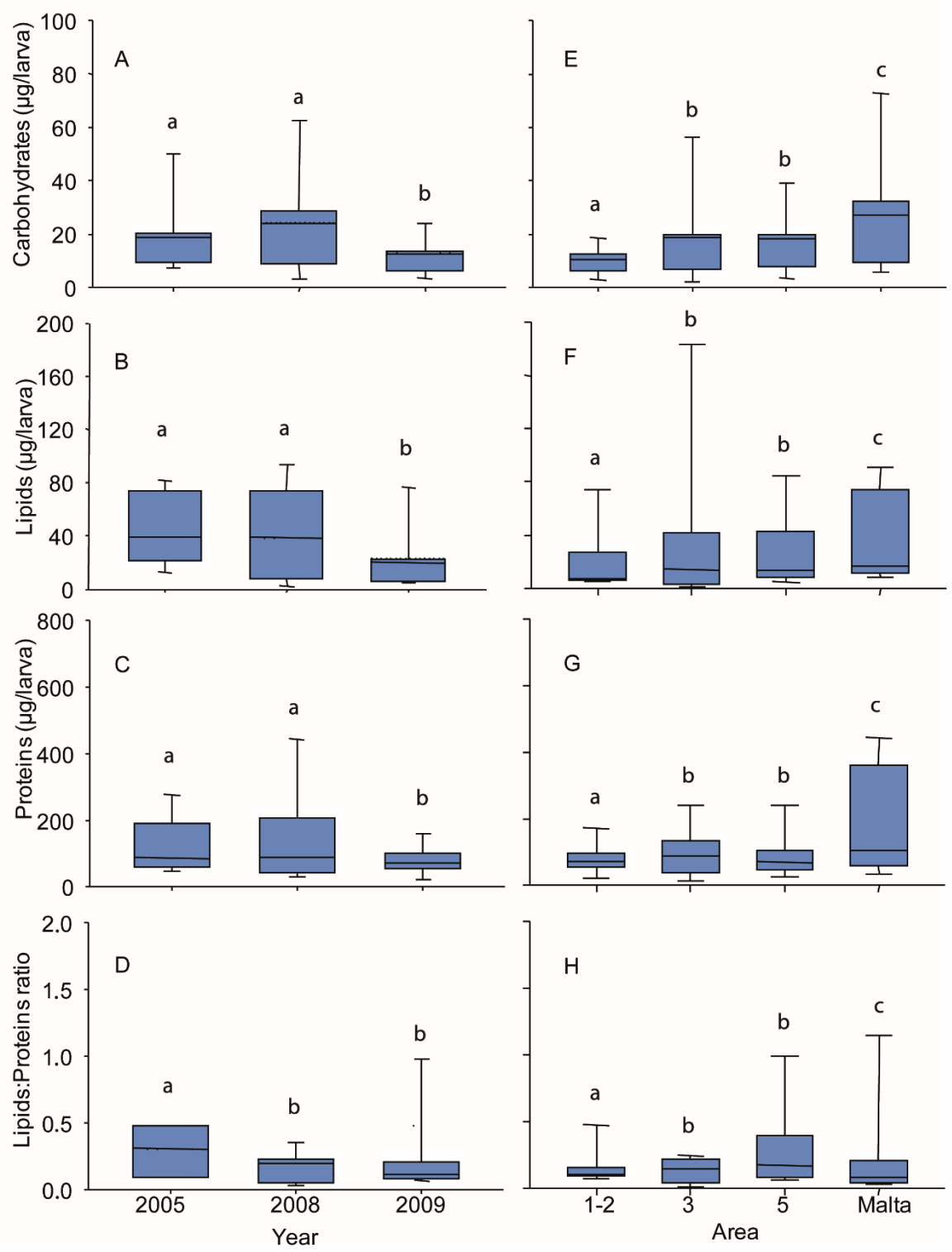

Figure 9. Biochemical content box plots. (**A**–**D**) Larval carbohydrates, lipids and proteins content and lipid–protein ratio observed in the different years. (**E**–**H**) Larval carbohydrates, lipids and proteins content and lipid–protein ratio observed in the different areas. In all cases, the bottom and top of the boxes are the first and third quartiles, while the whiskers indicate the 10 and 90 percentiles, and the band inside is the mean value. Lowercase letters (a–c) indicate statistically significant difference among groups (permutational *t* tests, p-value < 0.05).

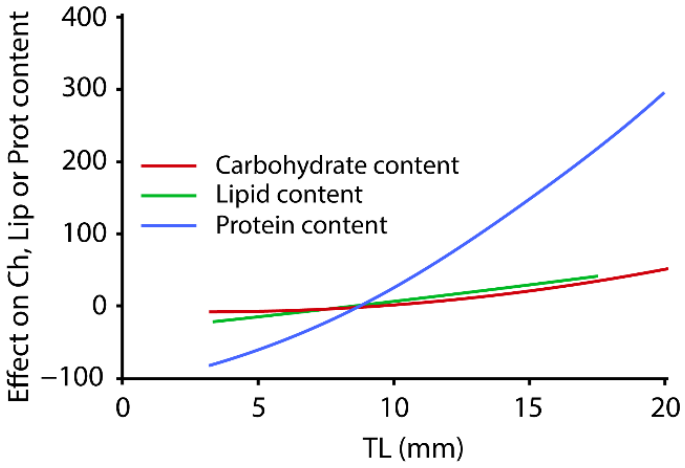

Figure 10. GAM model of organic compounds. Effect of the total length on the protein, carbohydrates and lipid content obtained with the best GAM models, using the interaction size-area as a variable.

4. Discussion

As it has been observed in previous studies [3,67] high anchovy larvae abundances were observed in the eastern part of the Sicilian Channel during the 2009 survey. The higher concentration was found in area 5, eastward to the spawning area on the Adventure Bank reported by Basilone et al. [17], in a highly oligotrophic region where larvae abundance is usually quite low. The presence of a cyclonic eddy next to the Island of Malta (see [68]) could have transported some larvae from area 3 or Malta to area 5, but most probably the high larval abundance in area 5 was linked to advection processes induced by the strong upwelling regimes in areas 1 and 2, as it is indicated by surface temperature [69].

It has been observed that wind-induced coastal upwelling along the southern Sicilian coast impacts the fate and distribution of European anchovy larvae within the Channel [70]. In agreement with the oceanographic conditions of this study, these authors showed that a coastal upwelling process around Sciacca (area 1–2 edge) occurred in July 2004 and promoted an offshore transport of anchovy larvae to the north of the island of Lampedusa, (area 5). In 2009. higher larval abundances were mostly found in the eastern part of area 5. This can be explained due to the upwelling event which also occurred off the coast of Licata, located south-eastward from Sciacca. In 2009, the observed larval distribution was inconsistent with the assumption that the south-eastern (SE) area off Capo Passero is the most favorable for feeding and growth of larvae due to a higher primary production, upwelling conditions and a retention mechanism driven by the presence of a cyclonic offshore vortex in the region [18].

It is not the first time that some discrepancies with the "ocean triad" hypothesis have been found in this area. Cuttitta et al. [3] observed that primary production was not higher in the SE region and other studies in the same area [17,69] indicated that the upwelling phenomena occurs mainly in the NW and Capo Passero area. In agreement with the results observed by Cuttitta et al. [3], the biggest larvae were found in the eastern sector of the study area (Malta and area 4), while larvae from the western side (area 1) were the smallest ones.

Two different reasons can explain this variation: the different age–length relationship between areas and diversity in the individuals' age. There were slight differences in the growth patterns between some sub-areas of the eastern sector of the Strait (Malta and area 3), but the age distribution confirms that the larvae found in eastern areas were older. Garcia Lafuente et al. [18] and Cuttitta et al. [16] showed that the branch of the Atlantic–Ionian Stream, running parallel to the southern Sicilian coast, acts as a transport mechanism for anchovy eggs and larvae towards the southernmost end of the island (Capo Passero). Therefore, larvae hatched in the western spawning area described by Basilone et al. [17] can be transported towards the south-east, thereby increasing the average age and size of

larvae in this region. In agreement with this observation, only small larvae were found in area 2, a non-retention area located in the western part of the Sicilian Channel. On the other hand, the presence of all sized larvae offshore in area 5 indicated a possible retention process in this region in agreement with the presence of an anticyclonic eddy in the region as it has been indicated by Bonanno et al. [68].

This larval drifting in the Strait promotes a modest level of morphometric discrimination among the areas, as it was indicated by Cuttitta et al. [34] for the Sicilian Channel and by Cotano et al. [71] for the Bay of Biscay; however, some clear differences can be observed, particularly in area 5 which presented the highest normalized values, suggesting better environmental conditions for larvae in 2009. This result disagrees with observations made by Cuttitta et al. [34] since they showed that BD was small in area 5; however, in the sampling period of that study there was no anticyclonic eddy in area 5 [68]. Different results in the patterns of morphometric parameters in the same area but in different time periods highlight how they respond to the diverse environmental conditions experienced by larvae.

The growth rates calculated in this study were within the range of this species for Mediterranean waters [9]. It is well known that larval growth rate is determined mainly by temperature and trophic conditions [72]. In the case of the genus *Engraulis*, it has been reported that a rise in the growth rate of larval stages is associated with increments in water temperature or food availability [8]. Our results are in agreement with this study, since the growth rate observed in Malta, where the higher temperature regime was observed, was higher than estimated for the adjacent area 3, where the average temperature was more than 2.5 °C lower; however, no statistical differences were spotted between Malta and area 5 despite the different temperature observed. Although, as it is suggested by Cotano et al. [71], we cannot reject the possibility that this homogeneity in growth rates could be due to the same average environmental history for larvae drifting between adjacent areas, and food availability could explain this homogeneity.

It is known that the amino acid composition of larvae is related to the biochemical composition of the available food [28], and larvae from area 5 presented a different amino acid composition compared to the ones sampled in the Malta area, indicating a diverse food condition. This possibility is also reflected in the morphometric measurements. In fact, the BD was higher in area 5 than in Malta and this parameter is the most responsive to starvation since it depends largely on the cumulative amount of muscle fibers that reflect muscular tissue stored as energy, as it has been indicated by Diaz et al. [73] and observed with different species such as, *Sparus aurata* [74], *Miichthys miiuy* [75] or *Seriolella violacea* [76]. Therefore, a better food availability could have compensated for the lower temperature regime in area 5.

The bigger size of the recently hatched larvae found in the eastern region of the Sicilian Channel indicates that eggs laid in this area could also have a higher dimension since size at-hatching is primarily influenced by egg size [77]. It has been observed that recently hatched larval size increment in small pelagic fishes is a maternal effect, intended to facilitate subsequent larval survival in sub-optimal environmental conditions [63,78]. In agreement, Schismenou et al. [79] found that anchovy size at hatching in the Mediterranean Sea was smaller when the summer temperature and plankton concentration were higher. According to 'bigger is better', larger larvae are more likely to survive because of higher chances of avoiding predation and encountering their prey as well [80], and this could indicate that the eastern region is more favorable for larvae survival. Apart from this chance, the size difference between the recently hatched larvae points towards the possibility that the spawning stock is divided into two different groups as it was indicated for Argentine anchovy *Engraulis anchoita* by Ciechomski [81], who observed differences in egg dimensions between the northern and the southern spawning sites.

In spite of larval advection along the AIS, which tends to transport and concentrate larvae eastward across areas with different physical and chemical characteristics, it is clear that there was a significant variability in the region in terms of morphometric parameters

and of AAC. The results obtained using these two different approaches complement each other, since there were no inconsistencies between them as it has been observed by other authors [23,24,32,35]. Area 5 larvae could be partially discriminated by their morphometric characteristics and were clearly differentiated by AAC. On the other hand, area 3 and Malta were confused between them (but not in terms of growth, which was much faster in the Malta area). In both cases the success of morphometric classification was similar to the one obtained in area 5, but in the amino acid case most of the larvae misclassified in area 3 were assigned to Malta, and vice versa. Therefore, at least in terms of discrimination, we detected two main groups: area 5, and area 3 and Malta. The main difference between these two groups is that area 5 was located offshore (depth > 200 m), where the oceanographic conditions and food composition should be different from the coastal areas.

In addition to area discrimination, in order to manage fishing resources, it is very important to determine the nutritional state of the analyzed larvae. Both morphometric and biochemical approaches have been used to determine the nutritional condition of larvae [73,82]. Morphometric indices can take a relatively long time to reflect—via the condition status of individuals—the effects of food intake [53] while biochemical condition estimators respond rapidly to variations in crucial environmental factors such as temperature or food availability [82]. As far as morphometry is concerned, a reduction in body diameter and standard length in starvation has been pointed out [73,83]. With these findings we can infer that larvae from area 5 presented an overall better condition since they had a higher BD value, while the larvae from area 3 presented a weaker TL–age relationship and, thus, a possible lower nutritional condition.

In the case of biochemical analysis, our result agrees with the general observation of a significant positive correlation between size and each organic component [84]. It is important to emphasize that the contents remain almost constant up to 13 mm of TL and grow faster for greater sizes. This difference between the small larvae and bigger ones has also been observed by Diaz et al. [44] for European anchovy from the Bay of Biscay, and they suggest that this could be due to the fact that pre flexion larvae (<11.5 mm standard length) are less metabolically active than post flexion larvae; this obviously agrees with our observations.

Moreover, there were differences among the protein contents throughout the years, reinforcing the importance of environmental conditions in the biology of the larvae of these small pelagic fish. Proteins and lipids have been used to analyze nutritional conditions in fish larvae. In the case of the total lipid content, the method does not distinguish between structural and reserve lipids, therefore it is difficult to interpret in terms of nutritional condition; however, Bonanno et al. [11], in a study performed with anchovies from the Sicilian Channel used the lipid–protein relationship as a nutritional indicator for comparison with larvae collected in Libyan waters. They observed higher lipid and lesser protein contents for Libyan larvae, suggesting a better body condition than in the Maltese–Sicilian waters. In our case, no differences were observed in this parameter within the Sicilian Channel and any assumption on nutritional condition can be brought forward. This is not the case of the protein content that should be a good indicator of larval condition under environmental fluctuations ([53] and references therein). Protein quantity and increment of this parameter with size was higher in Malta compared to the other areas, indicating a better condition in this area.

In conclusion, even considering the inter-annual variability observed in the organic content among the areas, from our results we can infer that area 5, and area 3 and Malta show two different larvae subpopulations in relation to morphometry and biochemical composition, an evidence also confirmed by the differences found in the recently hatched larvae from these regions. In addition, the lower protein growth rate estimated for area 3 compared to Malta suggests a lower conditional status in this area; however, this study highlighted also the presence of a more complex dynamic affecting the evaluation of the larval condition. Indeed, the Maltese area presented a higher protein content and protein growth with size compared to area 5, which in turn presented a higher body depth and

larval growth rate despite the lower temperature. In this case, the different contribution provided by several indicators makes the identification of the larval condition a challenging object that could be better defined through, for instance, mesocosm experiments. In this context, this work represents an important step derived from observations in the field and sheds light on the main factors for the assessment of the condition during the most vulnerable phase of fish life such as the early life stages.

Author Contributions: Conceptualization, A.C., B.P., I.M. and M.T.; methodology, I.M., F.F. and M.C.; software, M.T. and E.M.Q.; formal analysis, A.C., B.P. and M.T., investigation, S.R., B.P., M.M., T.M., F.P., M.D.N. and C.D.B.; resources, A.C. and B.P.; writing—original draft preparation, A.C., I.M. and M.T.; writing—review and editing, V.P., B.P., M.M. and T.M.; funding acquisition, A.C. and B.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the RITMARE project from the Italian government and ALIF project from the European Union. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Acknowledgments: We thank Salvo Mazzola for his contribution to scientific growth of the research team engaged in this study. We thank the captain and the crew of the "Urania" R/V, all the CNR team on board for the surveys and Donatella Spera for administrative support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO. Review of the state of world marine Fishery resources. In *FAO Fisheries and Aquaculture Technical Paper No. 569*; FAO: Rome, Italy, 2011.
2. SGMED; Study Group on Mediterranean Fisheries. *Assessment of Mediterranean Stocks Part I*; Scientific, Technical and Economic Committee for Fisheries (STECF): Luxembourg; Publications Office of the European Union: Heraklion, Crete, Greece, 2010.
3. Cuttitta, A.; Guisande, C.; Riveiro, I.; Maneiro, I.; Patti, B.; Vergara, A.; Basilone, G.; Bonanno, A.; Mazzola, S. Factors structuring reproductive habitat suitability of the European anchovy (*Engraulis encrasicolus*) in the south coast of Sicily. *J. Fish Biol.* **2006**, *67*, 1–12.
4. Fréon, P.; Cury, P.; Shannon, L.; Roy, C. Sustainable exploitation of small pelagic fish stocks challenged by environmental and ecosystem changes. *Bull. Mar. Sci.* **2005**, *76*, 385–462.
5. Borja, Á.; Fontán, A.; Sáenz, J.; Valencia, V. Climate, oceanography, and recruitment: The case of the Bay of Biscay anchovy (*Engraulis encrasicolus*). *Fish. Oceanogr.* **2008**, *17*, 477–493. [CrossRef]
6. Cuttitta, A.; Torri, M.; Zarrad, R.; Zgozi, S.; Jarboui, O.; Quinci, E.M.; Patti, B. Linking surface hydrodynamics to planktonic ecosystem: The case study of the ichthyoplanktonic assemblages in the Central Mediterranean Sea. *Hydrobiologia* **2018**, *821*, 191–214. [CrossRef]
7. Bakun, A. *Patterns in the Ocean: Ocean Processes and Marine Population Dynamics*; University of California Sea Grant: San Diego, CA, USA, 1996; p. 323, (in cooperation with Centro de Investigaciones Biologicas de Noroeste, La Paz, Baja California Sur, Mexico).
8. Takahashi, M.; Watanabe, Y. Effects of temperature and food availability on growth rate during late larval stage of Japanese anchovy (*Engraulis japonicus*) in the Kuroshio–Oyashio transition region. *Fish. Oceanogr.* **2005**, *14*, 223–235. [CrossRef]
9. Palomera, I.; Olivar, M.P.; Salat, J.; Sabatés, A.; Coll, M.; García, A.; Morales-Nin, B. Small pelagic fish in the NW Mediterranean Sea: An ecological review. *Prog. Oceanogr.* **2007**, *74*, 377–396. [CrossRef]
10. Houde, E.D. Emerging from Hjort's shadow. *J. Northwest Atl. Fish. Sci.* **2008**, *41*, 53–70. [CrossRef]
11. Bonanno, A.; Zgozi, S.; Cuttitta, A.; El Turki, A.; Di Nieri, A.; Ghmati, H.; Mazzola, S. Influence of environmental variability on anchovy early life stages (*Engraulis encrasicolus*) in two different areas of the Central Mediterranean Sea. *Hydrobiology* **2013**, *701*, 273–287. [CrossRef]
12. Patti, B.; Torri, M.; Cuttitta, A. General surface circulation controls the interannual fluctuations of anchovy stock biomass in the Central Mediterranean Sea. *Sci. Rep.* **2020**, *10*, 1554. [CrossRef]
13. Robinson, A.R.; Sellschopp, J.; Warn-Varnas, A.; Leslie, W.G.; Lozano, C.J.; Haley, P.J.J.; Lermusiaux, P.F.J. The Atlantic Ionian Stream. *J. Mar. Syst.* **1999**, *20*, 129–156. [CrossRef]
14. Beranger, K.; Mortier, L.; Gasparini, G.P.; Gervasio, L.; Astraldi, M.; Crepon, M. The dynamics of the Strait of Sicily: A comprehensive study from observations and models. *Deep-Sea Res.* **2004**, *51*, 411–440.

15. Macedo-Soares, L.; Eiras-Garcia, C.A.; Santarosa-Freire, A.; Muelbert, S.H. Large-Scale Ichthyoplankton and Water Mass Distribution along the South Brazil Shelf. *PLoS ONE* **2014**, *9*, e91241. [CrossRef] [PubMed]
16. Cuttitta, A.; Carini, V.; Patti, B.; Bonanno, A.; Basilone, G.; Mazzola, S.; Cavalcante, C. Anchovy egg and larval distribution in relation to biological and physical oceanography in the Strait of Sicily. *Hydrobiology* **2003**, *503*, 117–120. [CrossRef]
17. Basilone, G.; Bonanno, A.; Patti, B.; Mazzola, S.; Barra, M.; Cuttitta, A.; McBride, R. Spawning site selection by European anchovy (*Engraulis encrasicolus*) in relation to oceanographic conditions in the Strait of Sicily. *Fish. Oceanogr.* **2013**, *22*, 309–323. [CrossRef]
18. García-Lafuente, J.; Garcıa, A.; Mazzola, S.; Quintanilla, L.; Delgado, J.; Cuttita, A.; Patti, B. Hydrographic phenomena influencing early life stages of the Sicilian Channel anchovy. *Fish. Oceanogr.* **2002**, *11*, 31–44. [CrossRef]
19. Falcini, F.; Corrado, R.; Torri, M.; Mangano, M.C.; Zarrad, R.; Di Cintio, A.; Lacorata, G. Seascape connectivity of European anchovy in the Central Mediterranean Sea revealed by weighted Lagrangian backtracking and bio-energetic modelling. *Sci. Rep.* **2020**, *10*, 18630. [CrossRef]
20. Patti, B.; Zarrad, R.; Jarboui, O.; Cuttitta, A.; Basilone, G.; Aronica, S.; Mazzola, S. Anchovy (*Engraulis encrasicolus*) early life stages in the Central Mediterranean Sea: Connectivity issues emerging among adjacent sub-areas across the Strait of Sicily. *Hydrobiologia* **2018**, *821*, 25–40. [CrossRef]
21. Stephenson, R.L. Stock complexity in fisheries management: A perspective of emerging issues related to population sub-units. *Fish. Res.* **1999**, *43*, 247–249. [CrossRef]
22. Tzeng, T.D. Morphological variation between populations of spotted mackerel *Scomber australasicus* of Taiwan. *Fish. Res.* **2004**, *68*, 45–55. [CrossRef]
23. Félix-Uraga, R.; Quiñonez-Velázquez, C.; Hill, K.T.; Gómez-Muñoz, V.M.; Melo-Barrera, F.N.; García-Franco, W. Pacific Sardine (*Sardinops sagax*) stock discrimination off the west coast of Baja California and southern California using otholith morphometry. *CalCOFI Rep.* **2005**, *46*, 113–121.
24. García-Rodríguez, F.J.; García-Gasca, S.A.; De La Cruz-Agüeroa, J.; Cota-Gómeza, V.M. A study of the population structure of the Pacific sardine Sardinops sagax (Jenyns, 1842) in Mexico based on morphometric and genetic analysis. *Fish. Res.* **2011**, *107*, 169–176. [CrossRef]
25. Traina, S.; Basilone, G.; Saborido-Rey, F.; Ferreri, R.; Quinci, E.; Masullo, T.; Mazzola, S. Assessing population structure of European Anchovy (*Engraulis encrasicolus*) in the Central Mediterranean by means of traditional morphometry. *Adv. Oceanogr. Limnol.* **2011**, *22*, 141–153. [CrossRef]
26. Vergara-Solana, F.J.; García-Rodríguez, F.J.; La Cruz-Agüero, D. Comparing body and otolith shape for stock discrimination of Pacific sardine, *Sardinops sagax* Jenyns, 1842. *J. Appl. Ichthyol.* **2013**, *29*, 1241–1246. [CrossRef]
27. Joensen, H.; Grahl-Nielsen, O. Distinction among North Atlantic cod *Gadus morhua* stocks by tissue fatty acid profiles. *J. Fish Biol.* **2014**, *84*, 1904–1925. [CrossRef] [PubMed]
28. Riveiro, I.; Guisande, C.; Franco, C.; Lago de Lanzos, A.; Solá, A.; Maneiro, I.; Vergara, A.R. Egg and larval amino acid composition as indicators of niche resource partitioning in pelagic fish species. *Mar. Ecol. Prog. Ser.* **2003**, *260*, 252–262. [CrossRef]
29. Tanner, S.E.; Pérez, M.; Presa, P.; Thorrold, S.R.; Cabral, H.N. Integrating microsatellite DNA markers and otolith geochemistry to assess population structure of European hake (*Merluccius merluccius*). *Est. Coast. Shelf. Sci.* **2014**, *142*, 68–75. [CrossRef]
30. Falco, F.; Barra, M.; Wu, G.; Dioguardi, M.; Stincone, P.; Cuttitta, A.; Cammarata, M. Engraulis encrasicolus larvae from two different environmental spawning areas of the Central Mediterranean Sea: First data on amino acid profiles and biochemical evaluations. *Eur. Zool. J.* **2020**, *87*, 580–590. [CrossRef]
31. Abaunza, P.; Murta, A.G.; Campbell, N.; Cimmaruta, R.; Comesana, A.S.; Dahle, G.; Zimmermann, C. Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. *Fish. Res.* **2008**, *89*, 196–209. [CrossRef]
32. Baibai, T.; Oukhattar, L.; Quinteiro, J.; Mesfioui, A.; Rey-Mendez, M.; Soukri, A. First global approach: Morphological and biological variability in a genetically homogeneous population of the European pilchard, Sardina pilchardus (Walbaum, 1792) in the North Atlantic coast. *Rev. Fish Biol. Fish.* **2012**, *22*, 63–80. [CrossRef]
33. Zarraonaindia, I.; Iriondo, M.; Albaina, A.; Pardo, M.A.; Manzano, C.; Grant, W.S.; Irigoien, X.; Estonba, A. Multiple SNP markers reveal fine-scale population and deep phylogeographic structure in European anchovy (*Engraulis encrasicolus* L.). *PLoS ONE* **2012**, *7*, e42201. [CrossRef]
34. Cuttitta, A.; Patti, B.; Maggio, T.; Quinci, E.; Pappalardo, A.; Ferrito, V.; De Pinto, V.; Mazzola, S. Larval population structure of *Engraulis encrasicolus* in the Strait of Sicily as revealed by morphometric and genetic analysis. *Fish. Oceanogr.* **2015**, *24*, 135–149. [CrossRef]
35. Ryman, N.; Lagercrantz, U.; Andersson, L.; Chakraborty, R.; Rosenberg, R. Lack of correspondence between genetic and morphologic variability patterns in Atlantic herring. *Heredity* **1984**, *53*, 687–704. [CrossRef]
36. Waldman, J.R. The importance of comparative studies in stock analysis. *Fish. Res.* **1999**, *43*, 237–246. [CrossRef]
37. Ruggeri, P.; Splendiani, A.; Bonanomi, S.; Arneri, E.; Cingolani, N.; Santojanni, A.; Barucchi, V.C. Searching for a stock structure in *Sardina pilchardus* from the Adriatic and Ionian seas using a microsatellite DNA-based approach. *Sci. Mar.* **2013**, *77*, 565–574.
38. Yaisel, J.B.; Piñera, J.A.; Sánchez Prado, J.A.; Blanco, G. Mitochondrial DNA and microsatellite genetic differentiation in the European anchovy *Engraulis encrasicolus* L. *ICES J. Mar. Sci.* **2012**, *69*, 1357–1371. [CrossRef]
39. Turan, C.; Erguden, D.; Gurlek, M.; Basusta, N.; Turan, F. Morphometric structuring of the anchovy (*Engraulis encrasicolus* L.) in the Black, Aegean and northeastern Mediterranean Seas. *Turk. J. Vet. Anim. Sci.* **2004**, *28*, 865–871.

40. Chang, M.Y.; Tzeng, W.N.; You, C.F. Using otolith trace elements as biological tracer for tracking larval dispersal of black porgy, *Acanthopagrus schlegeli* and yellowfin seabream, *A. latus* among estuaries of western Taiwan. *Environ. Biol. Fish.* **2012**, *95*, 491–502. [CrossRef]
41. Cuttitta, A.; Basilone, G.; Patti, B.; Bonanno, A.; Mazzola, S.; Giusto, G.B. Trends in anchovy (*Engraulis encrasicolus*) condition factor and gonadosomatic index in the Sicilian Channel. *Biol. Mar. Medit.* **1999**, *6*, 566–568.
42. Coombs, S.H.; Giovanardi, O.; Halliday, N.C.; Franceschini, G.; Conway, D.V.P.; Manzueto, L.; McFadzen, I.R.B. Wind mixing.; food availability and mortality of anchovy larvae *Engraulis encrasicolus* in the northern Adriatic Sea. *Mar. Ecol. Prog. Ser.* **2003**, *248*, 221–235. [CrossRef]
43. Blackith, R.E.; Reyment, R.A. *Multivariate Morphometrics*; Academic Press: London, UK, 1971.
44. Diaz, M.V.; Pájaro, M.; Sánchez, R.P. Employment of morphometric variables to assess nutritional condition of Argentine anchovy larvae *Engraulis anchoita* Hubbs & Marini., 1935. *Rev. Biol Mar. Oceanogr.* **2009**, *44*, 539–549.
45. Sturges, H. The choice of a class-interval. *J. Am. Stat. Assoc.* **1926**, *21*, 65–66. [CrossRef]
46. Lleonart, J.; Sarlat, J.; Torres, G.J. Removin allometric effects of body size morphological analysis. *J. Theor. Biol.* **2000**, *205*, 85–93. [CrossRef] [PubMed]
47. Breiman, L. Random Forests. *Mach. Learn.* **2001**, *45*, 5–32. [CrossRef]
48. Liaw, A.; Wiener, M. Classification and Regression by randomForest. *R News* **2002**, *2*, 18–22.
49. Diaz-Uriarte, R.; Alvarez de Andres, S. Gene selection and classification of microarray data using random forest. *BMC Bioinform.* **2006**, *7*, 3. [CrossRef]
50. Morales-Nin, B. Review of the growth regulation processes of otolith daily increment uniformation. *Fish. Res.* **2000**, *46*, 53–67. [CrossRef]
51. Aldanondo, N.; Cotano, U.; Etxebeste, E.; Irigoien, X.; Álvarez, P.; Martínez de Murguía, A.; Herrero, D.L. Validation of daily increments deposition in the otoliths of European anchovy larvae (*Engraulis encrasicolus* L.) reared under different temperature. *Fish. Res.* **2008**, *93*, 257–264. [CrossRef]
52. Zweifel, J.R.; Lasker, R. Prehatch and posthatch growth offishes—A general model. *Fish. Bull. US* **1976**, *74*, 609–621.
53. Costalago, D.; Tecchio, S.; Palomera, I.; Alvarez-Calleja, I.; Ospina-Alvarez, A.; Raicevich, S. Ecological understanding for fishery management Condition and growth of anchovy late larvae during different seasons in the Northwestern Mediterranean. *Estuar. Coast. Shelf Sci.* **2011**, *93*, 350–358. [CrossRef]
54. Regner, S. Ecology of planktonic stages of the anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), in the Central Adriatic Sea. *Acta Adriat.* **1985**, *26*, 1–113.
55. Ré, P. Ictioplâncton estuarino da Península Ibérica. In *Guia de Identificação dos Ovos e Estados Larvares Planctónicos*; Câmara Municipal de Cascais: Cascais, Portugal, 1999.
56. Guisande, C.; Maneiro, I.; Riveiro, I. Homeostasis in the essential amino acid composition of the marine copepod *Euterpina acutifrons*. *Limnol. Oceanogr.* **1999**, *44*, 691–696. [CrossRef]
57. Brucet, S.; Boix, D.; Lopez-Flores, R.; Badosa, A.; Quintana, X.D. Ontogenic changes of amino acid composition in planktonic crustacean species. *Mar. Biol.* **2005**, *148*, 131–139. [CrossRef]
58. Lowry, O.H.; Rosenbraugh, N.J.; Farr, A.L.; Randall, R.J. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 256–275. [CrossRef]
59. Maxwell, M.A.K.; Haas, S.M.; Bieber, L.L.; Tolbert, N.E. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **1978**, *87*, 206–210.
60. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356. [CrossRef]
61. Zöllner, N.; Kirsch, K. Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophosphovanillin. *Z. Gesamte Exp. Med.* **1962**, *135*, 545–561. [CrossRef]
62. Díaz, E.; Txurruka, J.M.; Villate, F. Biochemical composition and condition in anchovy larvae *Engraulis encrasicolus* during growth. *Mar. Ecol. Prog. Ser.* **2008**, *361*, 227–238. [CrossRef]
63. Riveiro, I.; Guisande, C.; Maneiro, I.; Vergara, A.R. Parental effects in the European sardine *Sardina pilchardus*. *Mar. Ecol. Prog. Ser.* **2004**, *274*, 225–234. [CrossRef]
64. Hasties, T.J.; Tibshirani, R.J. *Generalized Additive MODELS*; Chapman and Hall: New York, NY, USA, 1990.
65. Wood, S.N. *Generalized Additive Models: An Introduction with R*; Chapman and Hall/CRC: New York, NY, USA, 2006.
66. Sakamoto, Y.; Ishiguro, M.; Kitagawa, G. *Akaike Information Criterion Statistics*; D. Reidel: Dordrecht, The Netherlands, 1986; Volume 81, p. 26853.
67. Cuttitta, A.; Quinci, E.M.; Patti, B.; Bonomo, S.; Bonanno, A.; Musco, M.; Mazzola, S. Different key roles of mesoscale oceanographic structures and ocean bathymetry in shaping larval fish distribution pattern: A case study in Sicilian waters in summer 2009. *J. Sea Res.* **2016**, *115*, 6–17. [CrossRef]
68. Bonanno, A.; Placenti, F.; Basilone, G.; Mifsud, R.; Genovese, S.; Patti, B.; Mazzola, S. Variability of water mass properties in the Strait of Sicily in summer period of 1998–2013. *Ocean Sci.* **2014**, *10*, 759–770. [CrossRef]
69. Torri, M.; Corrado, R.; Falcini, F.; Cuttitta, A.; Palatella, L.; Lacorata, G.; Santoleri, R. Planktonic stages of small pelagic fishes (*Sardinella aurita* and *Engraulis encrasicolus*) in the central Mediterranean Sea: The key role of physical forcings and implications for fisheries management. *Prog. Oceanogr.* **2018**, *162*, 25–39. [CrossRef]

70. Falcini, F.; Palatella, L.; Cuttitta, A.; Buongiorno Nardelli, B.; Lacorata, G.; Lanotte, A.S.; Santoleri, R. The role of hydrodynamic processes on anchovy eggs and larvae distribution in the Sicily Channel (Mediterranean Sea): A case study for the 2004 data set. *PLoS ONE* **2015**, *10*, e0123213. [CrossRef] [PubMed]
71. Cotano, U.; Irigoien, X.; Etxebeste, E.; Álvarez, P.; Zarauz, L.; Mader, J.; Ferrer, L. Distribution, growth and survival of anchovy larvae (*Engraulis encrasicolus* L.) in relation to hydrodynamic and trophic environment in the Bay of Biscay. *J. Plankt. Res.* **2008**, *30*, 467–481. [CrossRef]
72. Heath, M.R. Field investigations of the early life stages of marine fish. *Adv. Mar Biol.* **1992**, *28*, 1–174.
73. Diaz, M.V.; Arano, M.F.; Pájaro, M.; Aristizábal, E.O.; Macchi, G.F. The use of morphological and histological features as nutritional condition indices of *Pagrus pagrus* larvae. *Neotrop. Ichthyol.* **2013**, *11*, 649–660. [CrossRef]
74. Yufera, M.; Polo, A.; Pascual, E. Changes in chemical composition and biomass during the transition from endogenous to exogenous feeding of *Sparus aurata* L. (Pisces.; Sparidae) larvae reared in the laboratory. *J. Exp. Mar. Biol. Ecol.* **1993**, *167*, 149–161. [CrossRef]
75. Shan, X.J.; Cao, L.; Huang, W.; Dou, S.Z. Feeding, morphological changes and allometric growth during starvation in miiuy croaker larvae. *Environ. Biol. Fish.* **2009**, *86*, 121–130. [CrossRef]
76. Bustos, C.; Silva, A. Endogenous feeding and morphological changes in hatchery-reared larval palm ruff *Seriolella violacea* (Pisces: Centrolophidae) under starvation. *Aquac. Res.* **2011**, *42*, 892–897. [CrossRef]
77. Chambers, R.C. Environmental influences on egg and propagule sizes in marine fishes. In *Early Life History and Recruitment in Fish Populations*; Chambers, R.C., Trippel, E.A., Eds.; Chapman & Hall: London, UK, 1997; pp. 63–102.
78. Castro, L.R.; Claramunt, G.; Krautz, M.C.; Llanos-Rivera, A.; Moreno, P. Egg trait variation in anchoveta *Engraulis ringens*: A maternal response to changing environmental conditions in contrasting spawning habitats. *Mar. Ecol. Prog. Ser.* **2009**, *381*, 237–248. [CrossRef]
79. Schismenou, E.; Tsiaras, K.; Kourepini, M.I.; Lefkaditou, E.; Triantafyllou, G.; Somarakis, S. Seasonal changes in growth and condition of anchovy late larvae explained with a hydrodynamic-biogeochemical model simulation. *Mar. Ecol.* **2013**, *478*, 197–209. [CrossRef]
80. Anderson, J.T. A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. *J. Northwest Atl. Fish. Sci.* **1988**, *8*, 55–66. [CrossRef]
81. Ciechomski, J. The size of the egg of the Argentine anchovy *Engraulis anchoita* in relation to the season of the year of spawning. *J. Fish Biol.* **1973**, *5*, 393–398. [CrossRef]
82. Gisbert, E.; Conklin, D.B.; Piedrahita, R.H. Effects of delayed first feeding on the nutritional condition and mortality of California halibut larvae. *J. Fish Biol.* **2004**, *64*, 116–132. [CrossRef]
83. Torri, M.; Pappalardo, A.M.; Ferrito, V.; Giannì, S.; Armeri, G.M.; Patti, C.; Cuttitta, A. Signals from the deep-sea: Genetic structure, morphometric analysis, and ecological implications of Cyclothone braueri (Pisces, Gonostomatidae) early life stages in the Central Mediterranean Sea. *Mar. Environ. Res.* **2021**, *169*, 105379. [CrossRef]
84. Catalán, I.A.; Berdalet, E.; Olivar, M.P.; Roldán, C. Response of muscle-based biochemical condition indices to short-term variations in food availability in postflexion reared sea bass *Dicentrarchus labrax* (L.) larvae. *J. Fish Biol.* **2007**, *70*, 391–405. [CrossRef]