A STUDY ON POSSIBLE VARIANT FORMS OF ANCHOVY IN THE BLACK SEA

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Approval of the Graduate School of Marine Sciences

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Thesis to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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ABSTRACT

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The anchovy in the Black Sea is a commercially important fish source for Turkey, which is represented by two different subspecies in the basin. Spawning and feeding occurs in northern coasts where food supplies are more abundant. When the water temperature starts to decrease, the anchovy migrate toward the warmer waters of the southern coasts. The schools formed during this migration make them a primary target for fishing fleets. Due to morphological similarities, it is not possible to report the catches by subspecies which creates crucial problems in stock assessment and consequently fisheries management. Even if discrimination is carried out by methods such as genetics, blood types and parasitism rate, these are either impractical or very costly. Therefore, in the present study we aimed to establish more practical methods which can be applied for stock assessment studies. In order to achieve this purpose, sampling was carried out at overwintering grounds, with the notion that differences in feeding and nursery grounds may cause variation in size frequency distribution of age groups and length-weight relationships. Other than dissimilar environmental conditions, distances and different routes used to reach the wintering areas may affect the condition factor.

An additional aim of this study was to test if shape analysis of otoliths, generally used for ageing in stock assessment studies, could be an effective method to discriminate between mixed anchovy stocks. Furthermore, various otolith shape parameters were studied contrary to adopting only otolith length/width ratio (aspect ratio) for stock discrimination.

Biological data were collected by midwater trawls in Autumn-Winter (November to January), in 2011/12 and 2012/13 during the fishing season, in the area covering the southern Black Sea region from İğneada to Hopa. Length, weight and otolith shape variables were measured in the laboratory. In addition, environmental parameters; monthly averages of sea surface temperature (SST) and surface chlorophyll concentrations (Chl-a) were derived from satellite data for the entire study period.

Study results indicate dissimilarities among stations in the allometric growth coefficient of length-weight relationship- slope (b), an indicator of well-being of fish, condition factor (CF), and also length frequency distribution of individuals in anchovy schools sampled. However, the stations showing similarities did not always show geographic proximity, and significant geographical patterns could not always be observed. In general, results indicate the presence of two groups, even though condition factor analysis illustrated the existence of an alternative third group.

While, the clustering analysis (k-Means), which tests the feasibility of the applied methods for stock discrimination, supports the presence of two groups, ANOVA results indicate that the constants of length-weight relationship (a), and condition factor are not effective variables in grouping.

Otolith shape, body length and length-weight parameters were used for principal component analysis (PCA), and those variables justifying most of the variance were later used for discriminant analysis. PCA results indicate that several variables related to otolith shape, as well as aspect ratio, account for most of the variance. However the variables significantly contributing to the discrimination differed in different age groups. 84.15% and 88.51% of fish belonging to 0 and I age classes respectively, were successfully classified via discriminant analysis. Cluster analysis carried out over otolith shape variables evaluated independently from the rest, produced similar results. Although clustering pointed out the existence of two groups, individuals from the same station were not distributed in the same cluster. According to the results of cluster

analysis, clusters were explained by different parameters for each age group. So, within 0 age class, parameters such as aspect ratio and ellipticity did not significantly contribute grouping, while within the age I age class, total length of fish and roundness were excluded.

As observed in the literature, the results denote the presence of two distinct groups. Hence, approaches already being used could be effective in separating anchovy stocks. However, contrary to this, condition factor results point out the presence of another additional group which may represent a hybrid fraction in the stocks. Therefore, to test the accuracy of group separation and determine whether or not a hybrid stock does exist, it is suggested to support the sampling and analyses carried out in this study with further sampling from the feeding and spawning grounds (the north-western Black Sea basin and Sea of Azov) before wintering, where the subspecies would in theory be isolated.

Key Words: Black Sea anchovy, Azov anchovy, length-weight relationship, condition factor, length frequency distribution, otolith shape analysis

ÖZ

KARADENİZ’DE HAMSİNİN OLASI FARKLI FORMLARI ÜZERİNE BİR ÇALIŞMA

Şahin, Ezgi

Yüksek Lisans, Deniz Biyolojisi ve Balıkçılık Bölümü

Tez Yöneticisi: Doç. Dr. Ali Cemal Gücü

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Karadeniz’de hamsi Türkiye için ekonomik öneme sahip olup basende iki alt tür ile temsil edilen bir balıktır. Üreme ve beslenme, besin erişiminin daha yüksek olduğu kuzey sahillerinde gerçekleşmektedir. Su sıcaklığının düşmeye başlaması ile birlikte hamsi daha sıcak olan güney kıyılarına doğru göç etmektedir. Göç sırasında oluşturdukları sürüler hamsiyi balıkçılık filosu için hedef haline getirmektedir. Morfolojik benzerlikleri yüzünden avlanan hamsi miktarını alt tür bazında rapor etmek mümkün değildir. Bu durum balık stok miktarının belirlenmesinde dolayısı ile balıkçılık yönetiminde önemli problemler yaratmaktadır. Her ne kadar stoklar birbirinden genetik yöntemler, kan grupları ve parazitlenme oranları ile ayrılabilseler de bu metotlar uygulanması kolay olmayan pahalı metotlardır. Bu yüzden, gerçekleştirilen bu çalışmada stok miktarını belirleme çalışmalarında kullanılabilecek daha pratik metotlar bulunması hedeflenmiştir. Bu amaca ulaşmak için, alt türlerin beslenme ve büyüme bölgelerinde gözlenen farklılıkların yaş gruplarındaki boy frekans dağılımında ve boy ağırlık ilişkisinde farklılıklara sebep olabileceği düşünülerek kışlama alanında örneklemeler yapılmıştır. Farklı çevresel koşulların yanı sıra kışlama alanına gelirken kullandıkları farklı göç yolları ve kışlama alanına olan mesafelerinin de kondisyon faktörünü etkilemiş olabileceği düşünülmüştür.

Bu çalışmanın bir diğer amacı ise, genelde stok miktarını belirleme çalışmalarında yaş tahmininde kullanılan otolit organı üzerinde yapılan şekil analizinin türlerin karışım bölgesinde stokları birbirinden ayrılmasında etkili olup olmayacağını test etmektir. Ayrıca, otolit boy/en oranı dışında stok ayırım çalışmalarında etkili olabilecek farklı otolit şekil parametrelerinin varlığı da araştırılmıştır.

Biyolojik veriler 2011/12 ve 2012/13 yılları balıkçılık sezonunda sonbahar kış aylarında (Kasım ayından Ocak ayına kadar) İğneada'dan Hopa'ya kadar olan güney Karadeniz kıyılarından toplanmıştır. Boy-ağırlık ilişkisi ve otolit şekil parametrelerinin ölçümü laboratuvar ortamında yapılmıştır. Temel çevresel değişkenlerden deniz yüzey sıcaklığı ve klorofil-a konsantrasyonu (DYS, Chl-a) çalışmanın gerçekleştirildiği yıllar için uydu verilerinden aylık ortalamalar şeklinde elde edilmiştir.

Çalışma sonuçları istasyonlar arasında allometrik büyüme katsayısı (b), balığın iyi durumda oluşunun işareti olan kondisyon faktörü (KF) ve avlanan balık sürülerindeki bireylerin boy frekans dağılımları açısından istasyonlar arasındaki benzemezliği göstermektedir. Fakat birbirine benzeyen istasyonlar her zaman coğrafik olarak yakın yerlerde bulunmamakta ve belirgin bir dağılım paterni sergilememektedir. Her ne kadar KF alternatif üçüncü bir grubun varlığını gösterse de genel olarak, sonuçlar iki grubun varlığına işaret etmektedir.

Başvurulan metotların stok ayırımı için elverişli olup olmadığını test etmek için uygulanan kümeleme analizi (k-Means) sonuçları iki grubun varlığını desteklerken, ANOVA sonuçları, boy ağırlık ilişkisi sabiti (a) ve kondisyon faktörünün gruplandırmada etkin rol almadığını göstermektedir.

Otolit şekil, balık boy ve boy-ağırlık ilişkisi parametreleri temel bileşenler analizinde (TBA) kullanılmış ve varyansın çoğunu açıklamada yardımcı olan değişkenler diskriminant analizinde yer almıştır. TBA sonuçları otolit şekline bağlı, boy/en oranınında dahil olduğu bir çok değişkenin varyansı açıklamada yer aldığını göstermiştir. Fakat ayırımda önemli katkısı olan değişkenler farklı yaş gruplarında değişiklik göstermektedir. Diskriminant analizi ile birlikte 0 ve I yaş sınıfında ait bireyler sırası ile %84.15 ve %88.51'lik oranlarda başarıyla sınıflandırılmıştır. Diğer metotlardan bağımsız olarak sadece otolit şekil değişkenleri kullanılarak yapılan kümeleme analizi de elde edilen sonuçları desteklemektedir. Kümeleme analizi

sonuçları iki grubun varlığına işaret etsede, aynı istasyonu temsil eden bireyler aynı gruplarda yer almamaktadır.

Kümeleme analizi sonuçlarına göre gruplar her yaş grubu için farklı değişkenler ile açıklanmaktadır. 0 yaş sınıfında otolit boy/en oranı ve ovallik, I yaş sınıfında yuvarlaklık ve balık toplam boyu gruplandırmada yer almamıştır.

Literatürde de gözlendiği gibi, sonuçlar iki farklı grubun varlığına işaret etmektedir. Dolayısı ile kullanılan yaklaşımlar hamsi stoklarının ayrılmasında etkili bir yöntem olabilir. Yalnız, bu bulguların aksine kondisyon faktörü ek olarak başka bir grubun varlığına işaret etmektedir. Bahsi geçen bu grup stoklar arasında kesişimi temsil eden bir hibrit olabilir. Bu yüzden grup ayırımındaki hassasiyeti test etmek ve hibrit bir stoğun var olup olmadığını gözlemlemek adına bu çalışmada uygulanan örneklemenin ve analiz stratejilerinin kışlamadan önce alt türlerin izole bir şekilde olduğu düşünülen beslenme ve üreme alanlarında (Karadeniz'in kuzey-batı baseni ve Azak Denizi) da gerçekleştirilmesi önerilmektedir.

Anahtar Kelimeler: Karadeniz hamsisi, Azak hamsisi, boy – ağırlık ilişkisi, allometri, otolit şekil, kondisyon faktörü

To my family

Thank them for their invaluable love which makes me believe in miracles

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1 INTRODUCTION

Anchovy has a great importance in the Black Sea basin not just commercially but also ecologically. The large quantities of anchovy provides commercial support in the Black Sea. Besides, the species functions as a bridge between lower and higher trophic levels in energy flow process as being planktivorous fish. Therefore it has a supplementary role in the Black Sea ecosystem. In consequence, with its biology, ecology and management strategies anchovy has become subject of a remarkable amount of studies.

1.1 Physical Characteristic of the Black Sea

The Black Sea is a semi–enclosed inland sea which connects to the Mediterranean Sea through Bosporus and Dardanelles Straits called as Turkish Straits System (Oğuz et al., 2005) and the Sea of Azov with the Kerch Strait. It has a border with six countries – Turkey to the south, Bulgaria and Romania to the west, Ukraine to the north, Russia and Georgia to the northeast and east respectively (Figure 1.1).

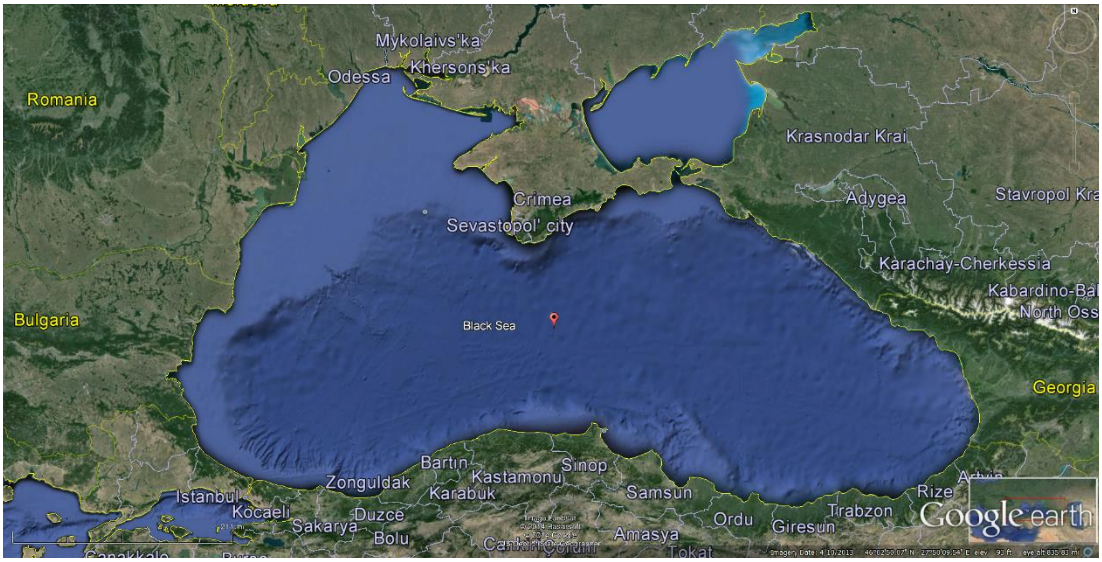


Figure 1.1 The Black Sea, its neighbours and adjacent seas retrieved from Google Earth (43°54'47.15'' N 34°14'23.39'' E. Google Earth, 4/10/2013. 6/23/2014).

The surface of Black Sea covers 423.000 km^2 areas and the sea contains total 547.000 km^3 water. The Sea of Azov has approximately 39.000 km^2 area with a maximum depth of 14 meter while the Black Sea has a maximum 2200 meter depth (Zaitsev & Mamaev, 1997).

The Black Sea belongs to the temperate zone and has subtropical character. While the summer sea temperature can exceed 25^oC, in winter at the open sea it can fall to 6-8^oC degrees. Only the south Crimea and Caucasian coasts are in the subtropical zone in the Black Sea. The Sea of Azov has a continental climate since it is relatively small and surrounded by land. In summer near the shores the temperature can be over 30^oC while on the open sea it can reach maximum 28^oC and its average water temperature in winter is 0-1^oC (Zaitsev et al., 2002; Dobrovolsky & Zalogin, 1982).

Because of the Black Sea is a landlocked basin, hydrologic elements control its total water mass and hydrochemical character. The Black Sea has positive water balance thanks to amount of influx via rainfall (~300 km^3 yr^{-1}) and river discharge (~400 km^3 yr^{-1}) is higher than outflux via evaporation (~350 km yr-1) and the rest water budget correspond to the net outflow from the Bosporus Strait (Ünlüata et al., 1990; Bingel et al., 1993). Besides, for this landlocked basin, the narrow and shallow Bosporus is the only connection with Mediterranean. Through the Bosporus salty water is transferred to the Black Sea basin and it increases the deep water salinity value to 22.33 ppt. On the other hand, the Black Sea is rich in terms of river discharge and thanks to influx of several European rivers (especially Danube, Dniester, Dnieper, Don, and Kuban) the salinity is low in the surface, in general it is approximately 18 ppt. The salinity difference cause strong stratification between surface and deeper waters. Therefore, there is high degree of density gradient (Murray et al., 2007).

Being an index of phytoplankton biomass, chlorophyll-a concentration is a common property which characterizes the productivity of an area. Even in all over the Black Sea basin chlorophyll-a concentration varies in a wide range according to the regions and seasons (0.01 to 100 $mg.m^{-3}$), in general higher concentrations are observed in the Sea of Azov (https://lpdaac.usgs.gov/data_access).

As a result of the density gradient, water exchange within vertical layers is limited. While oxygenated layer extends over 50 meter depth, in deeper layer there is anoxic environment consisting of hydrogen sulphide (100 - 2000 meters). Between these stratified surface and deep layers, there is transition called as suboxic zone which has both low O_2 and H_2S concentrations (Murray, et al., 1989; Codispoti et al., 1991; Jørgensen et al., 1991).

Besides lateral oxygen exchange between adjacent seas to the deep basin is limited due to landmasses. Because of this restriction, anoxic conditions surpass almost all over the basin (Oguz et al., 1998).

Although the Black Sea is semi – closed inland sea, it has wind driven circulation system as open oceans which consists of gyres, eddies, deep water thermohaline circulation and shallower ventilation into the thermocline (Murray et al., 2007). One of the major features of upper layer circulation is the cyclonic boundary flow system named as Rim Current which flows over the rapidly changing continental shelf topography (Oguz & Besiktepe, 1999). Rim Current branches into two near the southern tip of the Crimea, eventually converging on the Turkish and Bulgarian coast, one branch follows the topographic slope heading south-westward and the second branch follows a north-westward path, flowing into the inner shelf where it is involved with the inner shelf current system (Korotaev, Oguz, Nikiforov, & Koblinsky, 2003). Additionally the cyclonic central gyres (western and eastern), at the coastal side of the Rim Current there are several anti – cyclonic eddies named Kizilirmak, Sinop, Sakarya, Bosporus, Sevastopol, Crimea, Caucasus, Batumi, Kaliakra and quasi – permanent Batumi (Oguz et al., 1998).

1.2 Anchovy Fisheries in the Black Sea

Fishery in the Black Sea has a special place in Turkey's marine resources predominantly owing to anchovy. Although fisheries pressure is the most known reason of changes upon anchovy stocks, environmental conditions as eutrophication, climatic oscillations and internal dynamics of ecosystem have a role (Oğuz, 2010).

In the period of 1977-1981 sharp fluctuations experienced upon small pelagic fishes with the total effects of withdrawal of predator fishes and mammals from the system in 1970s, increasing fishing fleets with technological advancements, extensive eutrophication, and climatic cooling meanwhile severe winter conditions. Even though fisheries pressure continued to rise, high stock levels could be maintained thanks to productivity of the Black Sea basin until the end of 1980s (Oğuz, 2010).

Then as a result of heavy fishing, new generations of anchovy got weak and in 1990-1992 anchovy stocks were collapsed. Explosion of ctenophore *Mnemiopsis leidyi* accelerated the sharp decline establishing competition for food.

After this breaking point between 1993-2006 anchovy recovered slightly and reached up to half of the catch in 1980s (Daskalov et al., 2012; Oğuz, 2010). 2005 was a particular year as the bonito reached highest numbers of last half century in the Black Sea (www.tuik.gov.tr). Therefore fisherman turned towards bonito instead of anchovy which has higher market value. Eventually, fisheries over anchovy declined as an aftermath of such extraordinary year. Yet there might be relation between temperature increase, plankton productivity decline and dispersal of the anchovy schools which makes them hardly accessible by fish nets. In the following year, 2006, anchovy catch increased again (Daskalov et al., 2012).

In last few years, unstable conditions of anchovy stocks remained. In 2012, anchovy catch decreased till 126331 tones (Sampson et al., 2013) (Figure 1.2). As it stands, anchovy fisheries need better management regulation. Eventually, future problems can arise in the case of the capacity of fishing fleet is higher than catchable anchovy stocks in the Turkey. As expected, overfishing might results in bad conditions, successively anchovy stocks can collapse and other planktivorous organisms like ctenophores may suppress the Black Sea ecosystem (Oğuz, 2010).

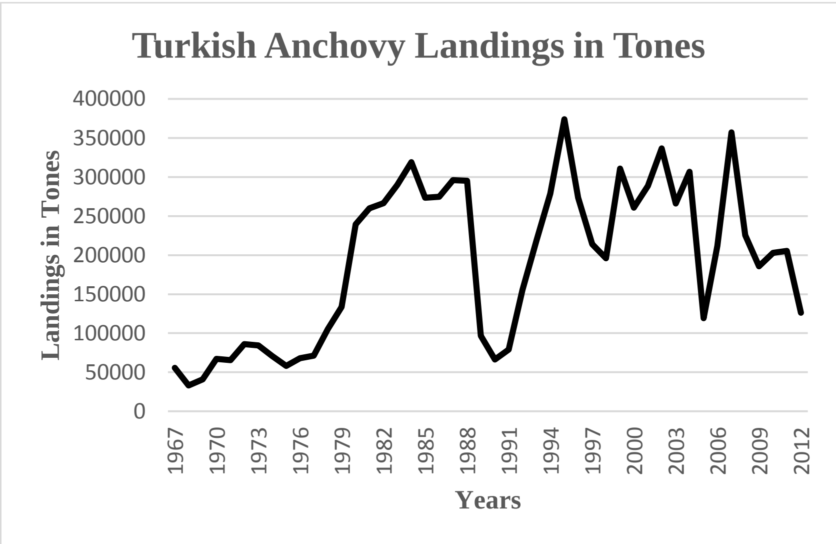


Figure 1.2 The Anchovy Landings of Turkey between 1960^{s} and 2010th; data obtained from Sampson et al., 2013.

1.3 Morphological Characteristics and Distribution of European Anchovy, *Engraulis encrasicolus* (Linnaeus, 1758)

European anchovy, *Engraulis encrasicolus*, is small pelagic fish which is one of eight Engraulis members. This ray finned fish is a part of Engraulidae family of Clupeiformes order (Whitehead P. , 1984).

The body of *Engraulis encrasicolus* is elongate, thin and oval in transverse. Its dorsal is generally green, greenish blue; its ventral and lateral are silver grey colour. Pearl grey scales are large according to the body. They are thin and flake off easily (Bat et al., 2008; Whitehead P. , 1984). The position of mouth is ventral and it is big according to the body size. Upper jaw is longer than lower jaw and the front edge of the lower jaw is at almost same level with nostril. The snout is pointed. There is a short rounded edge maxilla which lengthens nearly to the front side of pre-operculum (Figure 1.3). Its anal fin is short and remaining behind base of last dorsal fin ray. The number of rays in the fins is the following: Dorsal soft rays: 16 – 18, Anal soft rays: 13 – 15 Vertebrae: 46 – 47 (Whitehead P. J., 1985).

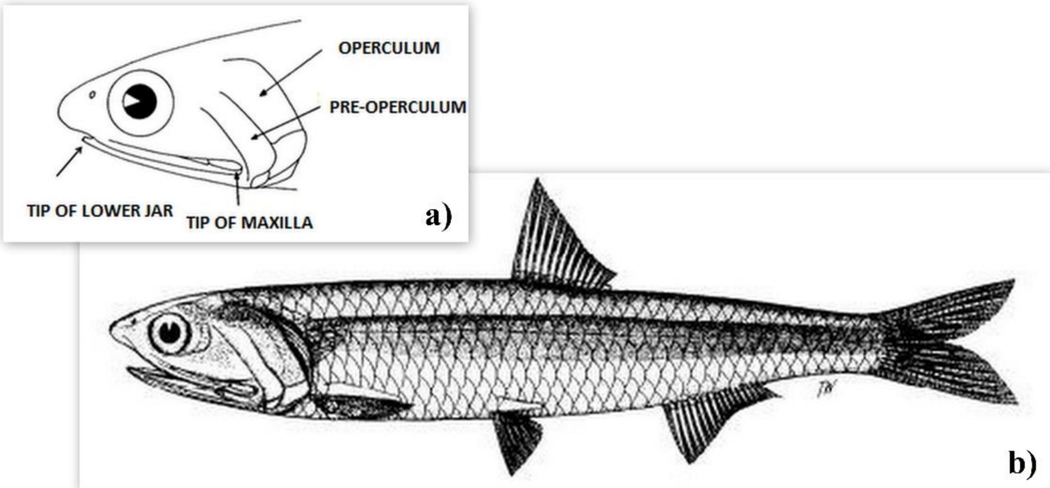


Figure 1.3 Morphological characteristic of the *Engraulis encrasicolus* adapted from Whitehead P. J., 1985; a) the position of mouth and shape of the snout b) shape of the body.

The European anchovy is distributed in the North Eastern - Central Atlantic, and Mediterranean reaching up to Black and Sea of Azov (Whitehead P. J., 1985). There is also evidence that its distribution extends over Western, Southern Africa and one part in Indian Ocean (Grant & Bowen, 1998; Borsa et al., 2004).

Within these different habitats anchovy has different races as Atlantic and Mediterranean, in Mediterranean subdivided as western and eastern (Fage, 1911 and 1920 as cited in Ivanova & Dobrovolov, 2006) and further in Black Sea; there are Sea of Azov and Black Sea races. While it was questionable that these two divided groups in the Black Sea are only different populations of the European anchovy (Lindberg, 1980; Tshashchin, 1985; Dobrovolov, 1988; Dobrovolov and Mikhailov, 1990 as cited in Prodanov, et al., 1997; Ivanova & Dobrovolov, 2006) or segregated subspecies (Aleksandrov, 1927; Pusanov, 1936; Rass, 1987; Prodanov et al., 1993 as cited in Prodanov et al., 1997), the recent genetic investigation carried out by Ivanova et al., 2013 proved that Black Sea *Engraulis encrasicolus ponticus* (Aleksandrov, 1927) and Sea of Azov *Engraulis encrasicolus maeoticus* (Pusanov, 1936) anchovies are subspecies.

1.4 Characteristic of the Black Sea Anchovy Populations

1.4.1 Feeding

Anchovy is a short lived small pelagic fish. Its average lifespan is 3 – 4 years in the Black Sea. This omnivorous fish mainly feeds on zooplankton species especially Copepoda and Cladocera. Species can change its food preference when the amount of zooplankton is low and eats mysids, fish eggs – larvae, polychaete larvae and phytoplankton (Whitehead P. J., 1985; Bulgakova, 1993). They can take nourishment both through filtering and particulate feeding (biting) mechanism, they change the feeding type depending on size and density of prey. Having two different feeding mechanisms provide an advantage to utilize maximum energy from different food supplies for anchovy (Bulgakova, 1993).

1.4.2 Reproduction

Engraulis encrasicolus is a batch spawner. In the Black Sea, spawning season takes place between the middle of May and at the second half of August with a peak from the middle of June to the end of July. During the spawning season temperature changes between approximately 16 and 28 ^oC and the maximum spawning occurs while the temperature is higher than 20 ^oC. The lowest temperatures (13 -16 ^oC) are seen only at the beginning of the spawning (Niermann et al., 1994; Lisovenko & Andrianov, 1996). The species prefers to spawn in coastal region optimally within the 5 – 10 meter depth range inclusively in areas influenced by rivers, estuaries (Slastenenko, 1955/56; Lisovenko & Andrianov, 1996). Correspondingly, the salinity value of spawning areas is low (7 – 18 $^o/_{oo}$). Individuals become mature and ready to spawn when they are one – year old. However, some findings indicate that young of the year can spawn few months after hatching (less than 1%). The Black Sea anchovy has a high reproductive capacity. It has a diel spawning rhythm and high number of spawns in short life cycle. Forming a new spawn, ripening and ovulation of eggs and resting periods take place in a one day (Lisovenko & Andrianov, 1996). While one average female can spawn approximately 13.000 – 40.000 eggs during a one spawning season (Bat et al., 2007), the number of batches can reach to 50 (Lisovenko & Andrianov, 1996). After hatching, around 25 – 35 % of laid eggs can survive during the breeding period. Both the survival of larvae and juvenile depends on abundance of planktons which are vital for their feeding (Pavlovskaya, 1955 as cited in Bat et al., 2007).

1.4.3 Habitat Preference and Migration

European anchovy prefers to live in coastal areas with some exceptions, its euryhaline character allows them to tolerate large salinity range from 5 to 41 and in some areas they enter lagoons, estuaries or lakes especially during the spawning season (Whitehead P. J., 1985). The species is not limited by the salinity but temperature, since it does not appear both in coldest and warmest waters. While the summer distribution of the species is mainly characterized by food availability, hence nutritional conditions and productivity, seasonal change in temperature cause migration from productive northern waters to warmer southern waters in the Black Sea basin (Reid, 1967; Whitehead P. J., 1985; Chashchin, 1996; Garcia & Palomera, 1996). Moreover, spawning time is also related to temperature and it occurs in warm summer months (Whitehead P. J., 1985; Chashchin, 1996; Garcia & Palomera, 1996).

Anchovies in the Black Sea follow same pattern with European anchovy. Their summer distribution take place in northern waters and even if different migration routes are predicted (Ivanov & Beverton, 1985; Chashchin, 1996; Shulman, 2002; Ivanova et al., 2013), in autumn season with increasing cold Sea of Azov and Black Sea dense anchovy schools begin to migrate approaching the warmer waters in the southern Black Sea coast (Chashchin, 1996). To begin wintering migration, initially temperature should fall till 9 – 12 ^{o}C and individuals should accumulate enough fat correspondingly (Vorobyev, 1945 as cited in Shulman, 2002).

The Black and Azov Sea subspecies disperse in different basins. The spawning and foraging area of Azov anchovy covers all over the Sea of Azov. In Sea of Azov water cooling happens earlier compared to the rest of the Black Sea. Therefore under the increasing cold wintering migration of Azov anchovy starts earlier. In September – October temperature decline stimulates the migration and Azov anchovy moves from Sea of Azov towards the Caucasus and also the southern part of the Crimea Peninsula. After cold winter days pass, at the middle of the April till end of the May the way back to the foraging area take place.

The Black Sea anchovy occupies almost all over the Black Sea predominantly the north-western shelf. In October – November, the wintering migration of the Black Sea anchovy starts towards Turkish waters and extends over Georgia. With increasing temperature in spring the Black Sea anchovy moves back to northern Black Sea.

Anchovies in the Black Sea do not have completely isolated habitats. During summer, juvenile Black Sea anchovies can enter the Sea of Azov and as opposite Azov anchovy members can be seen in less saline north-western Black Sea waters. The overlap is also observed on migration route. Lastly and most importantly there is mixing in the wintering ground (Chashchin, 1996; Ivanova et al., 2013) where they provide a great fishery source.

1.4.4 Differences in the Black Sea and Sea of Azov Anchovies

As being subspecies, the Black Sea and Sea of Azov anchovies exhibit differences in growth rates, otolith width / length ratios, parasitism rates, blood groups and some genetic sequences (Chashchin, 1996). The Black Sea anchovy is slightly longer than the Azov anchovy. This body length difference result in particular growth rates and The Black Sea anchovy has faster growth rate than the Azov Anchovy (Gubanov & Limansky, 1968; Shevchenko, 1980 as cited in Chashchin, 1996).

There is coherent difference in aspect ratio of anchovy otolith (length / weight). The Black Sea anchovy is equal to 2.15, whilst Azov anchovy is 1.96 (Skazkina, 1965 as cited in Chashchin, 1996).

The serological studies which were carried out during 1970s demonstrate three different blood types in terms of reaction between erythrocytes and normal pig / horse blood sera. Group A_1 blood cell react with the both types of sera, A_2 group has an interaction only with the pig serum. A negative blood group (A_0) does not trigger any reaction with both sera types. While Azov anchovy is represented by these three blood groups, Black Sea anchovy has only A_1 and A_2 blood groups. Besides observation frequency of the blood groups is different. Black Sea anchovy covered almost by A_1 group (96%) and just 4% takes place in A_2 group whereas Azov anchovy comprise of 63% - A_1, 16% A_2 and 21 % A_0 (Altukhov, Limansky, Payusova, & Truvaller, 1969 from Chashchin, 1996).

Moreover, incidence rate of *Contracaecum aduncum* has an important role on discrimination. Larvae of the nematode take part in Black Sea food. Thus, Black Sea anchovy is being exposed to infestation more than the Azov anchovy. However, age of fish and accumulation of the nematode during overwintering in the Black Sea should be taken into consideration if degree of infestation is used as a discrimination tool (Terekhov, 1971; Chashchin, 1981 from Chashchin, 1996).

Genetic is the last and promising trail for stock identification. Anchovies in the Black Sea demonstrate two distinct groups in terms of allozyme and mitochondrial DNA haplotype frequencies (Borsa et al., 2004; Ivanova et al., 2013).

1.5 Stock Discrimination Methods

Stock discrimination has a crucial role on stock assessment in turn manage rational and effective fisheries management, since each stock must be managed separately to optimize their yield (Grimes et al., 1987 from Cadrin et al., 2005). Under the assumption that stocks broadly represent populations, most population models accept that stock is a self-reproducing fish group whose members has constant vital parameters (e.g. growth, maturity, mortality) in particular geographic region and time. Moreover, for any kind of fisheries science studies (e.g. life history, growth, physiology, or diet) about living resources, sampling or analytical design are assumed as part of population. Hence, it might be considered as stock discrimination/identification underlies almost entire fisheries analysis (Thorpe et. al., 1995 from Cadrin et al., 2005).

To perform stock identification many approaches have been applied including life history traits, serology, marking techniques, internal and external tags, meristics and morphometrics, genetics, calcified structures and chemical analysis of different content structures or tissues (Kumpf et al., 1987 from Cadrin et al., 2005; Avşar, 1987). However none of them provides a practical solution to discriminate mingled anchovy stock in the wintering ground.

1.5.1 Allometry and Geographical proximity

In general, allometry expresses that how traits of objects (especially organisms) change with size. Origin of allometry is to observe scaling relationship between the size of the trait and the size of the whole object. However, nowadays the meaning of allometry can be extended and adapted for any kind of biological measurements to scale either morphological, physiological or ecological traits (Shingleton, 2010). The allometric equation is described as a power relationship;

$y = a.x^b$ where y is the trait of interest and x is the body size (Huxley, 1932).

Based on this description the length and weight relationship of fish expresses as;

$W = a.L^b$ (Le Cren, 1951)

In this respect, slope (b), the allometric exponent and the other component intercept (a), both have biological meaning in the equation. According to scaling relationship in the studied subject, the meaning of slope and intercept changes within each field.

As in the studies which carried out allometry, weight and length relationship covers wide field included fish biology, physiology, ecology and fisheries assessment studies. In this context, while the relation can be used for estimation of weight-at-age, it can be also applied on morphological and life history comparison between different species or populations living in different habitats (Petrakis & Stergiou, 1998; Gonçalves et al., 1997; Morato et al., 2001; Rosa et al., 2006; Pereira et al., 2011).

As it is known that the Black Sea anchovy is slightly longer but thinner than the Azov anchovy (Slastenenko, 1955/56), one may expect significant differences in the cubic relation between weight and length and particularly on the allometric exponent of the relation. Therefore, in the case of anchovies in the Black Sea, the slope assumed as a criteria of among group similarity/dissimilarity and it was used to make comparison between subspecies on traits of individuals at the same age.

1.5.2 Condition Factor

The length – weight relationship (LWR) of fish is applied to determine correlation between two variables and calculate the variation from the expected weight for the body length of fishes (Le Cren, 1951). The ratio between length and weight is the index of the fish condition and the indicator of well-being, fatness based on heavier fish of a given length has better condition hypothesis (Bagenal & Tesch, 1978). Mediately, condition factor takes place in determination of present and future population success by its influence on growth, reproduction and survival (Hossain et al., 2006). Condition factor can be also used for ontogenetic studies in the case that the length – weight parameters of the same species is different at the level of population because of feeding, reproduction activities fishing etc. (Safran, 1992). In addition, the relation is a useful tool to compare life history and morphological traits between different fish populations or species from different habitats and/or regions (Gonçalves et al., 1997).

As a migration strategy, anchovy stops to feeding before migration (Shulman, 2002). Regarding this, with the knowledge of growth is affected by temperature (Pauly, 1980), and fish production, partly determined by growth, is mainly controlled by primary production (Iverson, 1990) it is expected that differences in nourishment and hydrological conditions of the distinct nursery areas and distance between nursery and overwintering grounds (long for north-western shelf while shorter for Azov) should cause variation in the somatic condition of the zero age cohort anchovies when they arrive to the overwintering ground. Therefore, in order to observe potential effects of feeding in distinct distribution areas, condition factor comparison was carried out.

1.5.3 Age Determination and Otolith Shape Analysis

Age determination of fish is one of the most important element for stock assessments, management strategies, conservation plans (Helfman et al., 1997) and so evaluation of multiple biological aspects (for example growth and survival). Age of almost all fish species can be read from discontinuities in the skeletal structures such as scales, otoliths with the assumption that periodical morphological structures are generated proportionally fish growth (Campana & Neilson, 1985). Age reading of anchovy is generally carried out on otoliths because of practical usage of these ear stones (Robson & Jerome, 1969).

There are three pairs of otoliths named sagittae, lapilli and asterisci. Saggittae is the largest and most preferred pair for age reading studies (Holden & Raitt, 1974), however according to model organism and aim of a study the preference can be changed. As with the majority for anchovy age reading and shape analysis studies sagittae is used (Collins & Jerome, 1969) due to the size, easy removal and interpretation.

Anchovy has nearly oval sagittal otolith with an outer concave face (Figure 1.4). Its major axis elongates towards anterior-posterior direction. The dorsal and ventral edges merge at the front side close to the ventral edge and form “rostrum”. Adjacent to the rostrum there is less apparent “antirostrum” segregated from the rostrum by a deep cut. While the dorsal edge has slightly curved shape, there are saw-like teeth on the ventral which are more apparent and increased with age of fish. Anchovy otoliths are also characterized by diffuse zone in the centre of nucleus which generated in larval period and seem morphologically similar in each otolith (Pertierra, 1987 as cited in Giannetti & Donato, 2003).

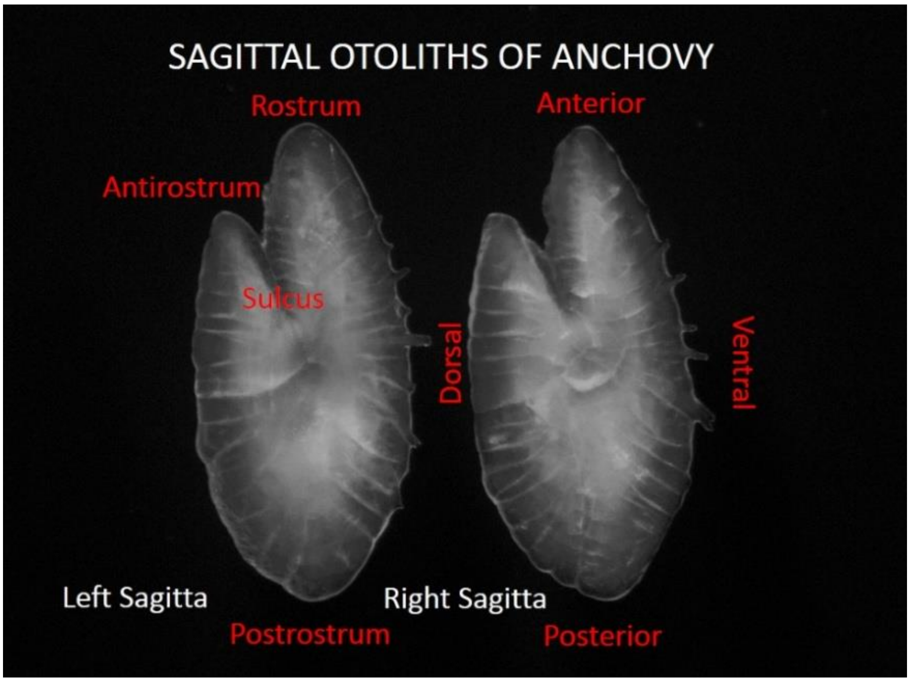


Figure 1.4 Anchovy' sagittal otolith. Image by METU-IMS (adapted by Giannetti & Donato, 2003).

Otoliths are composed of opaque and hyaline bands which formed by crystalline structure and protein matrix respectively (Dunkelberger et al., 1980 as cited in Cadrin et al., 2005). While opaque zones represents fast summer growth (May – November), hyaline zones represents slow winter growth (December-April) (Panella, 1971). The end of growth year is marked by thin hyaline edge at formation of each annulus. Age interpretation is carried out by counting these annuli deposition (Giannetti & Donato, 2003).

Otoliths are species specific organs (Morrow, 1976; Gaemers, 1984; L'Abbe´-Lund, 1988 as cited in Pothin et al., 2006) which have a crucial role as an indicator of stock identification. Its growth is more stable according to fish growth in all likelihood because of its function both as equilibrium and hearing (Campana & Casselman, 1993). Besides growth continues through lifetime of fish and once it stored, it is hardly decomposed or changed in contrast scales and bones (Campana & Neilson, 1985; Casselman, 1987). In consequence, otoliths are preserved from short term changes in fish conditions and so it is applicable for stock discrimination studies (Campana & Casselman, 1993; Zhang et al., 2013).

1.6 Motivation and Definition of the Problem

As mentioned above, anchovy is represented by two different subspecies in the Black Sea. Despite taking place in the same basin, they have different foraging and spawning areas with some overlaps both in summer distribution and overwintering area. Whilst they have tendency to be in northern waters in summer and distribute in surface water, with increasing cold they go downwards, accumulates dense schools and migrates towards warmer southern waters (Chashchin, 1996). This schooling behaviour makes them primarily target species on the Turkish Black Sea coast. The species occupies an important place in overall the Turkish landings as a sea resource and supplies more than 50% of the total catch of the country by alone (www.tuik.gov.tr). Even though there are small – scale artisanal fishery of the species mainly in Bulgaria, Romania and Ukraine with coastal trap nets or beach seines, on the wintering ground in Turkey and Georgia there is commercial fisheries with equipped purse seine vessels and as a recent method, midwater trawling has been performed. Therefore, with these large scale fisheries activities, Turkey has a great responsibility about anchovy's fisheries management. However, stock assessment for sustainable resource management does not pose a problem alone. The main concern arises from discriminating mingled anchovy stocks during overwintering for better assessment.

1.7 Objectives of the Study

In number of investigations, convincing features to distinguish Black Sea anchovies revealed. Yet, still there is not any practical method for stock discrimination in the wintering ground while anchovies merge. For this reason, the study aims to find out simple/ easily applicable methods to discriminate Black Sea anchovies in the southern Black Sea coasts through migration. For this purpose the objective of this study is to;

i) Test feasibility of allometric relationship to discriminate stocks
ii) Detect possible condition differences arising from different foraging ground
iii) Examine the potential use of the otolith shape to separate mixed stocks and investigate geographical differences in the otolith shape between individuals originating from different summer distribution area
iv) Evaluate applied methods together for clustering of anchovy stock

2 MATERIAL AND METHODS

2.1 Study Area

The study targets overwintering anchovies, therefore the study area is restricted to southern continental shelf of the Black Sea (İğneada to Hopa).

2.2 Field Study and Sampling

The samples used in the study were collected during four acoustical surveys conducted on the southern Black Sea. The areas covered during these surveys are given in Figure 2.1 and Figure 2.2. Table 2.1 displays the data inventory. While first two cruise were performed during 2011-2012 overwintering season (November – December 2011, January 2012) following two surveys were carried out in November and December 2012.

As the Azov anchovy arrives the southern Black Sea later in winter (Chashchin, 1996), the first part (November) of the successive cruises the target organism was the Black Sea anchovy, whilst in the second part (February) it was the Azov anchovy.

Throughout the observations total of 146 trawl hauls were performed and sufficient numbers of anchovies were caught at 56 stations.

Table 2.1 Sampling periods and number of stations (6 of 7 stations frozen samples were collected overlap with rest of the sampling stations).

Sampling Periods	Storage Type of Samples	Number of Stations
November – December 2011	In formaldehyde	21
January 2012	In formaldehyde	9
November 2012	In formaldehyde	14
December 2012	In formaldehyde	11
November – December 2012	Frozen	7

Trawl sampling was performed concurrently with hydroacoustic survey. Schools were first detected acoustically using scientific echo sounder SIMRAD EK60 (200 kHz, 120 kHz and 38 kHz). Later acoustically detected schools were sampled by a mid-water trawl equipped with depth sensors (attached to bottom line and head rope). In order to sample all size groups within a population the mid-water trawl net with a 14 mm (stretched) mesh size cod end was used. Some important technical information of the mid water trawl net used in this study are given in Table 2.2.

Table 2.2 Some features of the pelagic net design.

PARAMETER	**R/V BİLİM**
Type of trawl	Mid water trawl
Type of net	Combi Model / Midwater – Bottom
Length of foot rope (m)	28.44 m
Length of head rope (m)	28.60 m
Codend mesh size (mm)	14 mm
Mean trawling speed (nm/h)	3-5 knots

The trawl hauls were conducted in such a way to sample a single school at a time. Tows generally performed at a speed of 3 to 5 knots. According to size and depth of fish accumulations, the duration of the trawl operation was changed; particularly to avoid catching excessive amount of fish. The beginning of the haul was set at the moment when the warp is tightened. The moment that the vessel was slowed down and warp hauling started was set at the end of hauling.

Sampling stations were recorded on board by;

- Cruise Name
- Date
- Station number and Coordinates (Latitude and Longitude)
- Hauling period
- Starting and end depth

When the catch was taken on board all material were cleaned by sea water and species separated from each other in biology laboratory of the research vessel and then total weight and length measurements were taken. To obtain length frequency distribution the fishes were measured as soon as they were sorted out. These distributions usually cover all possible year classes (0 to 3). The length frequency distributions with standard deviations were therefore decomposed into age classes by fitting a normal distribution curve. Fitting was carried out by using NORMDIST function and SOLVER optimizer in EXCEL. In order to eliminate the potential impact arising from sex differences, only zero age class was taken into consideration for condition factor and otolith shape analyses.

Then according to the sample size, from each 0.5 cm length class five fish sample (throughout the available range) were stored for further analysis. To avoid decomposition of otoliths during preservation, the samples which were used for age reading and otolith shape analysis stored as frozen while rest was preserved with 4% formaldehyde solution (extracted from 10% formalin solution buffered with borax) – (Ferreiro & Labarta, 1988) for other studies. If the total number of anchovies sampled at a station did not reach sufficient number representing each length classes fishes were stored only in formaldehyde. Otherwise they were stored in both way. A total of 3006 anchovies were preserved in formaldehyde and 270 samples were stored frozen.

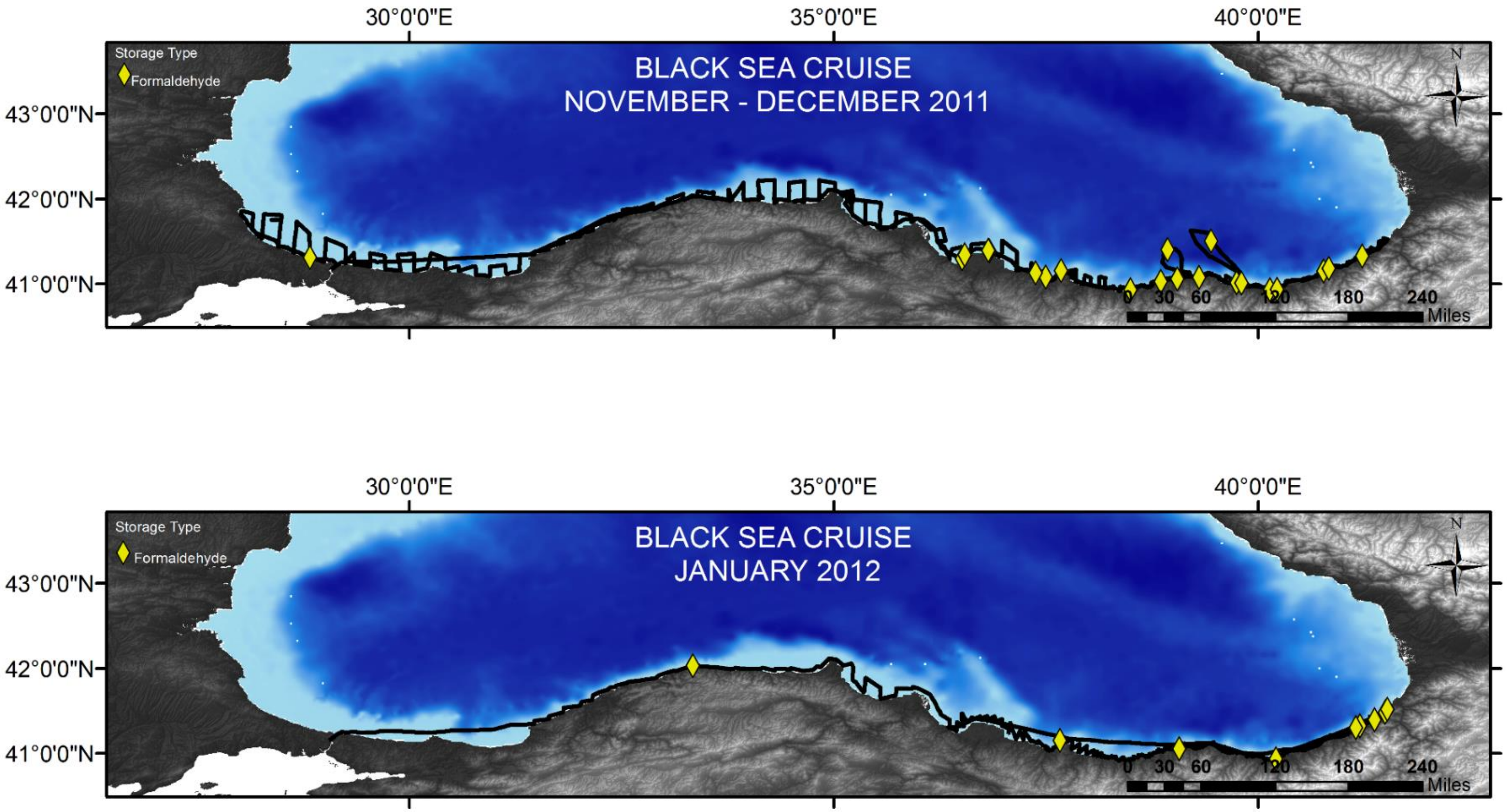


Figure 2.1 Locations of the sampling stations in November – December 2011 and January 2012 Cruises along the Turkish coast of the Black Sea including path of acoustic transects (black lines) where; symbols represent type of sample storage.

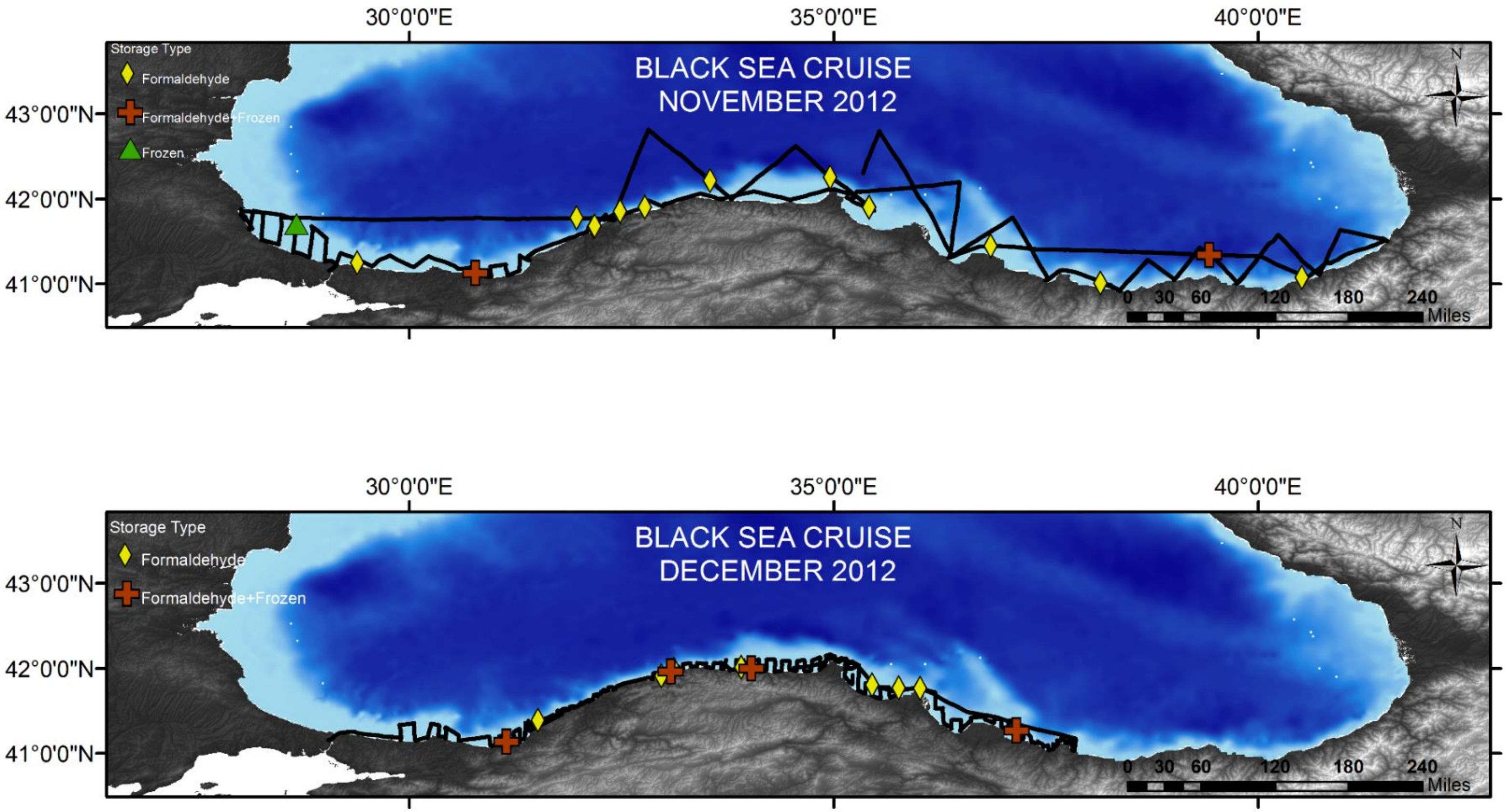


Figure 2.2 Locations of the sampling stations in November and December 2012 cruises along the Turkish coast of the Black Sea including path of acoustic transects (black lines) where; symbols represent type of sample storage.

2.3 Laboratory Studies

Samples preserved in formaldehyde were used for biometric measurements to apply on weight and length relationship based methods. Before the dissection, the fishes were washed with tap water to remove excessive formaldehyde solution. During this process water flow was kept at a minimum level in order to prevent any damage on the fish body. Total length (TL) to the nearest 0.1 cm and total body weight (W) to the nearest 0.01 g were measured in order to establish length – weight relationship.

The otolith and shape analysis were performed by using frozen samples in order to prevent possible chemical interaction between formaldehyde and calcium carbonate structure of otoliths. After total length and total weight of molten fishes were measured, samples were dissected and the sagittal otoliths were removed, cleaned and stored dry for age determination and shape analysis.

2.4 Chlorophyll-a Concentration and Sea Surface Temperature

The hypothesis of this thesis relies on the assumption that the differences in the two forms of anchovy are imprinted at the spawning/nursery grounds (Basilone et al., 2004). In order the check, whether or not, the north-western shelf and the Sea of Azov, which are suggested as the main anchovy spawning/nursery areas in the Black Sea (Ivanov & Beverton, 1985; Shulman, 1964) differs with respect to their ecologic and hydrologic conditions. These two factor would eventually determine limitations of a habitat for a species (Reid, 1967). As such, the mean monthly chlorophyll concentration and sea surface temperature data were used to associate the differences during the respective period of the study (2011-2012).

The satellite data that have been used to create monthly SST and chlorophyll maps were collected from NASA's MODIS (Moderate Resolution Imaging Spectroradiometer) (https://lpdaac.usgs.gov/data_access) satellite at 4 km resolution. Due to presence of cloud patches in the study region monthly temporal scale was used. The data was downloaded in ascii format from Giovanni online data system. Despite using monthly averaged composite format there were gaps mainly originated due to consistent cloud patches. In order to fill such gaps for better visual assessment, the IDW (inverse distance weighting) interpolation technique was used. IDW predicts the values at unknown points based on a technique which gives greater weights to the points that are closer to the prediction location and lesser to those farther away. For the SST maps two different scales was used for a clearer visual comparison by separating the monthly data into two groups as winter (November to April) and summer (May to October). The temperature scale that has been used for winter was between 4°C and 13 °C and for summer it was between 14°C and 26 °C. The extreme regional differences in chlorophyll data were hindering the visual comparisons. Thus, the chlorophyll data was transformed into logarithmic (log10) scale after the interpolations, nevertheless on the legends the scale was given in the back-transformed format.

With this object, The Chl-a data derived in ascii format was also used to observe monthly alterations between main distribution area of subspecies. The comparison was conducted specifically for the North-western shelf of the basin and Sea of Azov.

2.5 Stock Discrimination of Anchovies

In order to discriminate and classify anchovy stocks following methods were used;

- allometry and geographical proximity
- condition factor
- otolith shape analysis
- k mean clustering with all separation criteria

The statistical analyses and evaluations of applied methods were performed by using SPSS and EXCEL.

A school of a small pelagic fish is generally composed of individuals of the same cohort spawned at the same time and place and subjected to the same environmental conditions (Sparre & Venema, 1998). Based on this information, it was assumed that each anchovy school sampled during a trawl operation was composed of individuals of the same stock. So the discriminant methods were performed accordingly.

2.5.1 Allometry and Geographical proximity

The allometry in fish is expressed by the equation of length – weight relationship (Le Cren, 1951);

$W = a.L^b$ Equation 1

where, W is the body weight (gr), L is the total length (cm), ‘a’ is the intercept (initial growth coefficient) and the exponent ‘b’ is the slope (growth coefficient).

The equation was transformed into the logarithmic form to determine the coefficients empirically. Then using linear regression analysis (least-squares method) on log-transformed data, slope and intercept parameters were estimated (Zar, 1984) to obtain allometry coefficient, slope (b).

$\log W = \log a + b \log L$ Equation 2

In order to find (Ricker, 1975) out allometric correlation between two anchovy stocks firstly statistical correspondence comparison was carried out between slope values of each station. Significance of differences between two populations was tested by means of the Student’s t test (Equation 2). Then the obtained results were mapped to observe correspondence between stations within each cruise itself.

$t = \frac{b_1 - b_2}{S_{b_1 - b_2}}$ Equation 2

where, t = student t test value, b = slope and S = standard deviation

With the acceptance of calculated t values are the similarity index of stations, in order to observe effects of distance between stations on allometry, Pearson Correlation analysis was done. The degree of similarity (dissimilarity) between stations was examined in regard to geographic proximity of stations and demonstrated with scatter plots.

Geographical proximity of the stations with respect to each other was calculated with the following equation;

$D = 60 * \sqrt{(Lat1 - Lat2)^2 + (Lon1 - Lon2)^2 * cos^2}(0.50 * (Lat1 + Lat2))$ Equation 3

Lat1 and Lon1 are the latitude and longitude of the locations where the station “a” was sighted; Lat2 and Lon2 are the locations of the station in which “b” was sighted.

2.5.2 Condition Factor

Condition factor also called Fulton’s Condition factor (K_n) was calculated for each individual and was tested for significance between stations to compare well-being of anchovy stocks in overwintering ground. It was calculated by the following equation;

$$K = 100\ \frac{W}{L^3} \qquad \text{Equation 4}$$

where, K is condition factor, W is the body weight (gr) and L is the total length (cm); the factor 100 is to bring K close to unity.

The condition factor comparison was applied in two different ways. First comparison was carried out using t-test to observe K_n differences between predefined length groups of zero age class ($p<0.05$) (1.Group: L $\leq$6.5 cm, 2.Group: 6.5<L$\leq$9 cm, and 3.Group: 9<L cm). For this comparison, length groups were determined according to mean length of stations and each cruise was evaluated separately.

Second comparison was performed to test if there was a spatial pattern in the distribution of the condition factor levels. For that purpose the sets of sampling stations belonging to each cruise were separately grouped into two classes by dividing at the mean condition value of the particular cruise. Finally the classified sampling stations were plotted on the map to see if there was a spatial structuring based on the geographic locations.

2.5.3 Ageing and Otolith Shape Analysis

Independently from the rest of the study, otolith studies were carried out with frozen samples as formaldehyde can have erosive effect on the otolith. Within this study, a total of 270 individuals sampled at 7 stations were taken. However only those in intact conditions (240 individual) were used for further analysis.

After length – weight measurements, sagittal otoliths were removed from the individuals. These otolith pairs are located at the back part of the skull, in the otic capsule (Messersmith, 1969). To reach this part of the skull first gill arches were removed, then the skull was broken at the edge of head hence the rest of the body and otoliths were taken through capsules. The removed otoliths were cleaned by water gently to remove remaining tissue. To prevent damage upon otolith body and saw like teeth cleaning was done carefully. At last, otolith samples were stored in micro plates as dry with station labels.

Age determination and shape analysis were carried out with OLYMPUS SZX16 Stereo Microscope in the range of 20X and 64X magnification. The otoliths were placed in a black petri dish that filled with ethyl alcohol and observation was carried out under the reflected light to make zones apparent. Dense opaque zone prevent light transmission and appear white whilst hyaline bands allow the transmission and reflect the backgrounds colour as black (Giannetti & Donato, 2003). True annuli were determined from the post-rostrum region and ring continuity was checked on anterior and - if possible – on the dorsal-ventral edges as whole.

To estimate the age of an individual the following interpretation criteria were adopted (Giannetti & Donato, 2003);

- Anchovy otolith forms one opaque and one hyaline ring in each year.
- The birthday is accepted as 1st June based on spawning period of the anchovies in the Black Sea (within last half of May and August, (Lisovenko & Andrianov, 1996)).
- Opaque edges during the first six months of a year and hyaline edges during last six of the year were considered as belong to the previous age groups (according to anchovy’s birthday).
- While the fish becomes older, annual rings enlarge with decreasing intervals.

Otoliths were photographed by 3 megapixels CCD digital microscope camera and analysed with Olympus Stream Basic software. In order to minimize distortion caused by the concave shape of sagittas, samples were positioned on the petri letting the otolith siting on the sulcus (sulcus down). In contrary to the method used in age determination intensive reflected light was used for shape analysis so that contrast was achieved by bright 2D otolith image on the dark background.

Size descriptors otolith length, width, perimeter and area; and shape variables circularity, shape factor, ellipticity, roundness, aspect ratio, radius ratio, diameter ratio, ellipticity, sphericity and area/box area ratio were measured automatically with Olympus Stream Basic software (Table 2.3). After measurements, a t-test was applied on otolith pairs to observe the similarity/dissimilarity between right and left otoliths ($p<0.05$).

Table 2.3 Shape variables used in otolith shape analysis which were adapted from (Zhang et. Al., 2013; Tuset et. al., 2003).

Shape Variables	Description / Formula
Aspect Ratio	OL/OW
Roundness	$(4*A)/(\pi*L)$
Circularity	P/A^2
Shape Factor	$(4*\pi*A)/P^2$
Ellipticity	(L-W)/(L+W)
Sphericity	Approximately the squared quotient of width and length
Radius Ratio	Ratio between Min and Max ratio
Diameter Ratio	Ratio between Min and Max ratio
Area / Box Area	Ratio between surface area of object and area of its surrounding box

'L' length of otolith, 'W' width of otolith, 'A' surface area of otolith and 'P' perimeter of otolith

For each morphometric variable normality test was carried out using Anderson-Darling test. Variables that did not fit normality, were transformed according to type of skewness. In the case of positive skewness (skewed to right) square - square root of data was extracted, whilst for negative skewness (skewed to left) log transformation was done and the test was repeated.

Concurrently with age determination study, otolith shape analysis was done using multivariate techniques in order to find if there are meaningful parameters that can be used in discrimination of the anchovy stocks. First, a Principal Component Analysis (PCA) was performed to detect potential shape variations and reduce the dimensionality of data. The PCA was conducted including the otolith shape variables, body length and condition factor of fish. Age classes were evaluated separately. Variables for further multivariate analyses were established according to the significant principal component loadings as described in McGarigal, Cushman, & Stafford (2000).

Next, forward stepwise Canonical Discriminant Analysis (CDA) was applied to determine differences between anchovy stocks from different areas. The CD analysis was performed using PC residuals of selected variables (Me´rigot, Letourneur, & Lecomte-Finiger, 2007) according to significant PC loadings and the discrimination power was evaluated by Wilk's λ.

As the final step, k – means clustering was carried out to determine shape descriptors which maximize the class separation. As a similarity index among otoliths the Euclidian distance was chosen and classification accuracy was tested by v – fold cross validation.

2.5.4 k-Means Clustering

A general overview on discrimination success of applied methods and interregional grouping were evaluated using k-Means clustering. Distribution of stock members in generated classes and their frequency within each cluster were determined. To this end, 'v – fold cross validation' was used to choose optimum number of clusters data suggested. All evaluated stock discrimination criteria were taken into consideration for the final clustering.

3 RESULTS

The lengths of 33 013 individuals ranging from 2.5 cm to 15 cm were measured on board. During the on-board measurements, a total of 3007 individuals ranging 2.7 cm to 14.9 cm and 0.05g to 17.24 g were selected and set aside for further examination in the laboratory. Maximum and minimum values of length and weight parameters measured for each station, included all length classes, and the coefficient of determination (r^2) are given in Table 3.1. Linear regressions were statistically significant for all 52 stations (P<0.001) and r^2 values of 49 stations were greater than 0.95. Two of the remaining estimates were greater than 0.90 while one other was 0.80. The estimated slope values ranged from 3.0211 to 3.8973 with a mean value of 3.35 (StDev =0.21).

In November – December 2011 cruise, the highest slope of Length-Weight relationship (LWR) was calculated at the station KD11-031 as 3.5371 (41.51N-39.45E), while the lowest value was at the station KD11-038 (41.13N-37.38). The lowest and highest average condition factor values of *E. encrasicolus* were estimated in completely distinct regions. The individual having the highest condition factor (0.5786) was sampled at the station 18 in the eastern coast of Turkish Black Sea (41.03N-38.86E), while the lowest (0.4972) condition factor was calculated at the station KD11-061 (41.31N-28.82E). The largest (14.9 cm) and the smallest (4.6 cm) individuals were sampled at the station KD11-031 (41.51N-39.45E) and KD11-037 41.16N-37.68E) respectively. Both the lightest (0.34 gr) and heaviest (17. 24 gr) individuals were observed at the station KD11-037 (41.16N-37.68E).

In the following cruise which conducted during 2011-2012 fishing season (January 2012) from total of 9 stations, the highest slope of LWR (3.5338) was observed at the station KD12-012 (41.32N-41.20), whereas the lowest value was estimated as 3.2432 at the station KD12-010 close to eastern border of the Black Sea (41.39N-41.37E). The lowest average condition factor was calculated as 0.3831 at the station KD12-017 located along the border of Turkey-Georgia (41.52N-41.52E), while station KD12-002 had the highest value as 0.5072 (42.03N-33.34E). The shortest length, 5.1 cm, was at the station KD12-017 (41.52N-41.52E), while the longest at the station KD12-013 (41.30N-41.15). Both the lightest (0.48 gr) and heaviest (15.41 gr) individuals were observed at the same station (station KD12-012 at 41.32N-41.20E coordinates.

In the first cruise of 2012-2013 fishing season (November 2012), the lowest slope value of LWR was calculated as 3.2646 at the station KD12-029 (41.25N-25.38), whereas the highest value was at the station KD12-036 (42.22N-33.54). The station KD12-037 had the lowest condition factor (42.26N-34.95), whereas the highest was calculated at station KD12-042 (41.34N-39.42E). The shortest length was at the station KD12-042 (2.7 cm), while the longest was measured as 9.2 cm at the station KD12-052 (41.68N-32.18). The lightest individual was situated at the station KD12-036 (0.01 gr) and heaviest individual was observed at the station KD12-029 (4.11 gr).

At the last cruise, which was carried out in December 2012, the highest slope value of LWR was estimated at the station KD12-078 (41.77N-35.76E), while the lowest was at the station KD12-058 (41.77N-35.76E). In contrast to the slope values, the highest average condition factor was at the station KD12-058, while the lowest was at the station KD12-078. The smallest and largest individuals were observed at stations KD12-076 (41.80N-35.45E) and KD12-057 (41.14N-31.14E) respectively. The heaviest individual was at the station KD12-074 (42.00N-34.03E), whilst the lightest individual was observed at the station KD12-076 as parallel to the smallest length.

Table 3.1 Descriptive statistics and estimated parameters of the length–weight relationships ($W = a.L^b$) of each station along with coefficient of determination (R^2).

November - December 2011									
	Length and Weight Characteristics					**Parameters of the WLR**			
Station Code	**n**	**Min Length**	**Max Length**	**Min Weight**	**Max Weight**	**Slope (b)**	**Intercept (a)**	**Condition Factors**	**R^2**
KD11-009	76	5.8	14.1	0.9	15.87	3.2433	0.0034	0.5782	0.9917
KD11-010	70	6.1	12.8	1.25	11.63	3.235	0.0034	0.5699	0.9903
KD11-011	39	7.2	10.6	1.98	7.06	3.2608	0.0033	0.5747	0.9726
KD11-013	44	6.3	13.3	1.21	13.59	3.3504	0.0027	0.5739	0.9866
KD11-017	61	5.7	13.5	0.9	11.99	3.1177	0.0044	0.5669	0.9853
KD11-018	15	7.1	12.1	2.05	10.53	3.1387	0.0042	0.5786	0.9821
KD11-019	65	5.3	12.9	0.74	12.99	3.1651	0.0036	0.5038	0.9857
KD11-021	41	6.5	13	1.35	11.31	3.1609	0.0037	0.5242	0.9862
KD11-022	59	5.8	14.1	0.89	11.92	3.0907	0.0043	0.5246	0.9884
KD11-023	48	6.2	13	1.09	12.54	3.2174	0.0034	0.5436	0.9876
KD11-024	36	6.1	11.1	1.12	7.58	3.0735	0.0045	0.5163	0.969
KD11-025	81	5.8	13.2	1.01	13.34	3.1461	0.0039	0.542	0.9818
KD11-027	80	5.5	13	0.78	11.44	3.2187	0.0033	0.5321	0.9858
KD11-029	38	7.6	13	2.05	11.57	3.1131	0.004	0.5276	0.951
KD11-031	32	4.6	7.5	0.36	2.16	3.5371	0.002	0.5056	0.8798
KD11-032	78	6.6	13.9	1.16	15.2	3.261	0.003	0.5447	0.9862
KD11-034	60	5.4	13.7	0.67	15.08	3.2449	0.003	0.5159	0.991
KD11-037	65	4.7	14.9	0.34	17.24	3.1814	0.0034	0.5154	0.9899
KD11-038	58	6.2	14	1.24	16.04	3.0578	0.0048	0.5509	0.9636
KD11-040	71	5.7	13.6	0.88	14.69	3.2798	0.0029	0.5261	0.9867
KD11-061	54	5.2	11.3	0.5	9.1	3.425	0.0021	0.4972	0.9707
January 12									
	Length and Weight Characteristics					**Parameters of the WLR**			
Station Code	**n**	**Min Length**	**Max Length**	**Min Weight**	**Max Weight**	**Slope (b)**	**Intercept (a)**	**Condition Factors**	**R^2**
KD12_002	16	6.2	12.1	0.98	9.81	3.2971	0.0026	0.5072	0.9857
KD12_006	66	6.2	13.5	0.98	12.93	3.2848	0.0025	0.4736	0.9895
KD12_010	48	5.2	7.9	0.58	2.75	3.2432	0.0027	0.4245	0.9534
KD12_012	102	5.4	14.2	0.48	15.41	3.5338	0.0013	0.4916	0.9833
KD12_013	68	5.6	14.3	0.61	14.19	3.3299	0.0023	0.5045	0.9831
KD12_016	78	5.3	12.7	0.56	9.86	3.5102	0.0015	0.4474	0.9864
KD12_017	32	5.1	8.8	0.51	2.93	3.3454	0.002	0.3831	0.9711
KD12_021	62	5.7	12.7	0.71	10.15	3.3036	0.0023	0.4383	0.9864
KD12_025	52	5.5	10.2	0.59	6.01	3.5119	0.0015	0.4084	0.9676

Table 3.1 (continued). Descriptive statistics and estimated parameters of the length–weight relationships ($W = a.L^b$) of each station along with coefficient of determination (R^2).

November 12									
	Length and Weight Characteristics					Parameters of the WLR			
Station Code	**n**	**Min Length**	**Max Length**	**Min Weight**	**Max Weight**	**Slope (b)**	**Intercept (a)**	**Condition Factors**	**R^2**
KD12_029	41	5.5	8.9	0.76	4.11	3.2646	0.0031	0.5211	0.9683
KD12_034	32	3.8	7.3	0.2	1.97	3.5637	0.0018	0.4658	0.9883
KD12_036	77	2.9	6.8	0.01	1.6	3.8973	0.0009	0.4061	0.9269
KD12_037	92	2.8	5.8	0.06	0.93	3.2652	0.0028	0.3965	0.9462
KD12_038	40	3.3	5.8	0.11	0.94	3.5542	0.0018	0.4171	0.9778
KD12_039	55	4.6	7.6	0.33	2.89	3.7532	0.0013	0.506	0.9708
KD12_042	44	2.7	8.7	0.06	3.66	3.5111	0.0021	0.5278	0.9915
KD12_045	37	4.4	7.6	0.32	2.46	3.635	0.0016	0.5045	0.9883
KD12_046	70	3.3	7.3	0.13	2.06	3.4675	0.0021	0.4603	0.9849
KD12_048	33	4	6.6	0.26	1.62	3.6939	0.0016	0.4849	0.9821
KD12_050	32	4.7	8.1	0.4	3	3.6718	0.0014	0.4862	0.9774
KD12_051	34	5	8.4	0.58	3.59	3.5043	0.002	0.5252	0.9843
KD12_052	53	5	9.2	0.5	4.09	3.3822	0.0025	0.5165	0.9811
KD12_054	28	5.2	7.3	0.67	2.25	3.3129	0.0029	0.5056	0.9666

December 12									
	Length and Weight Characteristics					Parameters of the WLR			
Station Code	**n**	**Min Length**	**Max Length**	**Min Weight**	**Max Weight**	**Slope (b)**	**Intercept (a)**	**Condition Factors**	**R^2**
KD12_057	112	6.4	12.4	1.28	10.74	3.1089	0.0045	0.5707	0.9707
KD12_058	86	7.2	11.8	2.21	9.92	3.0211	0.0056	0.5905	0.9636
KD12_063	26	10	12.3	5.68	9.75	3.2254	0.0032	0.5468	0.8707
KD12_065	55	6.1	11.4	0.96	9.01	3.29	0.0029	0.5548	0.9851
KD12_066	40	7.9	12	2.3	9.66	3.1712	0.0037	0.5558	0.9516
KD12_072	52	5.6	12	0.81	8.51	3.1113	0.0043	0.5422	0.9768
KD12_074	84	7.1	12.3	1.67	10.97	3.1939	0.0036	0.5594	0.9664
KD12_076	63	3.5	8.2	0.16	3.12	3.6072	0.0016	0.4863	0.9905
KD12_078	48	4	7.2	0.21	2.07	3.8204	0.0011	0.4223	0.9857
KD12_079	38	3.8	10	0.2	5.13	3.4513	0.0021	0.5068	0.9893
KD12_080	40	4.2	8.3	0.26	3.66	3.7208	0.0013	0.517	0.9815

3.1 Chlorophyll-a Concentration and Sea Surface Temperature

The Chlorophyll-a concentration and SST images derived by satellite to associate differences in ecologic and hydrologic conditions of distinct nursery and spawning grounds were indicated significant differences between nursery areas (Figure 3.2, Figure 3.3). In late spring and summer high Chl-a concentrations were observed. However, it was clearly seen that Chl-a concentrations always higher in the Sea of Azov compared to the north-western shelf; 10-12 mg/m3 and 4mg/m3 respectively) (Figure 3.1).

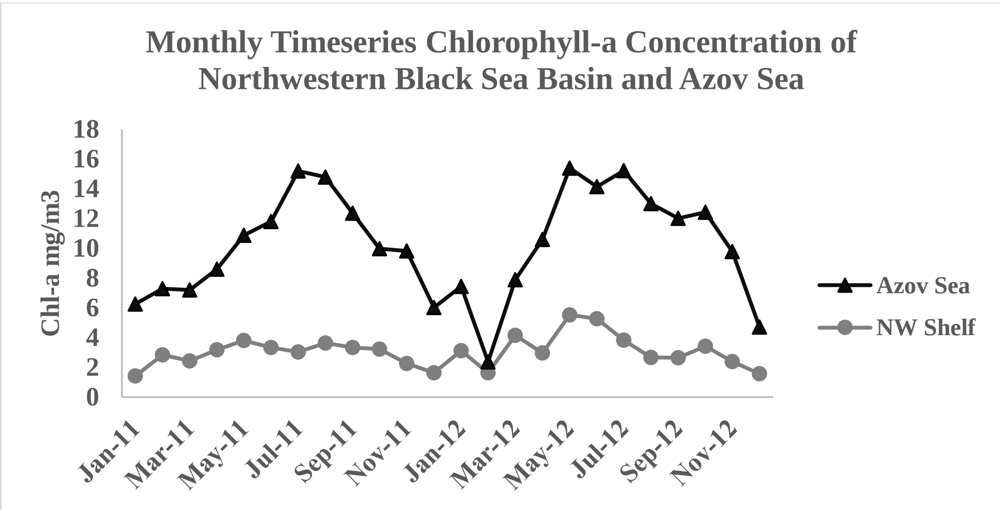


Figure 3.1 Annual chlorophyll-a (mg/m3) concentrations in the north-western shelf of the Black Sea and Sea of Azov as monthly intervals for January to June 2011 and 2012 – MODIS.

The SST images indicated temperature decreases in November and begins warming in April. It is recognisable that in winter months especially September to January southern coasts were warmer. Even summer temperature distribution seems uniform, relatively higher temperature was observed in the Sea of Azov which is under the influence of the continental climate. Besides, quite high temperature was observed between September and January months on the south-eastern Black Sea basin (Figure 3.4, Figure 3.5).

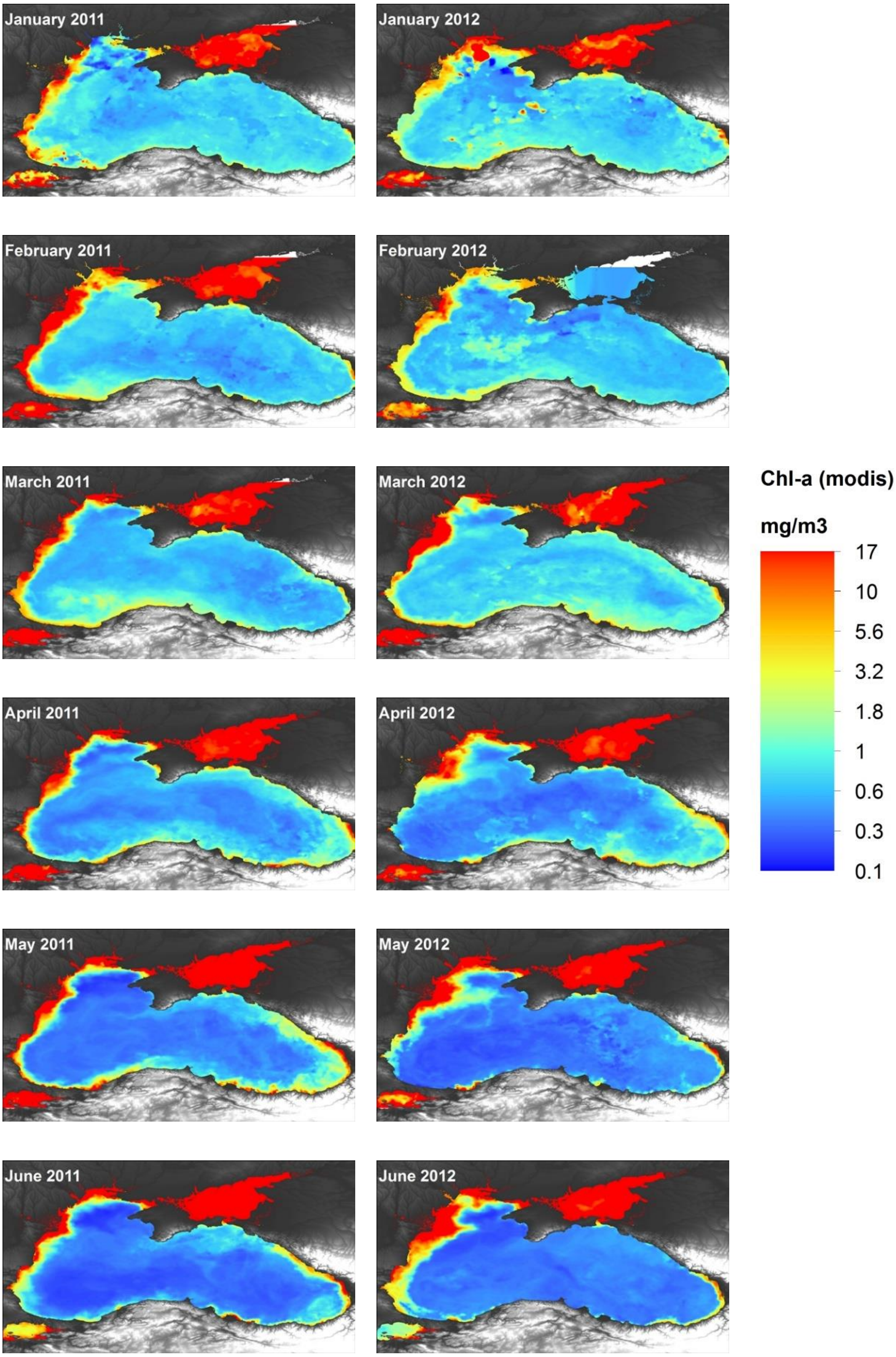


Figure 3.2 Distribution of mean Chlorophyll-a concentration (mg/m3) in the Black Sea basin for January to June 2011 and 2012 (derived by log transformed data) – MODIS.

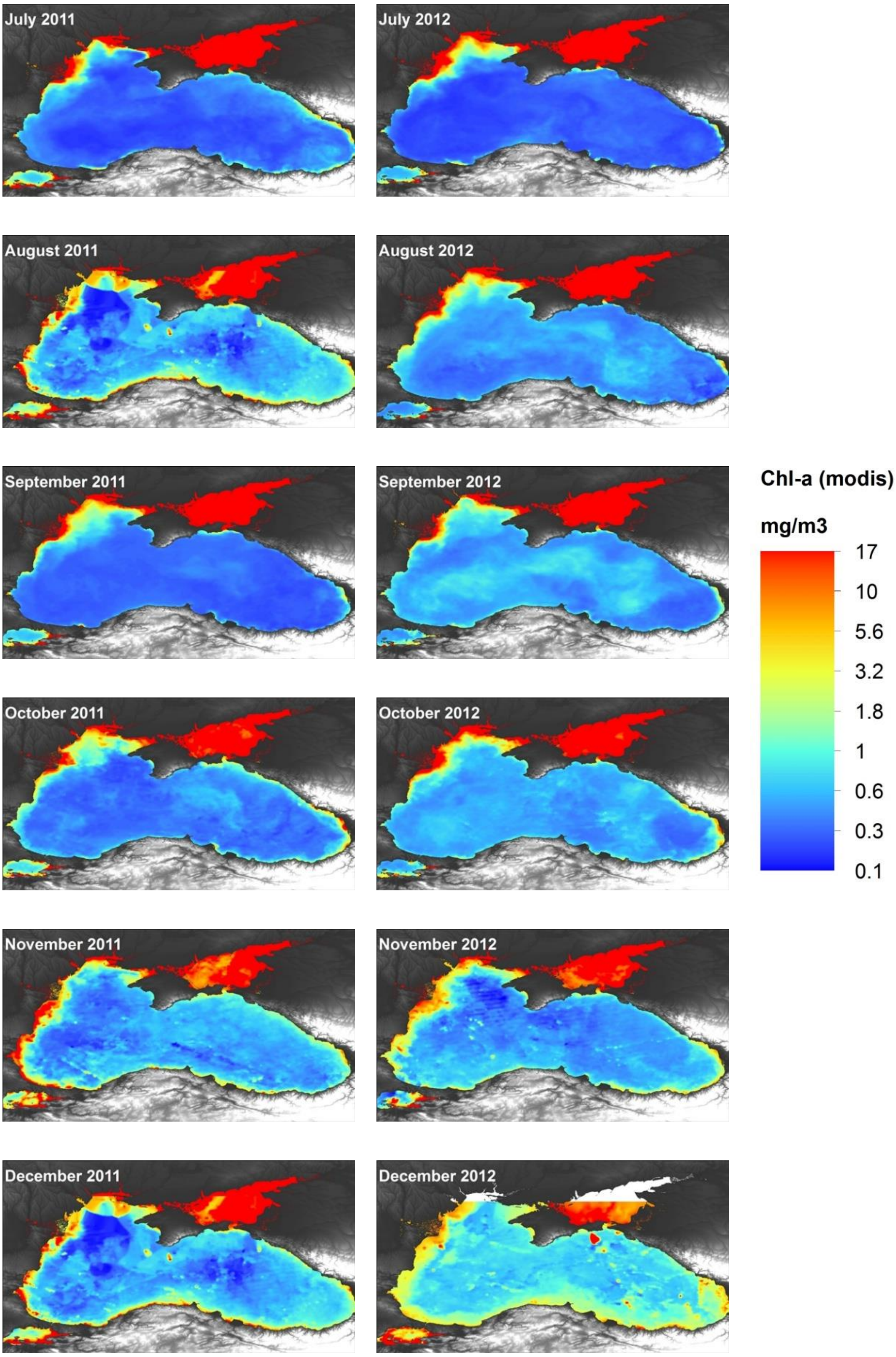


Figure 3.3 Distribution of mean Chlorophyll-a concentration (mg/m3) in the Black Sea basin for July to December 2011 and 2012 (derived by log transformed data) – MODIS.

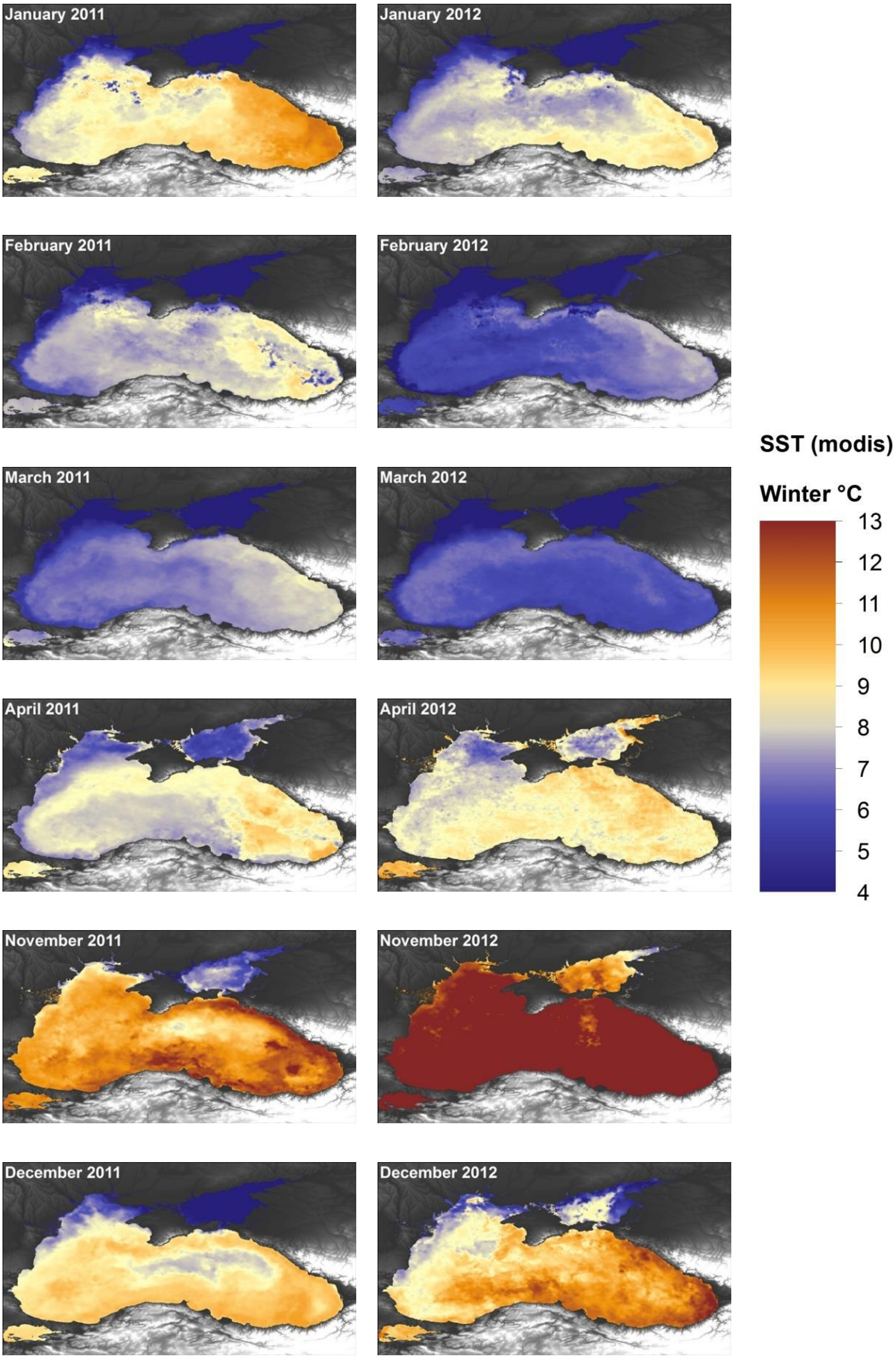


Figure 3.4 Sea surface temperature in the Black Sea basin for cold months (January to December 2011 and 2012) (range between 4 to 13°C) – MODIS.

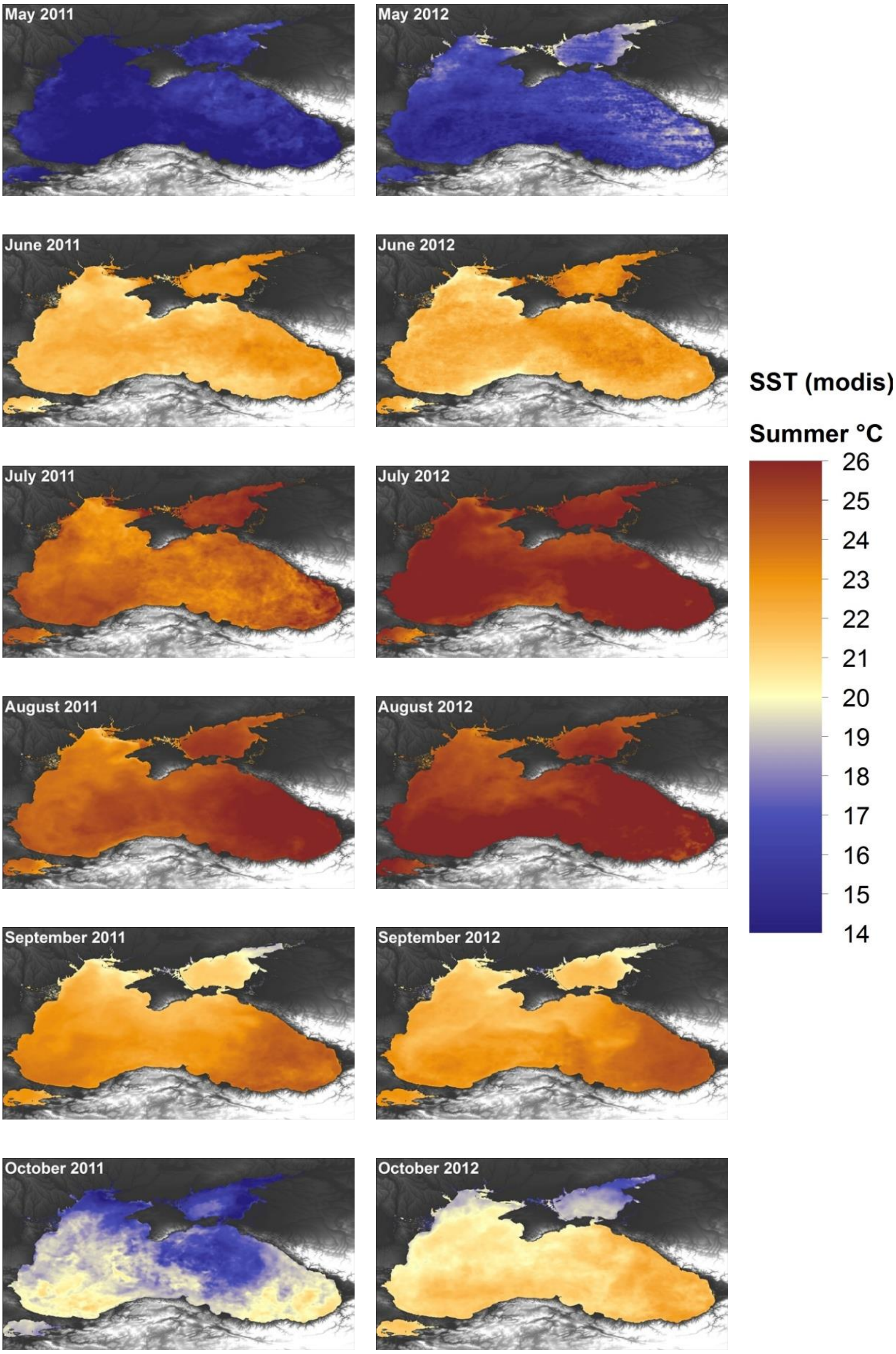


Figure 3.5 Sea surface temperature in the Black Sea basin for warm months (May to October 2011 and 2012) (range between 14 to 26ºC) – MODIS.

3.2 Length Frequency Distribution

The mean length of 0 age class varied between 3.15 cm to 8.09 cm in November – December 2011. Length frequency distribution of the same age class is shown in Table 3.2. Mostly, the stations displayed quite close mean lengths with similar standard deviations. However, in some cases, stations, although the mean lengths were identical there were significant differences in the standard deviations, indicating that the within group variation is greater in some stations. An example to this situation was observed in November – December 2011. The mean length of the 0 year class which was scattered around 7 cm, showed different variation of lengths. The station KD11-038 (41.13N-37.38E) differed significantly from the station KD11-013, KD11-023 and KD11-027, as can be seen from the significantly higher standard deviation.

Furthermore, the station KD11-025 (41.18N-40.84E) and the station KD11-032 (41.07N-39.30E) had 8 cm mean length, which was similar to the rest of the three stations (KD11-011, KD11-017 and KD11-061) regarding the average length. However the same set of stations displayed relatively higher standard deviation. Similar to the November – December 2011 cruise, remarkable variations in the standard deviation of the length of the 0 age class in the November 2012 cruise. The other two cruises (January and December 2012) did not show such variations. Among the stations having 6 cm mean length, the station KD12-045 (41.07N-40.52E) had the highest standard deviations, whereas the rest six stations had smaller variation. Lastly, within the stations of 7 cm mean length, KD12-042 (0.93) had wider distribution due to the higher variation among the individuals sampled at the station, whereas the station KD12-029 (41.25N-25.38E) had a narrower distribution.

Table 3.2 Mean length of 0 age class, standard deviations and condition factors.

November – December 2011				January 2012			
Station Code	**Mean Length at 0 Age**	**Standard Deviation**	**Condition Factor at 0 age**	**Station Code**	**Mean Length at 0 Age**	**Standard Deviation**	**Condition Factor at 0 age**
KD11-009	7.39	0.4813	0.5390	KD12_006	7.15	0.4614	0.4403
KD11-010	7.32	0.4170	0.5416	KD12_010	6.65	0.5255	0.4245
KD11-011	8.03	0.4565	0.5685	KD12_012	7.43	0.3493	0.3722
KD11-013	7.43	0.3493	0.5365	KD12_016	7.59	0.8596	0.4189
KD11-017	7.78	0.7210	0.5573	KD12_017	6.56	0.5165	0.3831
KD11-019	6.57	0.5174	0.4956	KD12_021	6.51	0.3393	0.4080
KD11-022	6.92	0.5583	0.5158	KD12_025	6.44	0.3157	0.3998
KD11-023	7.13	0.4247	0.5336				
KD11-024	6.93	0.5087	0.5153				
KD11-025	7.9	1.2871	0.5251				
KD11-027	7.26	0.6523	0.5104				
KD11-031	5.78	0.6182	0.5056				
KD11-032	7.5	0.9000	0.5047				
KD11-034	6.43	0.6815	0.4616				
KD11-037	5.96	0.6535	0.4650				
KD11-038	7.27	0.9043	0.5091				
KD11-040	6.69	0.3901	0.4902				
KD11-061	8.09	0.7544	0.4847				
November 2012				**December 2012**			
Station Code	**Mean Length at 0 Age**	**Standard Deviation**	**Condition Factor at 0 age**	**Station Code**	**Mean Length at 0 Age**	**Standard Deviation**	**Condition Factor at 0 age**
KD12_029	6.53	0.6064	0.5211	KD12_057	7.65	1.0231	0.5622
KD12_034	5.9	0.7743	0.4658	KD12_072	8.07	1.1260	0.5370
KD12_036	3.15	0.2979	0.3899	KD12_074	9.12	0.4269	0.5466
KD12_037	3.3	0.4100	0.3965	KD12_076	6.76	0.7834	0.4863
KD12_038	4.71	0.6950	0.4171	KD12_078	5.26	0.7326	0.4223
KD12_039	5.81	0.6638	0.5060	KD12_079	7.36	0.7023	0.5052
KD12_042	6.75	0.9274	0.5278	KD12_080	7.32	0.4795	0.5170
KD12_045	5.77	0.9958	0.5045				
KD12_046	4.96	0.7119	0.4603				
KD12_048	4.87	0.4059	0.4849				
KD12_050	6.24	0.6181	0.4862				
KD12_051	6.04	0.5035	0.5252				
KD12_052	6.02	0.4970	0.5177				
KD12_054	5.57	0.2992	0.5056				

3.3 Allometry and Geographical proximity

Length and weight relationship (LWR) was calculated as W= 0.003*$TL^{3.303}$ over the combined data (Figure 3.6). The allometric exponent of the length-weight relationship (b) calculated for combined sexes with juveniles was significantly different from 3 ($P<0.05$) indicating positive allometry.

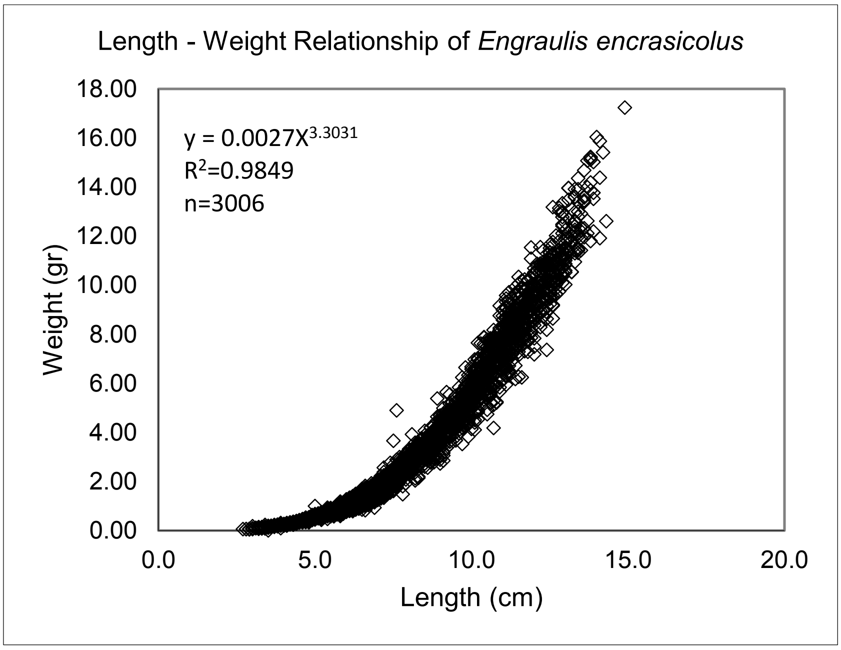


Figure 3.6 Calculated length-weight relationship (power function W= aL^b) of *E. encrasicolus*: for combined sexes with juveniles, where a: intercept; b: slope; R^2: correlation coefficient and n: sample size.

Slope (allometric exponent) estimated for each station separately was within the range of 3.0211 and 3.8973, while the intercept ranged between 0.0009 and 0.0056. The slope of the LWR of anchovy stocks was compared and similarities/dissimilarities were evaluated within each cruise itself (Figure 3.7, Figure 3.8, Figure 3.9, Figure 3.10, and Figure 3.11). Comparison of similarities in the estimated parameters did not indicate a clear and significant geographical pattern. More specifically, the stations KD11-011 and KD11-18 showed similarity with all others. It was also observed that the station KD11-61, which was located near Istanbul differ from almost all other stations (within November – December 2011 Cruise) (Figure 3.7, Figure 3.8). Likewise, in November 2012 cruise stations KD12-034, KD12-038 and KD12-051 showed high similarity with others, while station KD12-037 located offshore Sinop was different from almost all other stations (Figure 3.10).

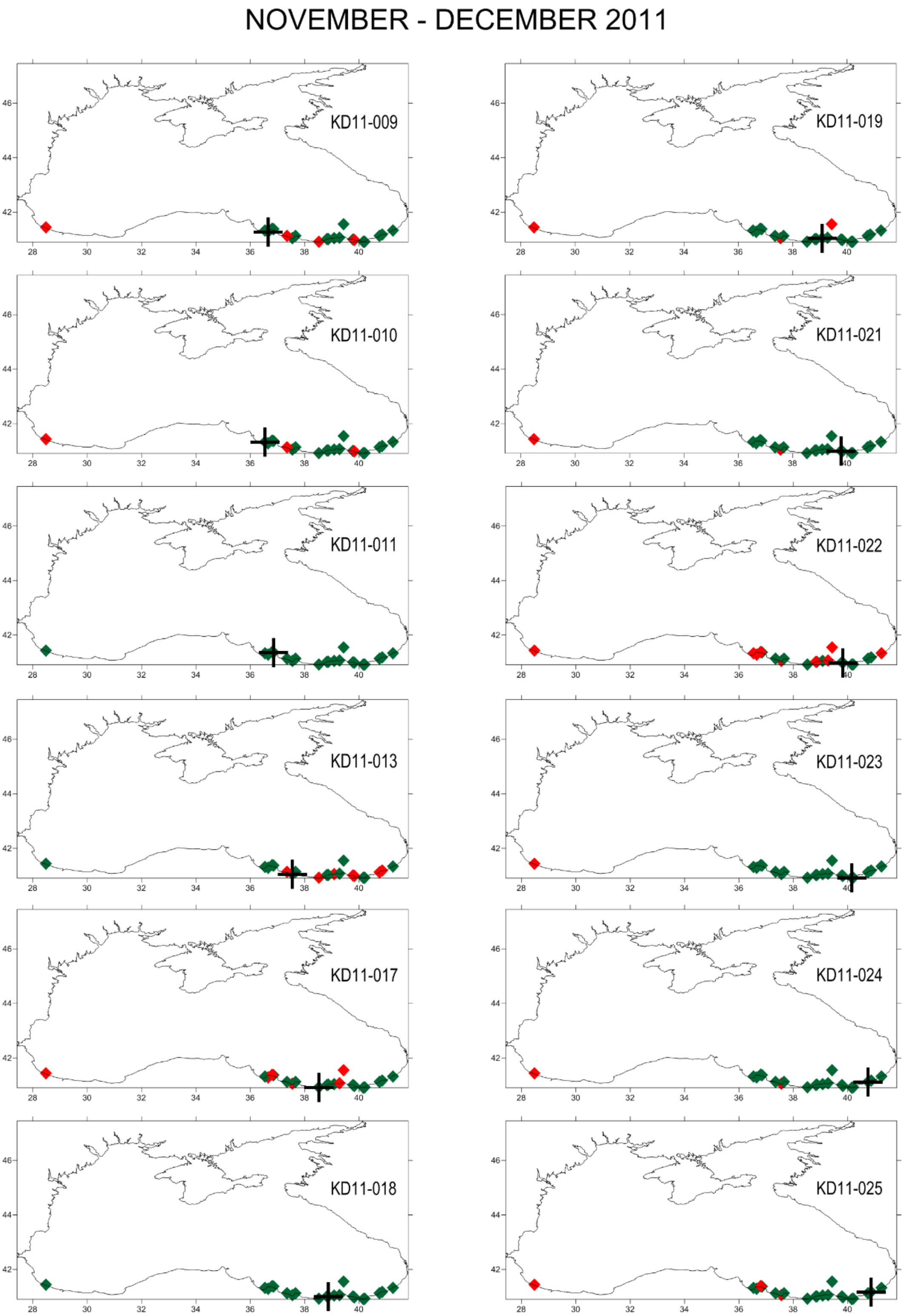


Figure 3.7 Evaluation of regression coefficient slope (b) accordance between stations for November – December 2011 Cruise. “+” = reference station, “◊” “green diamond symbol” = similar stations with reference stations, “◊” red diamond symbol = different stations with reference station.

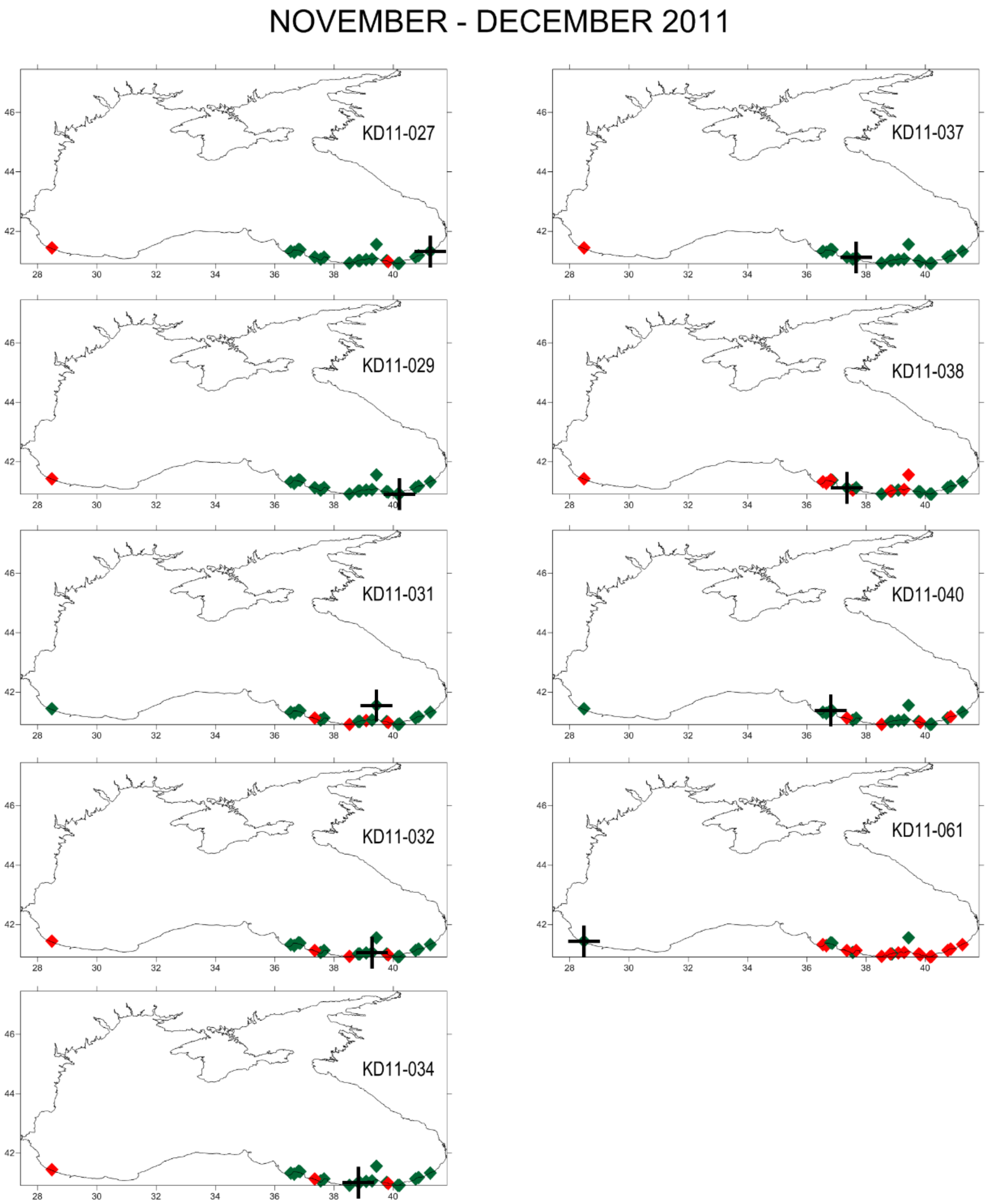


Figure 3.8 Evaluation of regression coefficient slope (b) accordance between stations for November – December 2011 Cruise. “+” = reference station, “◊” “green diamond symbol” = similar stations with reference stations, “◊” red diamond symbol = different stations with reference station.

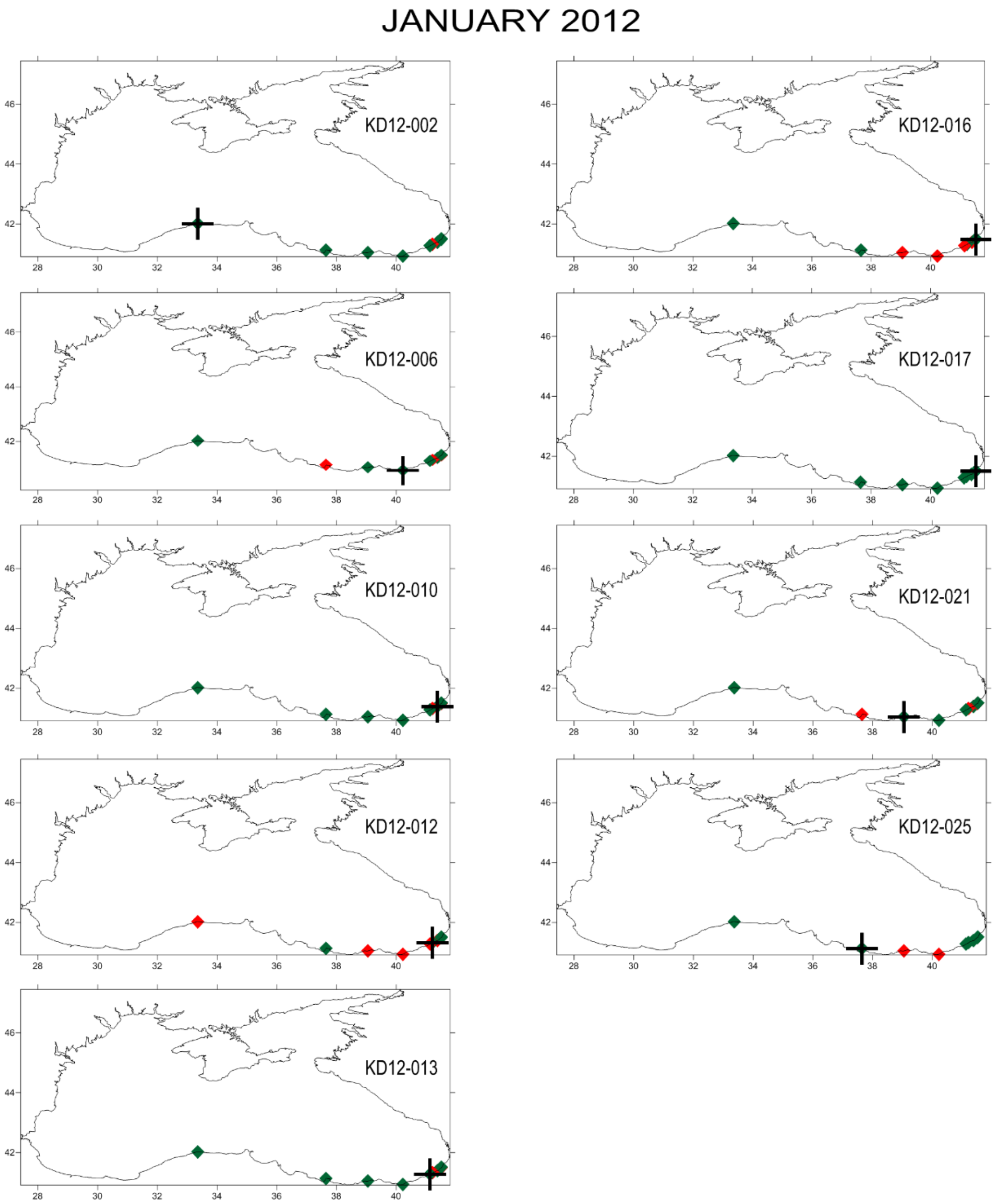


Figure 3.9 Evaluation of regression coefficient slope (b) accordance between stations for January 2012 Cruise. “+” = reference station, “◊” “green diamond symbol” = similar stations with reference stations, “◊” red diamond symbol = different stations with reference station.

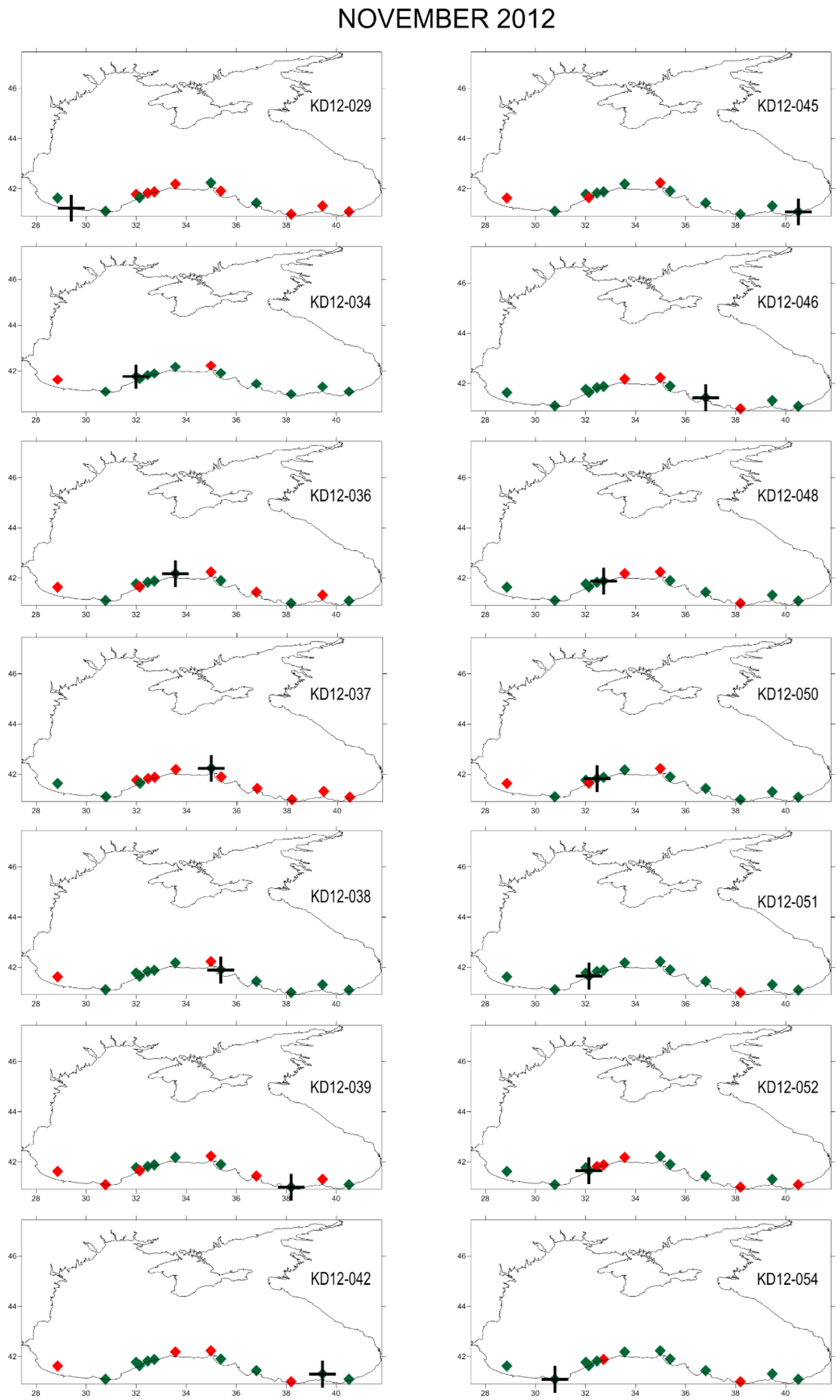


Figure 3.10 Evaluation of regression coefficient slope (b) accordance between stations for November 2012 Cruise. “+” = reference station, “◊” “green diamond symbol” = similar stations with reference stations, “◊” red diamond symbol = different stations with reference station.

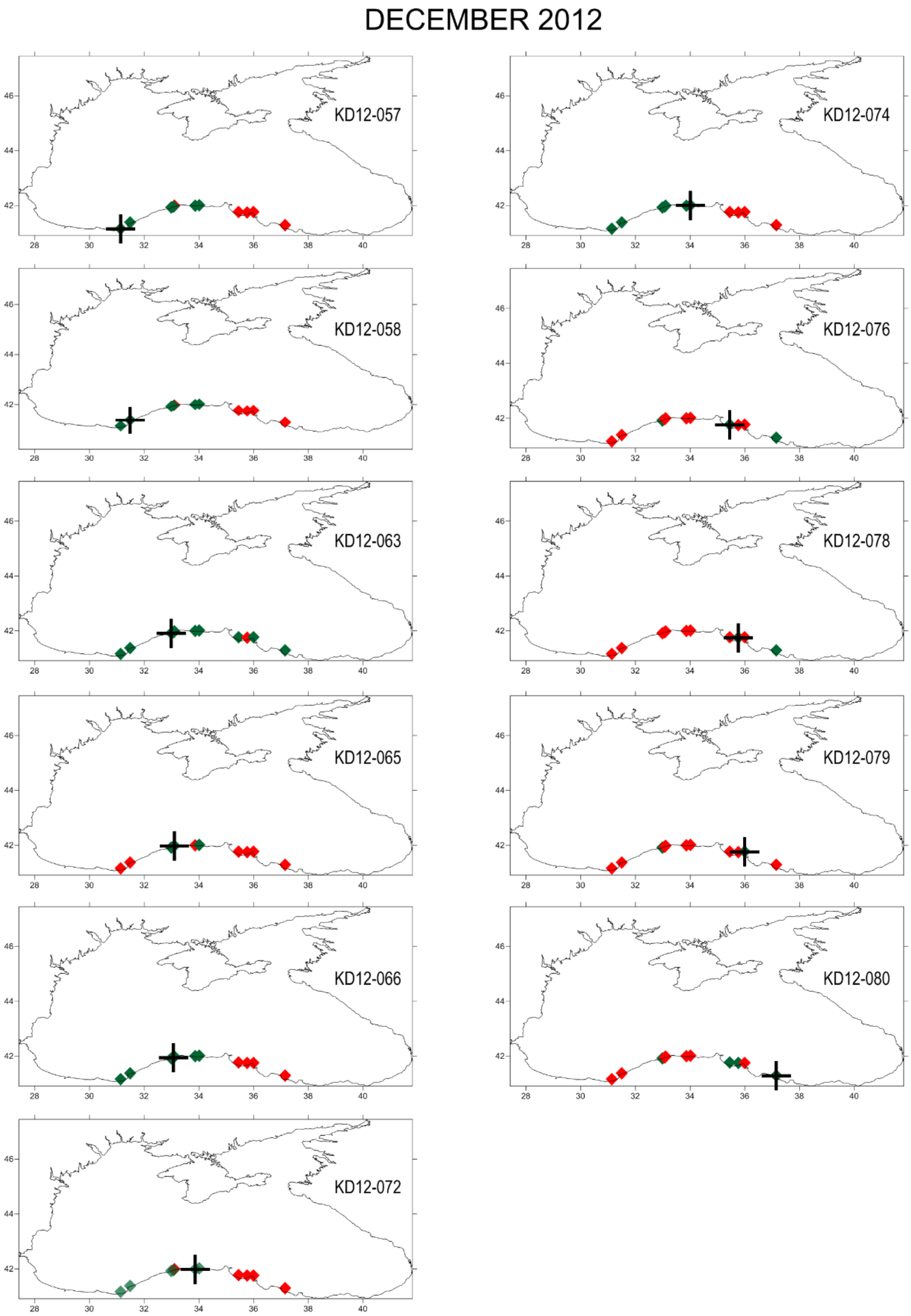


Figure 3.11 Evaluation of regression coefficient slope (b) accordance between stations for December 2012 Cruise. “+” = reference station, “◊” “green diamond symbol” = similar stations with reference stations, “◊” red diamond symbol = different stations with reference station.

The standardized differences between the sum of squared errors (SSE) of the slopes, which was assumed to represent similarity/dissimilarity between two stations, and geographical distance between stations were presented in Table 3.3. The results showed that in November-December 2011 and December 2012 with the increasing distance between stations, the dissimilarity between paired stations increased. However, this dissimilarity relation was not observed in the January and November 2012 cruises (Figure 3.12).

Table 3.3 Statistical fit between geographic distance and similarity index (t values). CC: Correlation Coefficient, ns: not significant.

Cruise	R^2	**Critical Values for CC**	**Corresponding Probabilities**
December 2011	0.208	0.113	0.05
January 2012	0.070	0.312	Ns
November 2012	0.006	0.197	Ns
December 2012	0.330	0.254	0.05

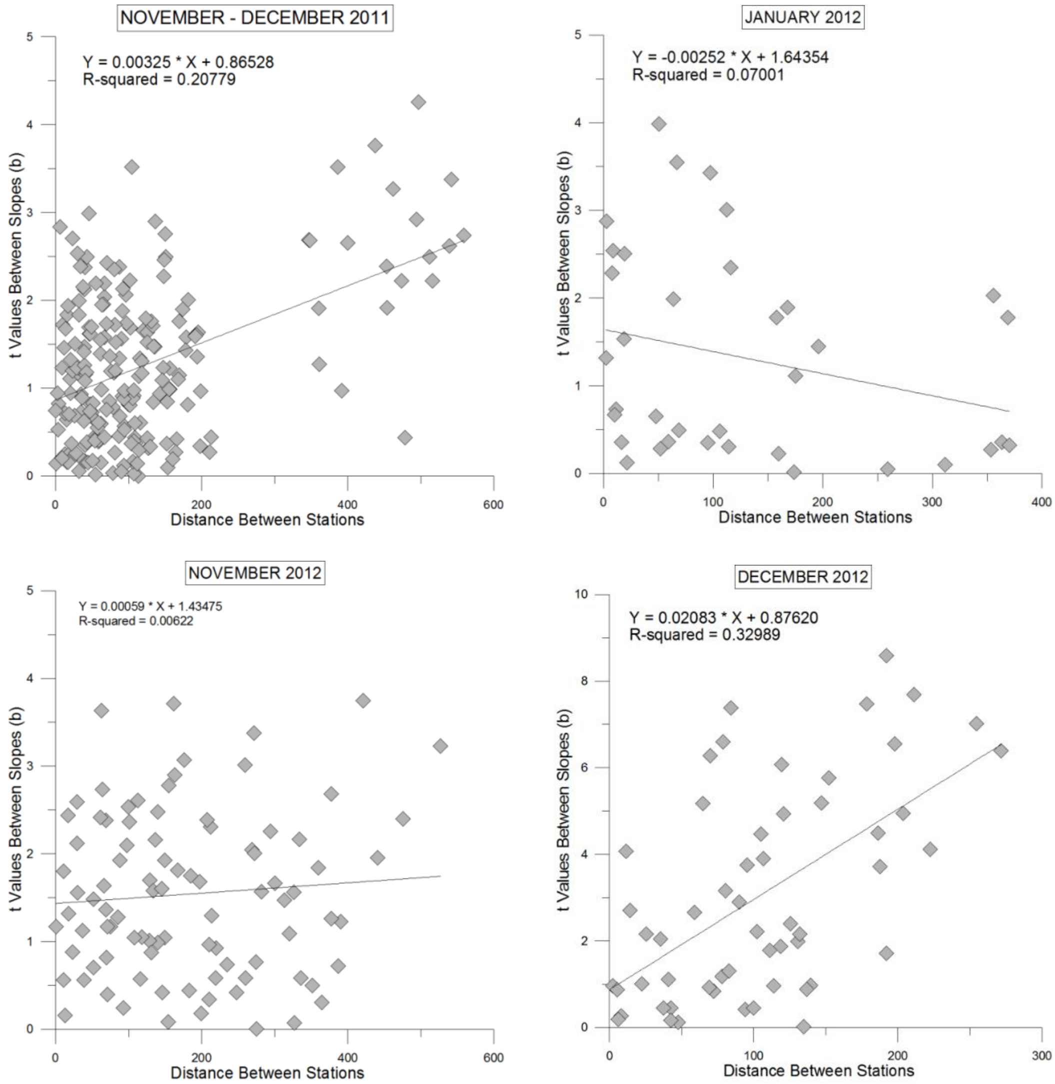


Figure 3.12 Comparison of distance between stations and similarity index at monthly cruises (November – December 2011, January, November and December 2012).

3.4 Condition Factor

The condition factor of the 0 age class varied between 0.37 and 0.59 with an average of 0.49 (Table 3.2). In the November-December 2011 cruise, the highest condition factor (0.57), was observed at the station KD11-011 (41.38N-36.83E), while the lowest was calculated at the station KD11-034 (41.40N-38.93E). In the January 2012 cruise, the maximum value was observed at the station KD12-002 (42.03N-33.34E) and the minimum value was observed at the eastern boundary of Turkey, at the station KD12-012 (41.32N-41.20E). In the first cruise of following fishing season (November 2012), the maximum condition factor was observed at the station KD12-042 (41.34N-39.42E) and the minimum value was recorded at the station KD12-036 (42.22N-33.54E). In December 2012 cruse, the highest condition factor was observed at the station KD12-058 (41.39N-31.51E) and the lowest value was at the station KD12-078 (41.77N-35.76E).

According to the condition factor comparison which carried out with predefined length groups (Figure 3.13), in November-December 2011 cruise first two length groups represented independent groups, while the CF significantly exceeded the first two length groups. In rest three cruises the same pattern was not observed. While in January 2012 cruise there were two segregated groups, November and December 2012 cruises represented by three.

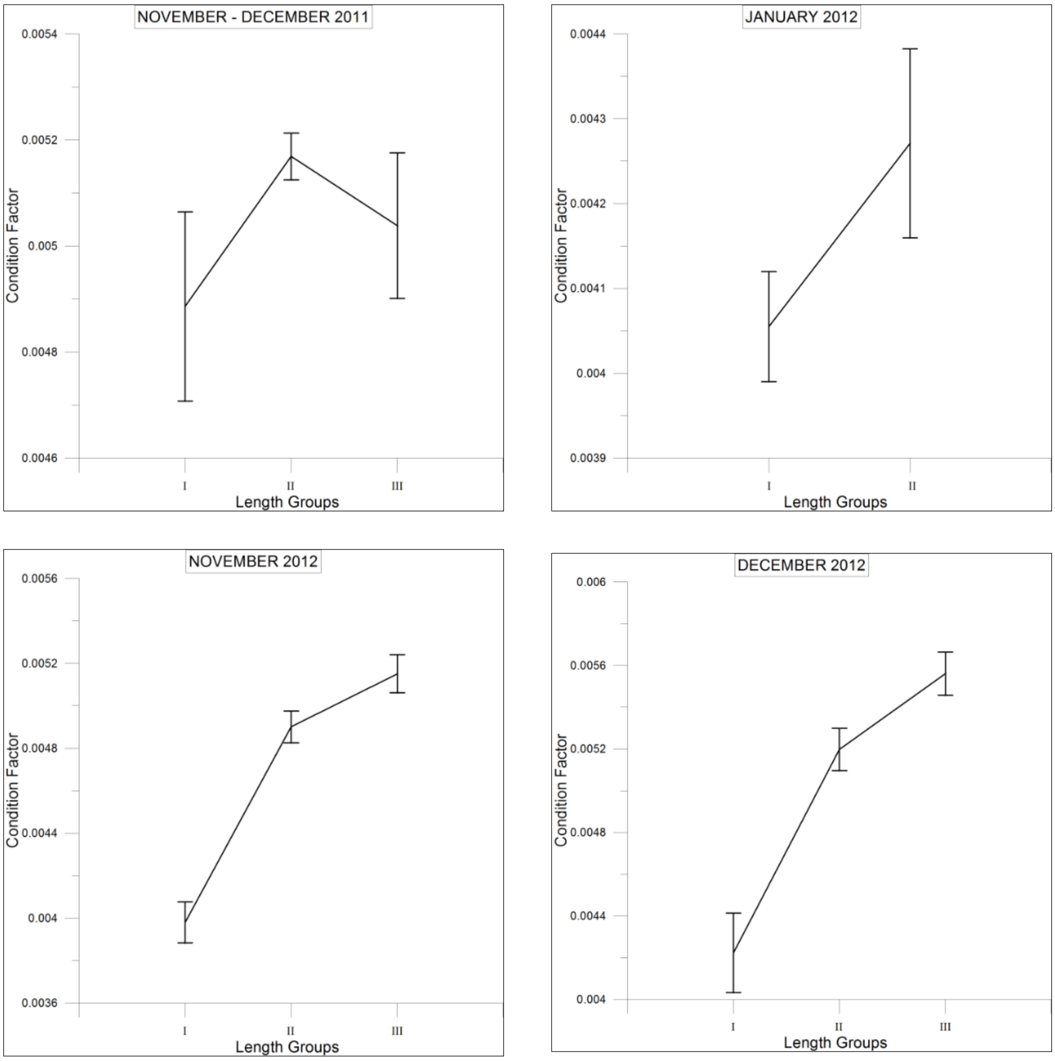


Figure 3.13 relationship between condition factors (W/L^3) and length groups determined according to mean length of zero – age –groups in 2011 – 2012 cruises.

As a results of the second comparison which had been carried out to test if there was a spatial difference in geographic distribution of anchovy stocks based on condition factor, the only significant regional difference was observed in December 2012 cruise. In the rest of the three cruises the mean condition values showed rather mixed pattern (November - December 2011, January and November 2012) (Figure 3.14).

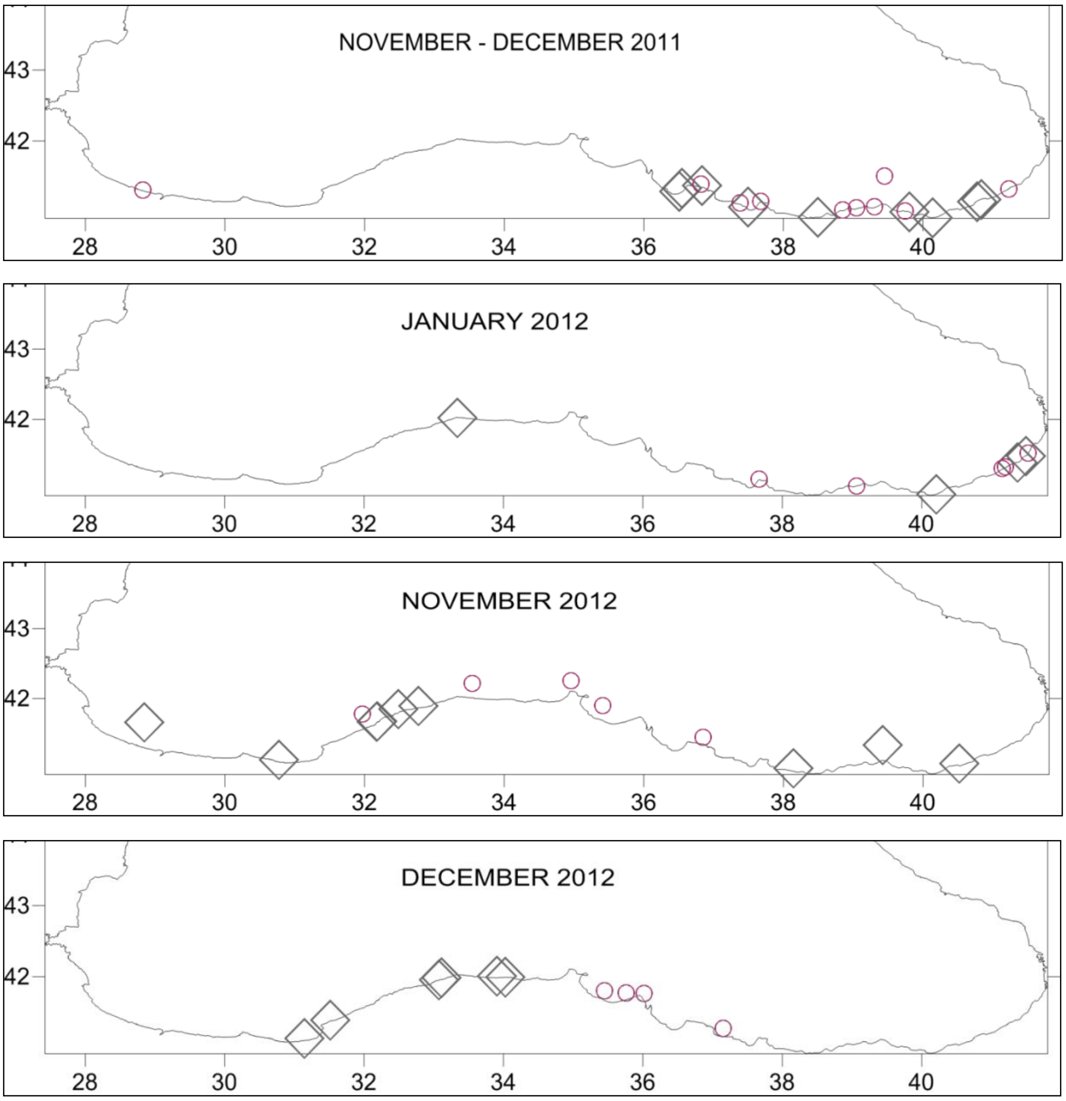


Figure 3.14 Comparison of groups formed according to mean condition factor value. O = group which has a lower value than mean length. ◊ = group which has a higher value than mean length.

3.5 Ageing and Otolith Shape Analysis

According to the t-test results, no significant difference were found between right and left otoliths ($P<0.05$). Therefore, shape analysis was applied with left otoliths, except it the left otolith was not available for the analysis due to the damage or crystallization, the right pairs were used.

The age reading results indicated that sufficient numbers of samples were obtained only from 0 and I age classes to capture statistically significant results for shape analysis. Hence, only 0 and I age classes were taken into account. To exclude the potential effects of the year – class growth differences, each year class evaluated within itself. In general, one year class formed majority of the samples. From 272 pairs of sagittal otoliths analysed, 155 individuals belong to I age class while 84 individuals belong to 0 age and II, III and IV age classes have total of 32 individuals.

The morphometric measurements and examined shape factors of otolith were given at Table 3.4. There was not any statistically significant correlation between fish length and the otolith shape variables.

Within the 0 age class, the fish length was varied between 4.6 cm to 7.5 cm with an average 5.9±0.62 cm. The largest otoliths were found in Samsun, whilst the smallest was at the station KD12-054 (41.13N-30-77E). According to the mean values of shape factors, the highest aspect ratio, ellipticity and circularity were observed at the station KD12-57 (41.14N-31.14E), while roundness at the station KD12-030 (41.70N-28.66E) and the other otolith shape variables at the station KD12-042 (form factor, diameter ratio, sphericity). Besides, while the smallest aspect ratio and circularity were observed at the station KD12-042 (41.34N-39.41E), the remaining shape values was found at the station KD12-057 (roundness, form factor, diameter ratio, box/area box and sphericity).

Within one age class length of fish was ranged between 6.7 cm to 12.5 cm with an average 8.8±1.32 cm. While highest aspect ratio was seen at the station KD12-057 same with 0 age class, circularity at the station KD12-066 (41.96N-33.07E), form factor at the station KD12-042, sphericity at the station KD12-054 besides roundness and area/box area were observed at the station KD12-042. The smallest otolith aspect ratio was found at the station KD12-054, whilst circularity at the station KD12-042 and the rest variables at the station KD12-057 (except ellipticity). Mostly the lowest values of 0 and I age classes were observed at the station KD12-057.

The Anderson-Darling test carried out on shape variables for each examined age groups confirmed that the variables are normally distributed ($P<0.05$).

The mean length-at-age differed greatly between stations. In general, the length-at-age values of western samples were greater than the eastern samples. Although only circularity and form factor showed differences within 0 age class, all shape variables among sampled schools showed significant differences within I age class. In contrast to length-at-age, the variables showed distinct irregular variation independent from latitude (Table 3.5)

Table 3.4 Age distributions, morphometric measurements (mean + SD) and determined shape factors of individuals sampled for shape analysis.

Station Code	Age Group	# of Ind	Length	±	Stdev	Weight	±	Stdev	Aspect Ratio	Ellipticity	Roundness	Circularity	Form Factor	Diameter Ratio	Area/Box Area Ratio	Sphericity
KD12-030	0 age	22	5.9	±	0.528	1.17	±	0.354	1.99	0.33	0.49	22.04	0.57	0.51	0.65	0.30
	1 age	2	7.2	±	0.075	2.30	±	0.075	2.07	0.35	0.47	23.16	0.55	0.49	0.66	0.26
KD12-042	0 age	19	5.7	±	0.753	1.07	±	0.505	1.98	0.33	0.48	20.79	0.61	0.51	0.66	0.30
	1 age	18	7.8	±	0.537	2.99	±	0.625	2.09	0.35	0.45	22.35	0.57	0.48	0.65	0.26
KD12-054	0 age	22	5.8	±	0.492	1.14	±	0.333	2.01	0.33	0.48	22.24	0.57	0.50	0.65	0.30
	1 age	5	6.9	±	0.148	1.87	±	0.196	2.06	0.35	0.45	22.41	0.56	0.49	0.65	0.28
KD12-057	0 age	2	6.5	±	0.495	1.56	±	0.394	2.15	0.36	0.46	23.80	0.53	0.47	0.64	0.26
	1 age	47	9.4	±	1.272	5.34	±	2.164	2.19	0.37	0.43	23.68	0.53	0.46	0.63	0.24
	2 age	7	10.8	±	1.157	8.03	±	2.256	2.21	0.37	0.42	23.94	0.53	0.45	0.63	0.24
	3 age	2	11.6	±	0.707	10.77	±	2.344	2.10	0.35	0.47	23.58	0.53	0.48	0.65	0.26
	4 age	2	11.5	±	0.141	10.40	±	0.493	2.14	0.36	0.45	23.49	0.54	0.47	0.64	0.25
KD12-066	1 age	33	9.3	±	1.136	4.87	±	1.855	2.15	0.36	0.44	23.99	0.53	0.47	0.64	0.25
	2 age	9	10.7	±	1.474	7.67	±	2.627	2.16	0.37	0.43	25.03	0.50	0.47	0.63	0.25
	3 age	1	11.4	±	-	8.93	±	-	2.20	0.38	0.42	29.10	0.43	0.44	0.62	0.23
KD12-074	1 age	28	9.4	±	1.178	5.11	±	1.892	2.13	0.36	0.44	23.24	0.55	0.47	0.64	0.25
	2 age	7	10.1	±	1.064	6.18	±	1.748	2.23	0.38	0.42	23.96	0.53	0.45	0.63	0.23
	3 age	2	11.3	±	0.212	9.68	±	0.129	2.09	0.35	0.47	20.96	0.60	0.48	0.65	0.26
	4 age	1	9.9	±	-	5.62	±	-	2.33	0.40	0.40	27.33	0.46	0.42	0.61	0.22
KD12-080	0 age	19	6.3	±	0.521	1.45	±	0.449	2.04	0.34	0.47	23.58	0.54	0.50	0.65	0.29
	1 age	22	8.6	±	1.287	4.08	±	1.877	2.13	0.36	0.45	22.70	0.56	0.48	0.64	0.26
	2 age	1	10.4	±	-	6.80	±	-	2.18	0.37	0.43	24.51	0.51	0.46	0.63	0.24

Table 3.5 ANOVA comparison of fish size and otolith shape variables (Mean±SD) across sampled regions.

	KD12-030	KD12-042	KD12-054	KD12-080	F	p
Length at 0 age	5.87 ± 0.53	5.65 ± 0.75	5.85 ± 0.49	6.35 ± 0.52	4.99198	0.003212
Aspect Ratio	1.99 ± 0.08	1.98 ± 0.11	2.01 ± 0.10	2.04 ± 0.10	1.62822	0.189605
Ellipticity	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.02	0.34 ± 0.02	1.63364	0.188372
Roundness	0.49 ± 0.02	0.48 ± 0.03	0.48 ± 0.02	0.47 ± 0.02	1.12294	0.344998
Circularity	22.04 ± 1.24	20.79 ± 0.97	22.24 ± 1.44	23.58 ± 2.15	10.99151	0.000004
Form Factor	0.57 ± 0.03	0.61 ± 0.03	0.57 ± 0.04	0.54 ± 0.05	11.66812	0.000002
Diameter Ratio	0.51 ± 0.02	0.51 ± 0.02	0.50 ± 0.02	0.50 ± 0.02	2.28593	0.085254
Area/Box Area Ratio	0.65 ± 0.01	0.66 ± 0.01	0.65 ± 0.01	0.65 ± 0.01	2.13978	0.101921
Sphericity	0.30 ± 0.03	0.30 ± 0.03	0.30 ± 0.03	0.29 ± 0.03	0.93405	0.428380

	KD12-042	KD12-057	KD12-066	KD12-074	KD12-080	F	p
Length at 1 age	7.83 ± 0.54	9.37 ± 1.27	9.31 ± 1.14	9.40 ± 1.18	6.35 ± 0.52	17.10787	0.000000
Aspect Ratio	2.09 ± 0.10	2.19 ± 0.11	2.15 ± 0.09	2.13 ± 0.12	2.13 ± 0.11	3.68445	0.006910
Ellipticity	0.35 ± 0.02	0.37 ± 0.02	0.36 ± 0.02	0.36 ± 0.02	0.36 ± 0.02	3.69511	0.006794
Roundness	0.45 ± 0.02	0.43 ± 0.02	0.44 ± 0.02	0.44 ± 0.02	0.45 ± 0.02	4.24616	0.002812
Circularity	22.35 ± 2.20	23.68 ± 2.05	23.99 ± 1.91	23.24 ± 2.62	22.70 ± 2.07	2.48690	0.046110
Form Factor	0.57 ± 0.05	0.53 ± 0.05	0.53 ± 0.04	0.55 ± 0.06	0.56 ± 0.05	2.86188	0.025585
Diameter Ratio	0.48 ± 0.02	0.46 ± 0.02	0.47 ± 0.02	0.47 ± 0.03	0.48 ± 0.02	2.97411	0.021424
Area/Box Area Ratio	0.65 ± 0.01	0.63 ± 0.01	0.64 ± 0.01	0.64 ± 0.02	0.64 ± 0.01	3.79110	0.005827
Sphericity	0.26 ± 0.02	0.24 ± 0.02	0.25 ± 0.02	0.25 ± 0.03	0.26 ± 0.02	2.89034	0.024460

In the PCA which was carried out with variables chosen based on model loadings (among 0 age class members), the first component explained 57.92% of total variance whilst the second component explained only 10.74%, indicating that the first value represented most of the variation among stocks (Figure 3.15, Table 3.6).

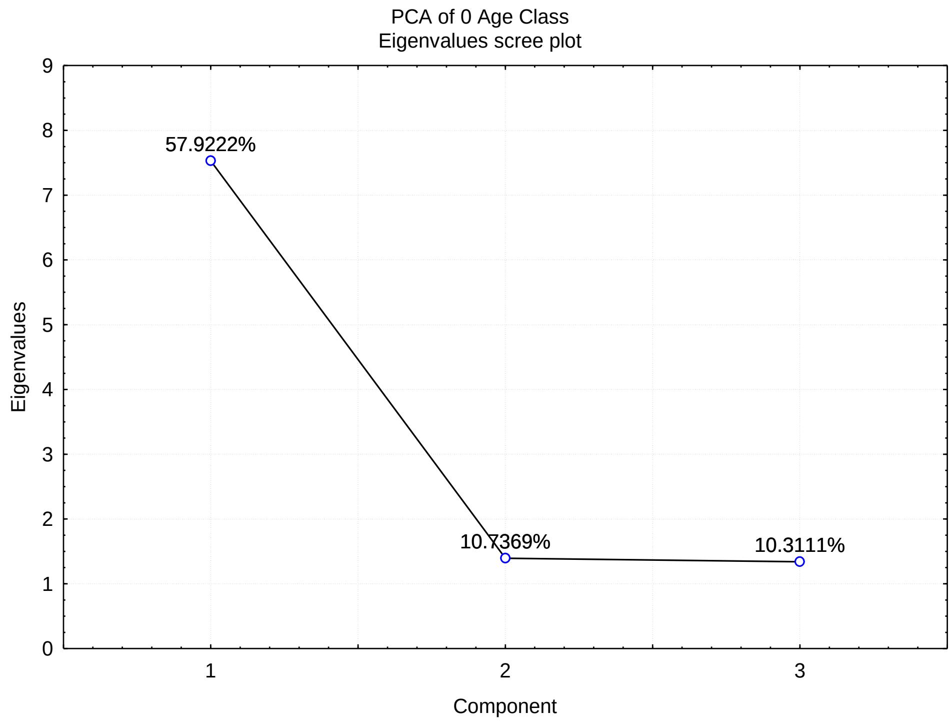


Figure 3.15 Explained variance percentages of components at 0 age class.

Table 3.6 PCA Eigenvalues at 0 age class.

	Eigenvalues	% Total variance	Cumulative eigenvalue	Cumulative %
1	7.529885	57.92219	7.52988	57.92219
2	1.395791	10.73686	8.92568	68.65905
3	1.340440	10.31108	10.26612	78.97013

PCA Eigenvalues, Number of Components is 3 PCA sum of variance 13.0000

Similar with the 0 age, within one age class, the variance explained by the first component was 53.70 %, while it was 11.17 % with the second component (Figure 3.16, Table 3.7).

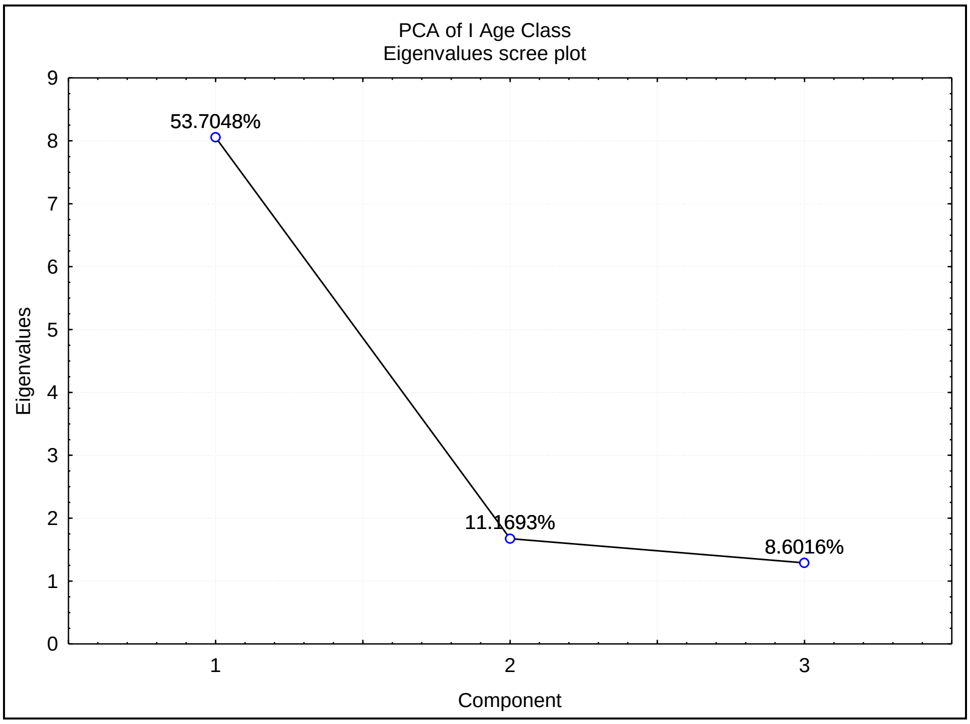


Figure 3.16 Explained variance percentages of components at I age class.

Table 3.7 PCA Eigenvalues at I age class.

	Eigenvalues	% Total variance	Cumulative eigenvalue	Cumulative %
1	8.0557228	53.70481847	8.055722771	53.70481847
2	1.6753967	11.16931125	9.731119459	64.87412972
3	1.2902372	8.601581423	11.02135667	73.47571115

PCA Eigenvalues, Number of components is 3, PCA sum of variance 15.0000

According to the principal component loadings, within 0 age class aspect ratio, ellipticity, roundness, diameter ratio, area/box area, elongation and sphericity were found feasible to apply in discriminant analysis among all examined otolith shape variables. The discriminant analysis carried out using PC residuals, indicated a strong discrimination between anchovy stocks (Table 3.8). Besides, the F-test associated with Wilks' lambda was found significant ($p<0.001$). In the classification matrix, each case was classified into the group where its value of classification is the largest. For the samples belong to the station KD12-030 and KD12-080 the success was 100% whereas at the station KD12-054 and KD12-042, there were only a few mixing between cases. The score plot clearly shows the discrimination among the stations (Figure 3.17).

Table 3.8 Classification matrix as a result of canonical discriminant analysis testing for differences in otolith shape between Black Sea anchovy cohorts at 0 age class using otolith shape variables.

	Percent	KD12-030	KD12-042	KD12-054	KD12-080
KD12-030	100.0000	22	0	0	0
KD12-042	68.4211	0	13	6	0
KD12-054	68.1818	0	7	15	0
KD12-080	100.0000	0	0	0	19
Total	84.1463	22	20	21	19

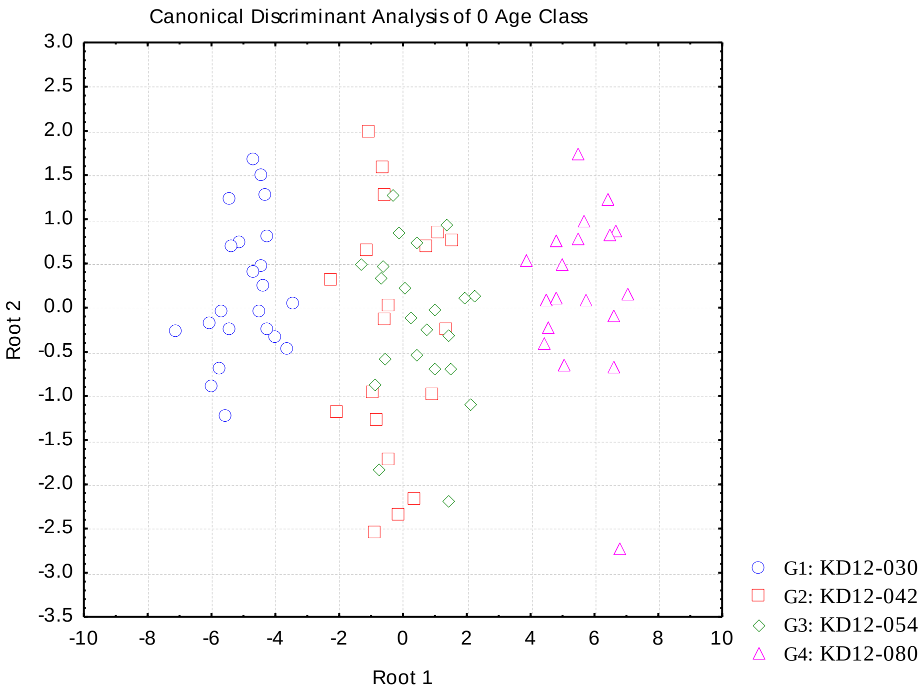


Figure 3.17 Canonical discriminant factor scores obtained from otolith shape variables at 0 age class.

The circularity, form factor and radius ratio were found as interpreting components within I age class. The discriminant analysis carried out with PC residuals showed quite high discrimination (Table 3.9). Significance of results were supported by F-test associated with Wilks' lambda ($p<0.001$). In the classification matrix, the classification success of cohorts was close to 100%. The only exception may be Trabzon samples which showed similarities with the KD12-074 station samples (see related figures for the location of the places). Thus, for I age class, 131 individuals were classified correctly while 17 were misclassified. Hence, in total, 88.51 % of individuals were correctly classified. According to the canonical discriminant analysis results the station KD12-066, KD12-057 and KD12-080 the origin of the fishes were closer to each other. These fishes were distinct from the samples collected at the stations KD12-074 and KD12-042 (Figure 3.18).

Table 3.9 Classification matrix as a result of canonical discriminant analysis testing for differences in otolith shape between Black Sea anchovy cohorts at I age class using otolith shape variables.

	Percent	**KD12-042**	**KD12-057**	**KD12-066**	**KD12-074**	**KD12-080**
KD12-042	50.00000	9	0	0	9	0
KD12-057	95.74468	0	45	1	0	1
KD12-066	96.96970	0	0	32	0	1
KD12-074	89.28571	3	0	0	25	0
KD12-080	90.90909	0	0	1	1	20
Total	88.51351	12	45	34	35	22

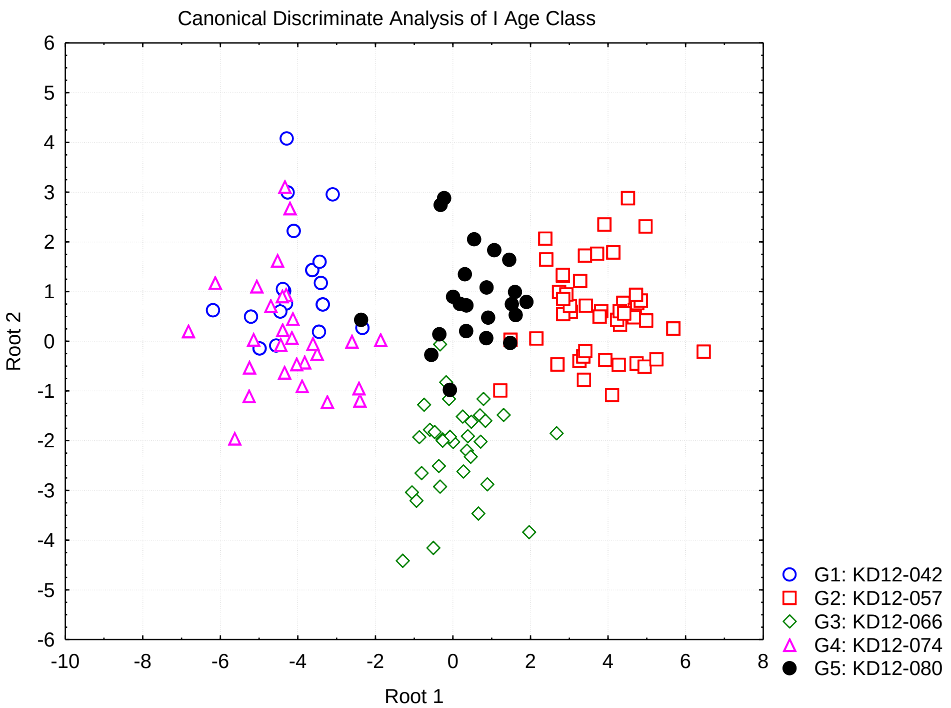


Figure 3.18 Canonical discriminant factor scores obtained from otolith shape variables at I age class.

Results of k-Means clustering carried out with PC residuals indicated that individuals were grouped into two clusters within 0 and I age classes (Figure 3.19, Figure 3.20). However, the classification frequencies showed that except for station KD12-066, none of the other stations nested in one single cluster (Table 3.10). This might indicated that the schools are composed of individuals of mixed origin.

Table 3.10 Classification frequencies of anchovy stocks belong to 0 and I age classes.

Classification Frequency in Percentage					
0 Age			**1 Age**		
Station	**Cluster 1**	**Cluster 2**	**Station**	**Cluster 1**	**Cluster 2**
KD12-030	64	36	**KD12-042**	67	33
KD12-042	47	53	**KD12-057**	34	66
KD12-054	45	55	**KD12-066**	94	6
KD12-080	47	53	**KD12-074**	71	29
			KD12-080	45	55

ANOVA results indicated that all examined variables except Aspect Ratio and Ellipticity was contributed to cluster analysis within 0 age group (Table 3.11). Besides, among the otolith shape variables introduced in k-Means clustering, total length of fish and roundness of otolith were not suitable to discriminate clusters within I age class (Table 3.12).

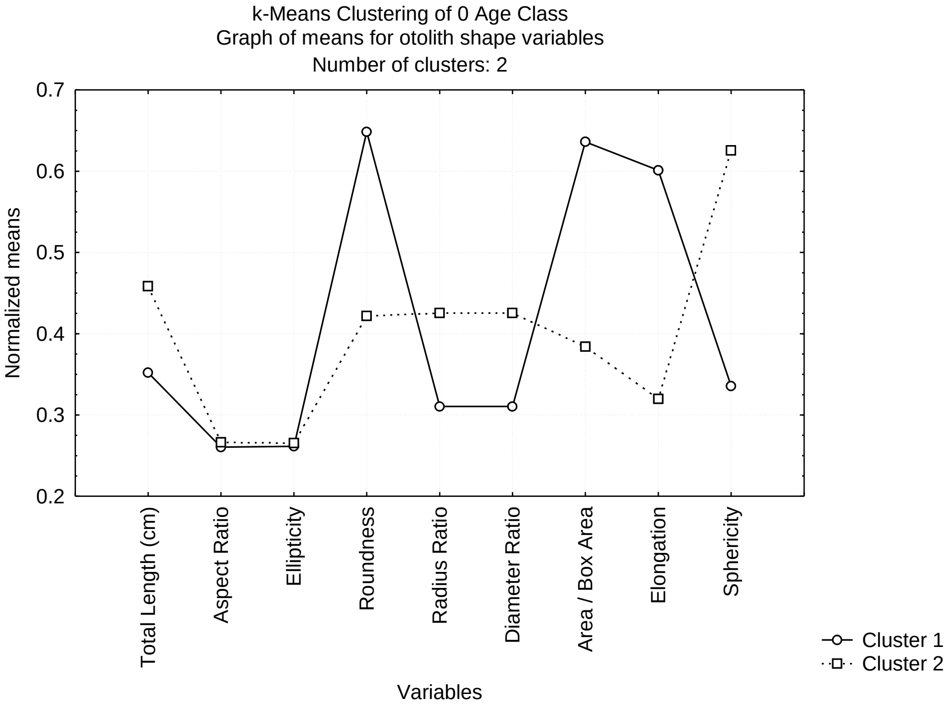


Figure 3.19 k-Means results of 0 age class, means of cluster for otolith shape variables.

Table 3.11 ANOVA results of k-Means clustering carried out with 0 age class otolith shape variables.

	Between	df	Within	Df	F	p value
Total Length (cm)	3.201399	1	41.70853	80	6.14052	0.015317
Aspect Ratio	0.003290	1	6.97573	80	0.03773	0.846479
Ellipticity	0.001305	1	6.69833	80	0.01559	0.900960
Roundness	2.496175	1	6.38702	80	31.26562	0.000000
Radius Ratio	0.406064	1	3.05369	80	10.63799	0.001629
Diameter Ratio	0.405974	1	3.05397	80	10.63468	0.001632
Area / Box Area)	7.356651	1	14.95803	80	39.34555	0.000000
Elongation	3.922689	1	4.58106	80	68.50273	0.000000
Sphericity	4.539722	1	5.38305	80	67.46687	0.000000

ANOVA for continuous variables, # r of clusters: 2, Total number of training cases: 82

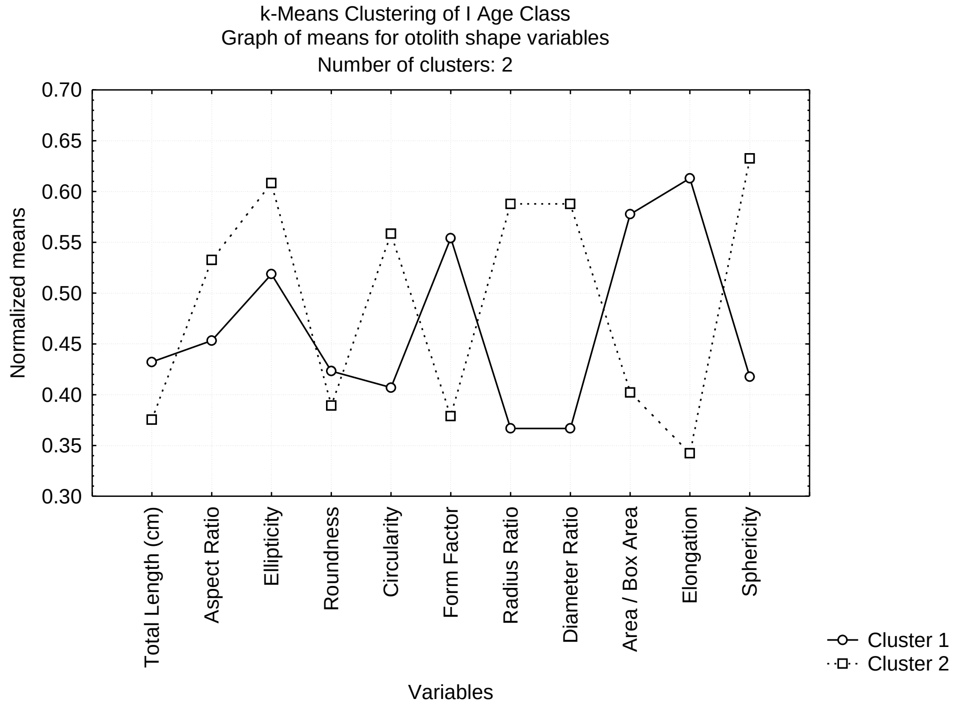


Figure 3.20 k-Means results of I age class, means of cluster for otolith shape variables.

Table 3.12 ANOVA results of k-Means clustering carried out with I age class otolith shape variables.

	Between	df	Within	df	F	p value
Total Length (cm)	1.087022	1	42.91690	146	3.6980	0.056426
Aspect Ratio	0.294896	1	7.27331	146	5.9196	0.016183
Ellipticity	0.376193	1	7.14558	146	7.6864	0.006289
Roundness	0.196092	1	20.75900	146	1.3791	0.242161
Circularity	4.819265	1	22.09091	146	31.8508	0.000000
Form Factor	4.652841	1	19.62964	146	34.6066	0.000000
Radius Ratio	1.855752	1	4.52190	146	59.9173	0.000000
Diameter Ratio	1.855775	1	4.52119	146	59.9273	0.000000
Area / Box Area	4.009633	1	16.22178	146	36.0877	0.000000
Elongation	4.820607	1	5.87337	146	119.8304	0.000000
Sphericity	3.570736	1	7.63493	146	68.2819	0.000000

ANOVA for continuous variables, # of clusters: 2, Total number of training cases: 148

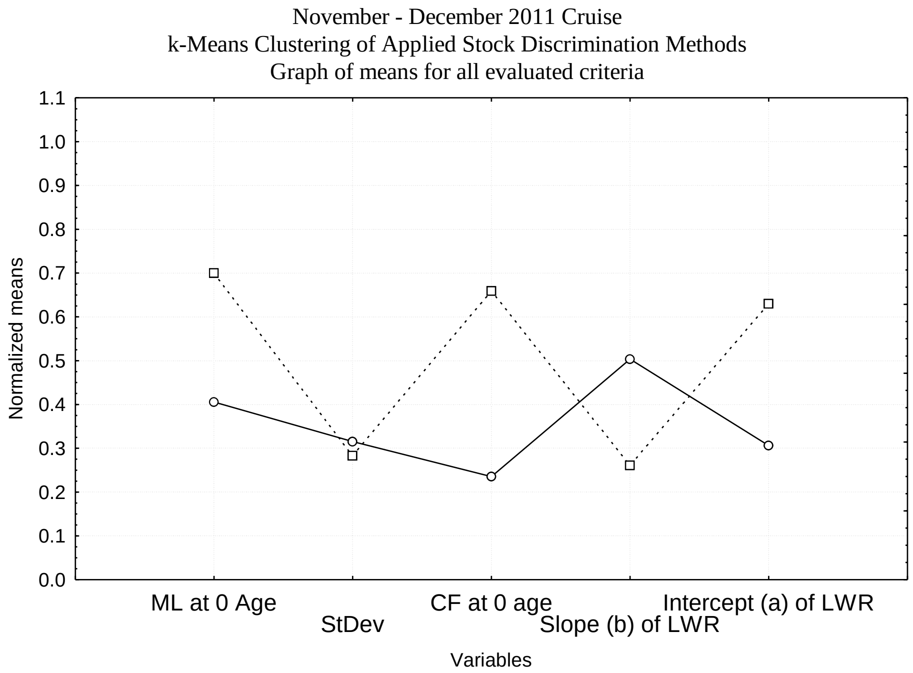
3.6 k-Means Clustering

The k-Means clustering carried out in addition to the other methods that had been applied to discriminate anchovy stocks. The clustering results confirmed that within each cruise individuals were grouped into two clusters (Figure 3.21). Such clustered distributions were observed slightly at cruises of November and December 2012 allowing geographical distinction, however, on the other hand, the results for cruises of November-December 2011 and January 2012 showed that the stocks had mixed distribution. According to the cluster distributions, schools had mixed distribution in November – December 2011 and January 2012 cruises.

The common threat of ANOVA results (Table 3.13) was that condition factor of fish and intercept of LWR were not good indicators for stock discrimination of anchovy, while mean length at zero age cohorts, its standard deviation and slope of LWR might be helpful tools to discriminate stocks ($p<0.05$).

Table 3.13 ANOVA results of k-Means clustering carried out with applied stock discrimination methods. ML: mean length, StDev: standard deviation of mean length, CF: condition factor of fish and LWR: length-weight relationship of fish.

		Between	df	Within	df	F	p value
November - December 2011	ML at 0 Age	910.4760	1	5.413197	16	2691	0.000000
	StDev	6.4408	1	0.911937	16	113	0.000000
	CF at 0 age	-0.0047	1	0.000001	16	-133597	1.000000
	Slope of LWR	184.4005	1	0.193749	16	15228	0.000000
	Intercept of LWR	-0.0032	1	0.000006	16	-7894	1.000000
January 2012	ML at 0 Age	327.8071	1	0.333880	5	4909.1	0.000000
	StDev	1.1814	1	0.169068	5	34.9	0.001974
	CF at 0 age	-0.0040	1	0.000000	5	-65041.8	1.000000
	Slope of LWR	77.1224	1	0.043575	5	8849.4	0.000000
	Intercept of LWR	-0.0019	1	0.000001	5	-10796.2	1.000000
November 2012	ML at 0 Age	407.7935	1	10.86704	12	450.3	0.000000
	StDev	4.4559	1	0.58680	12	91.1	0.000001
	CF at 0 age	-0.0045	1	0.00000	12	-32632.5	1.000000
	Slope of LWR	171.4709	1	0.30830	12	6674.2	0.000000
	Intercept of LWR	-0.0019	1	0.00000	12	-7193.7	1.000000
December 2012	ML at 0 Age	376.5348	1	4.041300	5	465.9	0.000004
	StDev	3.2781	1	0.338961	5	48.4	0.000945
	CF at 0 age	-0.0049	1	0.000001	5	-43475.8	1.000000
	Slope of LWR	79.3990	1	0.080047	5	4959.5	0.000000
	Intercept of LWR	-0.0026	1	0.000001	5	-12731.1	1.000000



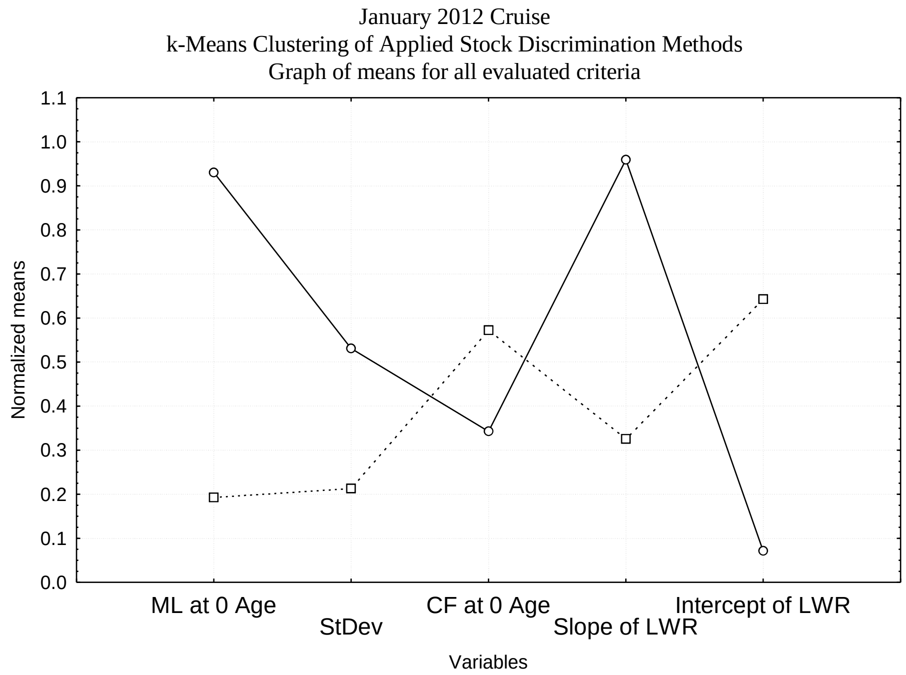
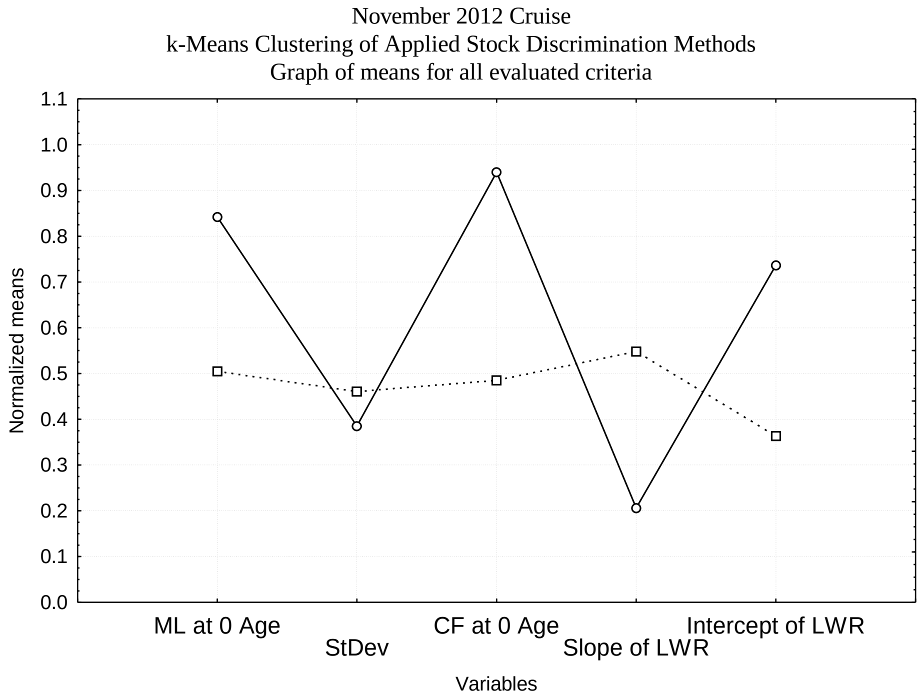


Figure 3.21 k-Means clustering carried out with applied stock discrimination methods. ML: mean length, StDev: standard deviation of mean length, CF: condition factor of fish and LWR: length-weight relationship of fish one age class, means of cluster for otolith shape variables.



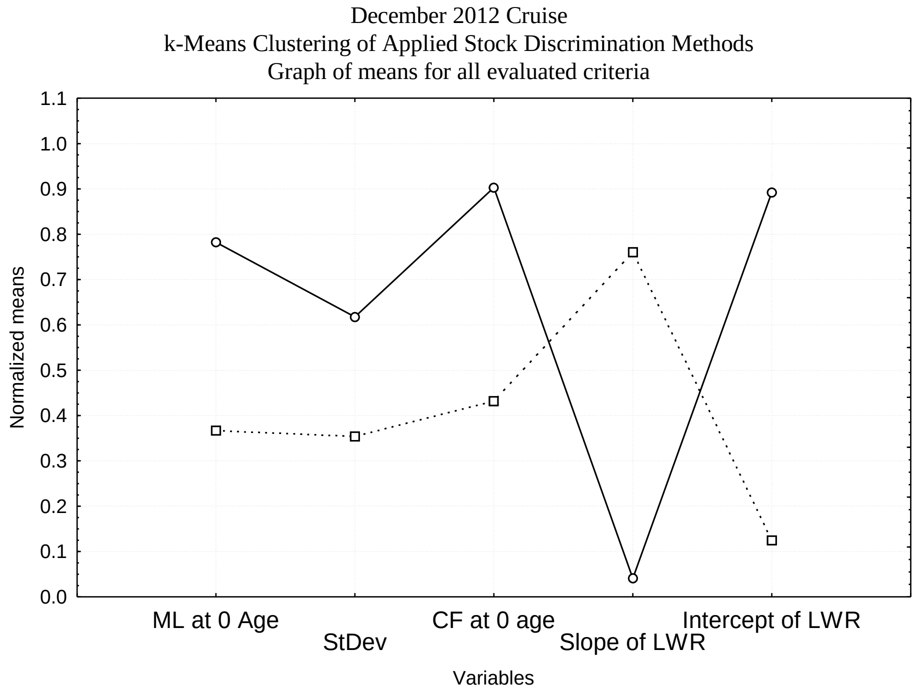


Figure 3.21 (continued). k-Means clustering carried out with applied stock discrimination methods. ML: mean length, StDev: standard deviation of mean length, CF: condition factor of fish and LWR: length-weight relationship of fish one age class, means of cluster for otolith shape variables.

4 DISCUSSION

Being the greatest contributor of the overall Turkish landings from sea and comprising over 50 % of the total national fish catch (www.tuik.gov.tr), Black Sea anchovies play a commercially extremely important role in Turkey. Therefore, managing anchovy fisheries is essential for both the economy of the country and the sustainability of the species. A sound management strategy targeting maximum sustainable yield should rely on stock assessment and the first step towards defining a scientifically sound stock assessment is to determine and define the stock units. Unit stocks are discrete entities which have their own physical life cycle under their own natural demographic influences and show their own response to unnatural interventions with their abundances and life history traits. Within these units, partial or entire isolations may lead to small changes in their morphological and genetic properties to specific environmental conditions (Waldman, 2005).

As previously mentioned, anchovy are represented by at least two different stocks in the Black Sea. They spawn and nurse on two separate and ecologically different geographical areas and are exploited by two different fisheries (Chashchin, 1996). On the other hand, the overwintering grounds of these two stocks may overlap during which time they may also be exploited together. Additionally, due to climatic, ecological, and biological reasons, the rate of mixing between the stocks appears to have increased during the last decade (Gucu et al., 2014). Due to identical morphologies, the fishermen cannot recognise the sub- species reporting their catches as the same species. Therefore, total Black Sea anchovy landings are reported as one value disregarding the stock units.

As the aim of the study is to develop a practical methodology to distinguish between these stocks especially when they are exploited together in the overwintering grounds, some widely accepted facts about the subspecies were adopted. The success of discrimination methods applied through these facts are discussed below.

4.1 Allometry and Geographical Proximity

In this study, the length and weight relationship indicated positive allometry contrary to some previous studies. It may be seen that the relation changed from negative to positive over time. While the allometric coefficient, b, indicated negative relation from the 1985/86, to 1987/88, 1990/91 to 1991/92, 1993/94 to 1995/96 fishing seasons; after 1997/98, including 2001/2002 seasons, the allometry became positive (Table 4.1). Sağlam & Sağlam (2013) suggested that the effects of environment and climate change on growth of anchovy populations and its food abundance within habitats result in variation.

As presented in the results of comparison carried out with coefficient of length-weight relationship, there were statistically significant differences in the allometry of the anchovy schools (Figure 3.7, Figure 3.8, Figure 3.9, Figure 3.10, and Figure 3.11). However the schools with different allometries were not geographically distinct. In some cases two separate schools with differing allometry were observed next to each other. In some other cases, two schools with identical allometries were observed at great distances. The comparison carried out to observe the allometric dissimilarity between stations supported these findings. In the cruises held in November-December 2011 and December 2012, increased dissimilarity versus distance was clear. However, in the remaining two cruises, the pattern was less clear (Figure 3.12). This irregularity may indicate that there are no geographical limits, but the subspecies occupy exactly the same areas during winter. This is not a surprise finding since the main drive for both subspecies is temperature and both are migrating to the Turkish coast during winter to reach the warmest water masses which are located on the southeastern Black Sea.

Table 4.1 Estimated length – weight relationship parameters and age frequencies from previous surveys (as cited in Sağlam & Sağlam, 2013).

Mean		Coefficients of LWR		Age-Frequency in Percentage				Fishing Season
L	W	A	B	0	1	2	3	
11.3	10.5	0.0023	3.4120	24.19	24.91	47.17	3.73	1985/1986 [1]
10.8	8.7	0.0025	3.3830	20.14	51.55	22.54	5.77	1986/1987 [2]
9.3	6.6	0.0025	3.3870	33.94	48.93	14.22	2.91	1987/1988 [3]
10.7	8.1	0.0064	2.9740	2.39	53.33	42.49	1.70	1988/1989 [4]
8.9	4.2	0.0065	2.9780	69.40	29.00	1.20	0.40	1989/1990 [5]
8.5	3.9	0.0049	3.1230	39.60	56.60	3.80	-	1990/1991 [6]
9.1	5.1	0.0055	3.0360	41.56	41.62	16.76	0.06	1991/1992 [7]
9.5	5.2	0.0053	2.9990	39.27	30.61	27.39	2.73	1992/1993 [8]
10.4	6.8	0.0051	3.0480	14.29	66.43	16.79	2.50	1993/1994 [9]
9.0	4.8	0.0047	3.0980	63.28	23.24	10.86	2.62	1994/1995 [10]
10.7	7.3	0.0052	3.0300	23.87	49.27	20.93	5.95	1995/1996 [11]
9.6	7.2	0.0057	3.1200	-	-	-	-	1996/1997 [12]
10.7	7.9	0.0073	2.9030	22.95	47.37	18.48	11.20	1996/1997 [13]
10.8	8.1	0.0055	3.0270	23.88	47.75	19.52	8.85	1997/1998 [14]
10.7	7.5	0.0118	2.7100	-	-	-	-	2000/2001 [15]
11.3	9.1	0.0051	3.0570	-	-	-	-	2001/2002 [16]
10.2	6.5	0.0075	2.8950	-	-	-	-	2002/2003 [17]
11.4	9.3	0.0066	2.9670	8.34	54.04	22.88	14.74	2004/2005 [18]
11.3	9.2	0.0101	2.7900	8.20	10.60	60.80	20.40	2004/2005 [19]
11.6	10.0	0.0110	2.7420	1.02	33.12	64.06	1.80	2010/2011 [20]

(Özdamar et al., 1991), (2) (Karaçam & Düzgüneş, 1998), (1988), (3) (Düzgüneş & Karaçam, 1989), (4) (Ünsal, 1989), (5) (Okur, 1989), (6) (Genç & Başar, 1991), (7) (Genç & Başar, 1992a), (8) (Genç & Başar, 1992b), (9) (Mutlu, 1994), (10) (Özdamar et. al., 1995), (11) Mutlu (1996), (12) (Kayalı, 1998), (13) (Mutlu, 2000), (14) (Mutlu, 2000), (15,16,17) (Samsun et. al., 2006), (18) (Şahin et. al., 2006), (19) (Bilgin et. al., 2006), (20) (Sağlam & Sağlam, 2013)

4.2 Condition Factor

The average condition factor (CF) of the present study (0.51) was slightly lower than earlier studies. While, Özdamar et. al. (1991) reported CF as 0.724 for the 1985/86 fisheries season, in the study results of Sağlam & Sağlam (2013) this value was calculated as 0.63. The difference may not be due to growth difference in the anchovy but, can probably be explained by the length range of the individuals sampled. Indeed, the present study comprises a wider length range, hence small size individuals may reduce the final average.

The condition factor (CF), to some extent, reflects the welfare of the fish hence giving information about the physiological state of the species. In terms of nutritional condition, the CF can be seen as the accumulation of fat and gonad development (Le Cren, 1951). The results of CF comparison indicated that 0 age class were represented by different groups in the wintering grounds. They displayed dissimilar average conditions which may reflect the nutritional conditions of the specific nursery. The distances between their summer distribution area and the over-wintering grounds of the two anchovy forms and therefore in the total energy cost of migration may also be a reason for the above mentioned fluctuations in average conditions. Besides, the results indicated that the groups having similar condition factors did not have a clear geographical boundary as was observed in allometric relation. The only exception which indicated geographic discrimination refers to the cruise carried out in December 2012 (Figure 3.14).

Moreover, it was seen that the two distinct groups discriminated by average condition factors may represent the Sea of Azov and Black Sea stocks. As shown in Chashchin (1996), Black Sea anchovy grow faster than the Azov anchovy. In this respect the group which displays higher CF might represent the Black Sea stocks and the group with lower CF would presumably be the stock originated from the Sea of Azov (Figure 3.13).

According to some researchers (Chashchin, 1985; Ivanova P. P., et al., 2013), the two subspecies exhibit a remarkable degree of mixing in nursery grounds, such as the presence of *E. encrasicolus ponticus* in the Sea of Azov and *E. encrasicolus maeoticus* spawning on the Bulgarian coast. The mixing may lead to hybridization among the subspecies. The confidence interval of the CF of the third group sampled in the November – December 2011 cruise may represent such hybrids. However, in November and December 2012 cruises, the third group showed no statistical overlap with group I or II and stand alone as a separate group. Gordina et. al. (1997), questioned the occurrence of a third anchovy race in the Black Sea based on the aspect ratios (length – width) of the eggs. As the size and morphometry of the eggs of the third group showed remarkable similarity to those of the anchovies inhabiting the Sea of Marmara, the authors claimed that there may be a substock of Marmara anchovy in the Black Sea. However, it should be noted that this inference of a third group was not deduced through scientifically precise experiments as the separation of length groups was performed visually (Figure 3.13).

4.3 Length Frequency Distribution

Because of the mutual attraction between individuals in pelagic fish populations, this physical grouping results in similar size assemblage in schools (Shaw, 1980 as cited in Fréon P., 1983). Hence, individuals in a cohort/school have similar length spread around certain mean length. The length frequency measurements (Table 3.2) may indicate that at least some of these variations arise from mixed cohort schools of different origin. The differences in the ecological and hydrological conditions in the geographically distinct spawning and nursery areas seen in Figure 3.2, Figure 3.3, Figure 3.4, and Figure 3.5, might also lead to variations in time of spawning, hatching and larval growth rates. These differences would eventually be reflected in the size distribution of the juveniles on their arrival at the overwintering grounds. Therefore, the stations displaying higher standard deviation, might be the 0 age class originated from the Sea of Azov. Because of the less saline and shallower characteristics of the basin, the cost of adaption to the Black Sea, which is saltier, might result in high variations in growth rate, hence the mean size attained.

4.4 Otolith Shape Analysis

The results of PCA showed that within different age groups variation was explained by different variables. Aspect ratio, ellipticity, roundness, radius and diameter ratio, area/box area ratio, elongation and sphericity were useful variables for both age classes. Additionally in I year class, significant results were obtained from circularity and form factor. According to PCA loadings only condition factor and rectangularity were variables not statistically significant for classification.

Discriminant analysis carried out based on otolith shape variables selected according to their PCA loadings (weights) enabled differentiation and classification of 84% and 89% of 0 and I year class individuals, respectively (Table 3.8 & Table 3.9). Friedland & Reddin (1994) and Simmoneau et al. (2000) applied similar tests of discriminant analysis on otoliths shapes of six population of lake trout, (Walbaum) in Canadian Rivers and achieved >75% and 70% accuracy in classification. Similarly, Begg & Brown (2000) reported classification success greater than 56-81% for the identification study of haddock *Melanogrammus aegiefinus* (L.) stocks on George Bank. The shape analysis study carried out with European anchovy in the Bay of Biscay indicated fairly high rates of discrimination with classification from 96 to 100% (Gonzalez-Salas & Lenfant, 2007). In this study, data showed relatively high rates of discrimination with classifications success of 84-89% of individuals. These results suggest that variability in otolith shape could be a good tool for stock discrimination of anchovies in the Black Sea. It should be noted that the classification percentage achieved in the present study is comparable and even higher than studies using the same type of otolith shape analysis in literature.

Although, general classification success of discriminant analysis was high, there were signs of mixing at some stations. Regarding the 0 age class, the samples of station KD12-030 and KD12-080 were classified completely. However, in stations KD12-042 and KD12-054, only part of the samples were classified, and the rest displayed a mixed pattern. The samples belonging to I age class were successfully classified at station KD12-057. However, there were some interactions between the stations KD12-042 and KD12-074 and between stations KD12-066 and KD12-080. The individuals/samples which remained outside the classified groups, may point to mixing between stocks (Figure 3.17 & Figure 3.18).

It is generally accepted that individuals which inhabit the same geographical area and are exposed to equivalent environmental conditions possess identical otoliths on which similar patterns of development (calcium deposition) are printed on their shape. As both k-Means clustering results pointed out the existence of two segregated classes, particular hydrological and nutritional conditions in the feeding and spawning areas, would eventually lead to changes in the growth rates of individuals. As the growth rate determines deposition rate and otolith formation, the shape of otoliths in distinct areas would therefore result in remarkable shape variations.

The results showed that not all the parameters are equally important in all age classes examined (Table 3.11, Table 3.12). Within 0 age class aspect ratio and ellipticity did not contribute significantly in clustering, while total body length of fish and roundness were not important in I age class. This is not surprising because in addition to environmental influence on the otolith formation, otolith shape may also change with age. An otolith grows on the anterior-posterior axis, faster than it grows dorsa-ventrally. Therefore, an otolith of a young individual would be rounder (displays higher roundness) than that of an aged individual.

Otolith shape analysis could be used as a potential tool to discriminate anchovies in the Black Sea as it has been used for many fish species. Yet, the result should be supported with the samples from nursery areas (mainly north-western shelf and Sea of Azov).

4.5 k-Means Clustering

As the overall evaluation, the k-Means clustering carried out with all applied methods showed that for each cruise, individuals were grouped into two clusters (Figure 3.21). Even the classification success of each variable changed from cruise to cruise, the distinguished patterns common to all results are: the condition factor and intercept of length weight relationship were by no means significant descriptors of the stocks (Table 3.13). The reason might be that the observed changes were not permanent.

Considered individually, all applied methods suggest that the Black Sea anchovies are members of two distinct stocks as presented in the literature (Aleksandrov, 1927; Pusanov, 1936; Rass, 1987; Prodanov, Ivanov, & Dencheva, 1993 as cited in Prodanov, et al., 1997 and Ivanova P. P., et al., 2013). However, biological significance of the distinction with applied methods and interpretation of results for fisheries management were not as clear as expected. Therefore, even though this study gives promising results, validation is necessary with samples collected from the nursery ground where the stocks can be observed separately.

5 CONCLUSION

Subspecies discrimination of anchovies in the Black Sea is key information for stock assessment and fisheries management, but at present there is no simple method applied on a large scale to assess this feature.

This study conducted an investigation on different subspecies of anchovies in the Black Sea in the wintering grounds, using innovative methods, based on the combination of otolith shape and length-weight parameters. The strength of this method is the use of a simple approach, based on easily applied parameter measurements using a set of basic instruments, in order to be easily applied by the fishery industry.

Investigations carried out, during two consecutive winters (2010-2011 and 2011-2012) in the southern coasts of the Black Sea, showed that differences imprinted on the investigated features of the populations in summer could be traced in wintering grounds. Whilst those differences pointed out two distinct groups in terms of allometric growth coefficient (b), length frequency distribution and otolith shape results; condition factor results indicated the existence of a third group. This group may imply a hybrid stock which is mentioned in some previous studies or it may be caused by oversensitivity of selection in the separation of the groups under evaluation.

One of the important results of this study is related to the distribution at the overwintering grounds of the two subspecies of Black Sea anchovy. The current hypothesis on migration of subspecies assumes that the Black Sea and Azov Sea anchovies, while migrating southward, target the warmer waters of the south-eastern region where stock mixing occurs. The present study, designed to observe different forms of anchovy sampled along the hypothetical migration route at different times, did not agree with such a clear migration pattern. The dissimilarities among stations were not observed specifically in the warmest region of the overwintering grounds, nor on the migration route. The stations showed similar patterns to be distributed throughout the region, so it is possible to conclude that mixing of the two species happened over the entire southern Black Sea coast.

The otolith shape analysis showed promising results. Hence, it can be concluded that this approach is a helpful tool to discriminate anchovy stocks. However, this method should not be used alone to identify different subspecies due to dynamic shape changes with the age. So, in order to avoid errors in subspecies identification, either all applied methods should be used together or more detailed sampling should be conducted both in feeding and wintering grounds during warmer months before migration to define the discrimination criteria more precisely.

Even if applied methods generated significant results, in order to use them for stock assessment studies, more accurate results need to be accessed. It is recommended for future studies to design a sampling strategy that covers both feeding and wintering grounds, post migration and during warmer months.

REFERENCES

Aleksandrov, A. I. (1927). Anchovies in the Sea of Azov and Black Sea, their origin and taxonomic status. Tr. Kertch Fish Farm. St., *1*, 1-3.

Avşar, D. (1987). Application of Mahalanobis Distance Function for the Morphometric Separation of Some Demersal Fish Stocks in the Gulf of Mersin (Master Thesis, Institute of Marine Sciences - METU, Mersin, Turkey). Mersin, Turkey.

Bagenal, T. B., & Tesch, F. W. (1978). Methods for assessment of fish production in freshwaters. (T. B. Bagenal, Ed.) IBP HandBook, 3. Edition, 101-136.

Basilone, G., Guisande, C., & Patti, B. (2004). Linking habitat conditions and growth in the European anchovy (*Engraulis encrasicolus*). Fisheries Research, 68, 9-19.

Bat, L., Erdem, Y., Ustaoğlu Tırıl, S., & Yardım, Ö. (2008). Systematic of Pisces (Balık Sistematiği) (1 ed.). Ankara, Turkey: Nobel (in Turkish).

Bat, L., Şahin, F., Satılmış, H. H., Üstün, F., Özdemir, B. Z., Kıdeys, A. E., & Shulman, G. E. (2007). Karadeniz'in Değişen Ekosistemi ve Hamsi Balıkçılığına Etkisi. Journal of Fisheries Sciences, 1 (4), 191 – 227 (in Turkish).

Begg, G., & Brown, R. (2000). Stock identification of Haddock, *Melanogrammus aeglefinus* on Georges bank based on otolith shape analysis. Transactions of the American Fisheries Society, 129, 935-945.

Bingel, F., Kıdeyş, A. E., Özsoy, E., Tuğrul, S., Baştürk, Ö., & Oğuz, T. (1993). Stock Assessment Studies for the Turkish Black Sea Coast. Institute of Marine Sciences.

Borsa, P., Collet, A., & Durand, J. D. (2004). Nuclear - DNA markers confirm the presence of two anchovy species in the Mediterranean. *327*, 1113-1123.

Bulgakova, Y. V. (1993). Diurnal Dynamics of Feeding in Anchovy, *Engraulis encrasicolus*, and Factors Determining It. J. Ichthyol., 33(7), 395-400.

Cadrin, S. X., Friedland, K. D., & Waldman, J. R. (2005). Stock Identification Methods - Applications in Fishery Science. Elsevier Academic Press.

Campana, S. E., & Casselman, J. M. (1993). Stock Discrimination Using Otolith Shape Analysis. Can. J. Fish. Aquat. Sci., 50, 1062 - 1083.

Campana, S. E., & Neilson, J. D. (1985). Microstructure of fish otoliths. 42, 1014-1032.

Casselman, J. M. (1987). Determination of age and growth. In A. H. Weatherley, & H. S. Gill, The biology of fish growth (pp. 209-242). New York: Academic Press.

Chashchin, A. K. (1985). Changes in the population structure of anchovy *Engraulis encrasicolus* of the Azov-Black Sea Basin. J. Ichthyol, 25(5), 9-15.

Chashchin, A. K. (1996). The Black Sea Populations of Anchovy. (I. Palomera, & P. Rubies, Eds.) Scientia Marina, 60, 219-225.

Codispoti, L. A., Friederich, G. E., Murray, J. W., & Sakamoto, C. M. (1991). Chemical variability in the Black Sea: implications of continuous vertical profiles that penetrated the oxic/anoxic interface. Deep-Sea Research, 38 (Suppl. 2), S691 - S710.

Collins, R. A., & Jerome, D. S. (1969). Age Determination of Northern Anchovies, *Engraulis mordax*, from Otoliths. In J. D. Messersmith, A Review of the California Anchovy Fishery and Results of The 1965-66 and 1966-67 Reduction Seasons (Vol. 147, pp. 39-55). California Sacramento: State of California, Dept. of Fish and Game.

Daskalov, G., Osio, C., & Charef, A. (2012). Scientific, Technical and Economic Committee for Fisheries (STECF) – 2013 Assessment of Black Sea stocks (STECF 12-15). Brussels: Publications Office of the European Union.

Dobrovolsky & Zalogin, 1982 from http://en.wikipedia.org/wiki/Sea_of_Azov (in Russian).

Sağlam, E. N., & Sağlam, C. (2013). Age, growth and mortality of anchovy *Engraulis encrasicolus* in the south-eastern region of the Black Sea during the 2010-2011 fishing season. Journal of the Marine Biological Association of the United Kingdom, 93(8), 2247-2255.

Ferreiro, M. J., & Labarta, U. (1988). Distribution and abundance of teleostean eggs and larvae on the NW coast of Spain. Marine Ecology - Progress Series, 43, 189 - 199.

Fréon P., 1983. Body length variability in fish schools and cohorts; application to Fisheries Biology in Tropical Waters (Paris, France). In: Proceedings ; FAO Fisheries Report (FAO) , no. 291, v.2-3; Expert Consultation to Examine Changes in Abundance and Species Composition of Neritic Fish Resources, San Jose (Costa Rica), 18 Apr 1983 Csirke, J. (ed.) Sharp, G.D. (ed.) / Rome (Italy), FAO, 1983, p. 438-442.

Garcia, A., & Palomera, I. (1996). Anchovy Early Life History and Its Relation to Its Surrounding Environment in the Western Mediterranean Basin. Scientia Marina, 60 (Supl. 2), 155-166.

Giannetti, G., & Donato, F. (2003). Age Determination Manual. Termoli: FAO - MIPAF Scientific Cooperation to Support Responsible Fisheries in the Adriatic Sea.

Gonçalves, J. M., Bentes, L., Lino, P. G., Ribeiro, J., Cana´rio, A. V., & Erzini, K. (1997). Weight–length relationships for selected fish species of the small-scale demersal fisheries of the south and south-west coast of Portugal. Fish. Res., 30, 253-256.

Gonzalez-Salas, C., & Lenfant, P. (2007). Interannual variability and intraannual stability of the otolith shape in European anchovy *Engraulis encrasicolus* (L.) in the Bay of Biscay. Journal of Fish Biology, 70(1), 35-49.

Gordina, A. D., Nikolskiy, V. N., Niermann, U., Bingel, F., & Subbotin, A. A. (1997). New data on the morphological differences of anchovy eggs (*Engraulis encrasicolus* L.) in the Black Sea. Fish. Res., 31, 139-145.

Grant, W. S., & Bowen, B. W. (1998). Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. 89, 415-426.

Gucu, A.C., İnanmaz, Ö. E., Ok, M., & Sakınan, S. (2014). Anchovy spawning grounds on the southern half of the Black Sea. ICES, Journal of Marine Sciences (submitted).

Helfman, G. S., Collette, B. B., & Facey, D. E. (1997). The Diversity of Fishes. Blackwell Science.

Holden, M. J., & Raitt, D. F. (1974). Manual of Fisheries Science Part 2 - Methods of Resource Investigation and their Application. Rome: Food and Agriculture Organization of the United Nations.

Hossain, M. Y., Ahmed, Z. F., Leunda, P. M., Jasmine, S., Oscoz, J., Miranda, R., & Ohtomi, J. (2006). Condition, length–weight and length–length relationships of the Asian striped catfish Mystus vittatus (Bloch, 1794) (Siluriformes: Bagridae) in the Mathabhanga River, southwestern Bangladesh. J. Appl. Ichthyol., 22, 304-307.

https://lpdaac.usgs.gov/data_access. (n.d.). Retrieved from Moderate Resolution Imaging Spectroradiometer (MODIS): http://modis.gsfc.nasa.gov/

Huxley, J. S. (1932). Problem of Relative Growth.

Ivanov, L., & Beverton, R. J. (1985). The fisheries resources of the Mediterranean. Part two: The Black Sea. Studies and Reviews (60 ed.). General Fisheries Council for the Mediterranean.

Ivanova, P. P., & 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(1)*, 13-22.

Ivanova, P. P., Dobrovolov, I. S., Bat, L., Kideys, A. E., Nikolsky, V. N., Yuneva, T. V., Shchepkina, A. M., Shulman, G. E. (2013). Application of Esterase Polymorphism to Specify Population Genetic Structure of *Engraulis encrasicolus* (Pisces: Engraulidae) in the Black and Azov Seas. Marine Ecological Journal, 12 (4), 45 - 52.

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

Jørgensen, B. B., Fosing, H., Wirsen, C. O., & Jannasch, H. W. (1991). Sulfide oxidation in the anoxic Black Sea chemocline. Deep-Sea Research, 38 (Suppl. 2), S1083 - S1104.

Korotaev, G., Oguz, T., Nikiforov, A., & Koblinsky, C. (2003). Seasonal, interannual, and mesoscale variability of the Black Sea upper layer circulation derived from altimeter data. Journal of Geophysical Research, 108 (C4).

Le Cren, E. D. (1951). The length-weight relationship & seasonal cycle in gonad weight & condition in perch (*Perca fluviatilis*). J. Anim. Ecol., 20, 201-219.

Lisovenko, L. A., & Andrianov, D. P. (1996). Reproductive biology of anchovy (*Engraulis encrasicolus ponticus* Alexandrov 1927) in the Black Sea. Scientia Marina, 60, 209-218.

McGarigal, K., Cushman, S., & Stafford, S. (2000). Multivariate Statistics for Wildlife and Ecology Research. Amherst, USA: Springer.

Me´rigot, B., Letourneur, Y., & Lecomte-Finiger, R. (2007). Characterization of local populations of the common sole Solea solea (Pisces, Soleidae) in the NW Mediterranean through otolith morphometrics and shape analysis. Mar. Biol., 151, 997-1008.

Messersmith, J. D. (1969). The northern anchovy (*Engraulis mordax*) and its fishery, 1965-1968. Scripps Institution of Oceanography Library; http://scilib.ucsd.edu/sio/.

Morato, T., Afonso, P., Lourinho, P., Barreiros, J. P., Santos, R. S., & Nash, R. D. (2001). Length-weight relationships for 21 coastal fish species of the Azores, north-eastern Atlantic. Fish. Res., 50, 297-302.

Murray, J. W., Jannasch, H. W., Honjo, S., Anderson, R. F., Reeburgh, W. S., Top, Z., Friederich G. E., Codispoti L. A., Izdar, E. (1989). Unexpected changes in the oxic/anoxic interface in the Black Sea. Nature, 338 (6214), 411 - 413.

Murray, J. W., Stewart, K., Kassakian, S., Krynytzky, M., & DiJulio, D. (2007). Oxic, Suboxic and Anoxic Conditions in the Black Sea. In V. Yanko - Hombach, A. Gilbert, N. Panin, & P. M. Dolukhanov, The Black Sea Flood Question. Seattle: Springer.

Niermann, U., Bingel, F., Gorban, A., Gordina, A. D., Gücü, A. C., Kideys, A. E., . . . Zaika, V. E. (1994). Distribution of anchovy eggs and larvae (*Engraulis encrasicolus* Cuv.) in the Black Sea in 1991 - 1992. *ICES J. Mar. Sci, 51*, 395 - 406.

Oğuz, T. (2010). Anchovy Fisheries - Ecosystem Interactions in the Black Sea (Karadeniz'de Hamsi Balıkçılığı - Ekosistem Etkileşimleri). 1st National Anchovy Workshop: Sustainable Fisheries (1. Ulusal Hamsi Çalıştayı: Sürdürülebilir Balıkçılık), 19-29 (in Turkish).

Oğuz, T., & Beşiktepe, S. (1999). Observations on the Rim Current structure, CIW formation and transport in the western Black Sea. Deep Sea Res., 1, 46, 1733 - 1753.

Oğuz, T., Ivanov, L. I., & Beşiktepe, S. (1998). Circulation and hydrographic characteristics of the Black Sea during July 1992. In T. Oguz, & L. I. Ivanov (Eds.), Ecosystem Modeling as a Management Tool for the Black Sea, NATO ASI Series 2: Environment (Vol. 47, pp. 69 - 92). Dordrecht, the Netherlands: Kluwer Academic Publisher.

Oğuz, T., Tuğrul, S., Kıdeyş, A. E., Ediger, V., & Kubilay, N. (2005). Physical and biogeochemical characteristics of the Black Sea. In A. R. Robinson, K. H. Brink, & eds., the Global Coastal Ocean - Interdisciplinary Regional Studies and Synthesis (Vols. The Sea Series, 14(33), pp. 1331-1369). Cambridge, MA: Harvard University Press.

Panella, G. (1971). Fish otoliths: daily growth layers and periodic patterns. Science, 173, 1124-1127.

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.

Pereira, J. N., Simas, A., Rosa, A., Aranha, A., Lino, S., Constantino, E., Monteiro, V., Tariche O., Menezes, G. (2011). Weight–length relationships for 27 demersal fish species caught off the Cape Verde archipelago (eastern North Atlantic). Journal of Applied Ichthyology, 28, 156-159.

Petrakis, G., & Stergiou, K. I. (1998). Weight-length relationships for 33 fish species in the waters of Cape Verde. *Fish. Res., 21*, 465-469.

Pothin, K., Gonzalez-Salas, C., Chabanet, P., & Lecomte-Finiger, R. (2006). Distinction between *Mulloidichthys flavolineatus* juveniles from Reunion Island and Mauritius Island (south-west Indian Ocean) based on otolith morphometrics. Journal of Fish Biology, 69, 38-53.

Prodanov, K., Mikhailov, K., Daskalov, G., Maxim, C., Chashchin, A., Arkhipov, A., Shlyakhov V., Ozdamar, E. (1997). Environmental management of fish resources in the Black Sea and their rational exploitation. Rome: FAO.

Pusanov, I. I. (1936). The anchovy. In *Uchanyia zapiski.* Gorkovskogo Gos. Unta (in Russian).

Reid, J. L. (1967). Oceanic Environments of the Genus Engraulis around the World. 11, 29-33.

Ricker, W. E. (1975). Computation and Interpretation of Biological Statistics of Fish Populations (191 ed.). Ottawa: Bulletin of the Fisheries Research Board of Canada.

Robson, A. C., & Jerome, D. S. (1969). Age Determination of Northern Anchovies, *Engraulis mordax*, From Otoliths. In J. D. Messersmith, The northern anchovy *(Engraulis mordax) and its fishery, 1965-1968* (Vol. 147). California Sacramento: State of California, Dept. of Fish and Game.

Rosa, A., Menezes, G., Melo, O., & Pinho, M. R. (2006). Weight–length relationships for 33 demersal fish species from Azores archipelago. Fish. Res., 80, 329-332.

Safran, P. (1992). Theoretical analysis of the weight-length relationships in the juveniles. 112, 545-551.

Sampson, D., Charef, A., & Osio, C. (2013). Report of the Scientific, Technical and Economic Committee for Fisheries (STECF) - 2013 Assessment of Black Sea Stocks (STECF 13-20). Luxembourg: Publications Office of the European Union.

Shingleton, A. W. (2010). Allometry: The Study of Biological Scaling. Nature Education, 3(10), 2 Retrieved from: http://www.nature.com/scitable/knowledge/library/allometry-the-study-of-biological-scaling-13228439

Shulman, G. E. (1964). Life Cycles of Fish. Physiology and Biochemistry. Hulsted Press, John Wiley and Sons, 253.

Shulman, G. E. (2002). Anchovies of the Sea of Azov and The Black Sea: Regularities of wintering migrations (Brief review). Marine ecological journal, 1, 67 - 77.

Simmoneau, M., Casselman, J. M., & Fortin, R. (2000). Determining the effect of negative allometry (length/height relationship) on variation in otolith shape in lake trout (*Salvelinus namaycush*), using Fourier-series analysis. Canadian Journal of Zoology, 78, 1597-1603.

Slastenenko, E. (1955/56). Karadeniz havzası balıkları. (H. Altan, Trans.) İstanbul: EBK Umum Müdl. Yay. (in Turkish).

Sparre, P., & Venema, S. C. (1998). Introduction to tropical fish stock assessment Part I. Manual. Rome: FAO Fisheries Technical Paper.

TUIK, 2003-2012. Fishery Statistics. State Institute of Statistics, Uploaded from its official web site on 05.06.2014: http://www.tuik.gov.tr/PreTablo.do?alt_id=1005

Tuset, V. M., Lozano, I. J., Gonzalez, J. A., Pertusa, J. F., & Garcia - Diaz, M. M. (2003). Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L ., 1758). J. Appl. Ichthyol, 19, 88-93.

Ünlüata, U., Oğuz, T., Latif, M. A., & Özsoy, E. (1990). On the physical oceanography of the Turkish Straits. In L. J. Pratt (Ed.), In the Physical Oceanography of the Sea Straits (Vols. NATO/ASI Series, 318, pp. 25-60). Dordrecht: Kluwer Academic Publishers.

Waldman, J. R. (2005). Definition of Stocks: An Evolving Concept. In S. X. Cadrin, K. D. Friedland, & J. R. Waldman, Stock Identification Methods: Applications in Fisheries Science (pp. 7-16). California, USA: Elsevier Academic Press.

Whitehead, P. (1984). Fishes of the North-eastern Atlantic and the Mediterranean (Vol. 1). (P. Whitehead, M. Bauchot, J. Hureau, J. Nielsen, & E. Tortonese, Eds.) Paris, France: Unesco.

Whitehead, P. J. (1985). Clupeoid fishes of the world (Suborder CLUPEOIDEI) An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, anchovies and wolf - herrings. Part 2, Engraulidae. In FAO Species Catalogue (Vol. 7, pp. Pt. 2: 305 - 579). FAO Fish. Synop.

Zaitsev, Y. P., Alexandrov, B. G., Berlinsky, N. A., & Zenetos, A. (2002). The Black Sea - an oxygen poor sea. Copenhagen: European Environment Agency (EEA). Retrieved from http://reports.eea.eu.int/report_2002_0524_154909/en.

Zaitsev, Y., & Mamaev, V. (1997). Marine Biological Diversity in the Black Sea: A Study of Change and Decline. In Black Sea Environmental Series (Vol. 3, p. 5). New York: United Nations Publications.

Zar, J. H. (1984). Biostatistical Analysis. New Jersey: Prentice Hall.

Zhang, C., Ye, Z., Panhwar, S. K., & Shen, W. (2013). Stock discrimination of the Japanese Spanish mackerel (*Scomberomorus niphonius*) based on the otolith shape analysis in the Yellow Sea and Bohai Sea. Applied Ichthyology, 29, 368-373.

TEZ FOTOKOPİSİ İZİN FORMU

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Deniz Bilimleri Enstitüsü ■

<u>YAZARIN</u>

Soyadı : Şahin
Adı : Ezgi
Bölümü : Deniz Biyolojisi ve Balıkçılık

<u>TEZİN ADI</u> (İngilizce) : A Study on Possible Variant Forms of Anchovy in the Black Sea

<u>TEZİN TÜRÜ</u> : Yüksek Lisans ■ Doktora ☐

1. Tezimin tamamı dünya çapında erişime açılsın ve kaynak gösterilmek şartıyla tezimin bir kısmı veya tamamının fotokopisi alınsın. ☐
2. Tezimin tamamı yalnızca Orta Doğu Teknik Üniversitesi kullancılarının erişimine açılsın. (Bu seçenekle tezinizin fotokopisi ya da elektronik kopyası Kütüphane aracılığı ile ODTÜ dışına dağıtılmayacaktır.) ☐
3. Tezim bir (1) yıl süreyle erişime kapalı olsun. (Bu seçenekle tezinizin fotokopisi ya da elektronik kopyası Kütüphane aracılığı ile ODTÜ dışına dağıtılmayacaktır.) ■

Yazarın İmzası: .... Tarih: ....