THE EFFECTS OF THE ENVIRONMENT ON THE BLACK SEA ANCHOVY - SIGNALS ON THE GROWTH

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ABSTRACT

THE EFFECTS OF THE ENVIRONMENT ON THE BLACK SEA ANCHOVY - SIGNALS ON THE GROWTH

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The Black Sea anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), is a fast-growing, short-lived small pelagic fish species. This fish stands out economically as it alone accounts for more than 60% of the total landings in the Black Sea. Being one of the most targeted species in commercial fisheries of the Black Sea, the sustainable exploitation of anchovy is aimed. Accordingly, it has been managed by the recommendations given as a result of age-based scientific stock assessment models in recent years. For these age-based models, age data representing the population is of great importance. The accuracy of age determinations and their consistency across countries in the region have always been a source of concern, from the earliest assessment studies to the present day. In addition, parameterizing the growth of the fish in a way that reflects the stock's overall health and resilience is also an important factor in order to deliver more reliable results for the models. Indeed, these models are already operating by assuming that these data are completely correct and reflect the stock. Therefore, estimating these age and growth, from which the biological life history of the fish is derived, is consistent and the margin of error is minimized, also makes it possible to reduce the uncertainties in the model.

In addition, the 'Unit Stock' concept is another presumption that assessment models assume to be accurate. For this reason, a correct definition of the stock to be managed brings this assumption closer to the truth. The ongoing assumption in the management of Black Sea anchovy is to accept that the Black Sea anchovy and the Azov anchovy are separate stocks and they never mix. However, considering the anchovy in the Black Sea,

the discussions regarding the separation of Black Sea and Azov anchovy stocks, which have been going on since the early 20th century, have not yet reached a clear conclusion. Nevertheless, according to the literature, it can be said that there are at least two separate stocks in the Black Sea region. However, while recent genetic studies emphasize that there is no genetic difference between these two groups, they attribute their differentiation to the effects of environmental factors. As a result, regional stock differences have come to the fore. But, particularly when anthropogenic and climate-induced changes are taken into account, regional differentiation has become more difficult with the disappearance of environmental barriers between them. On the other hand, the information that these two groups grow differently is underlined in the literature. Yet, a comprehensive study on this subject is not available.

The ultimate aim of this thesis is to find a way to obtain accurate information reflecting the stock by minimizing the uncertainties arising from the estimation of parameters such as age and growth to contribute to the sustainable management of anchovy in the Black Sea. In addition, the method of separating the different stocks mentioned in the Black Sea based on the growth information has been questioned. The otoliths, an important tool in fisheries science, are used to understand fish age, growth rate, fish response to environmental conditions, and population dynamics in general. This information obtained is of great importance for sustainable fisheries management.

In this study, sagittal otoliths from 3150 anchovy specimens collected during scientific surveys conducted in 2012, 2013, 2014, 2015, 2016, 2018, and 2020 covering the entire Turkish Exclusive Economic Zone were used to get both age and growth information. It was revealed that there is a statistically significant relationship between the otolith radius and the total fish length (R^2=0.92; p-value < .001). Opaque and hyaline rings, traces of fast and slow growing periods, respectively, were used for age determination. The radius of these increment rings from the otolith center were measured. From here, the growths for each age were calculated. A total of 3960 incremental radii data were measured for this aim.

As a result of this thesis, two separate outcomes were produced that will directly affect the model results. The first is the "Black Sea anchovy age reading protocol". This protocol is currently used throughout the Black Sea region. In this way, standard and comparable age-length keys can be produced. Another is the more accurate growth parameters that reflect the stock. So far, growth parameters for anchovy in the Black Sea have been obtained using the mean size at age method. These were generally estimated using samples collected at different times of the year. This method causes a shift in the age-corresponding length information depending on the time of the year that the sample is collected, thus causing errors in the estimations. In addition, t0 values that are too small to be true are estimated while constructing the growth curve. This reduces the reliability of the growth prediction. In order to avoid all these sources of error, in this study the growth parameters were estimated with the von Bertalanffy approach by using the growth increment radii-at-age as a proxy for the exact total length of the fish-at-age. Utilizing this method, L_{inf}=12.9 cm, K=0.765, and t_0= -0.134 were estimated for anchovies in the southern Black Sea region. This proposed method makes it possible to obtain growth information from otoliths that have already been collected to get age information, as well as to predict retrospective growth parameters.

In the last decades, very significant ecological changes have occurred in the Black Sea, and these changes have had a significant impact on many living beings. Anchovy is known to be severely affected by these changes. The existence of anchovy subgroups in the Black Sea region, which makes the definition of "unit stock" difficult, is also discussed as a part of these changes. In this study, the studies that have been done to date, which are still available, are compiled and presented in Chapter 2. According to the conclusion drawn from this review, anchovy groups in the Black Sea were defined as environmental ecotypes. In this thesis, the existence of different groups was investigated by their growth rates in the first year of their life, which corresponds to ~72% of their total asymptotic growth, with the knowledge that different forms grow differently and with the reference that growth is a response to the environment of the fish. The first-year growth information was obtained by measuring the distance from the center of the otolith to the edge of the increment corresponding to the first age. Accordingly, two different anchovy groups labeled as “Group 1” and “Group 2” that grow differently (ϕ_1=2.05, ϕ_2=2.29) have been detected in the southern Black Sea region (likelihood ratio test; chi-square statistics: p-value < .001, df=3). So, estimated growth parameters according to the method proposed in Chapter 3 for Group 1 and Group 2, respectively: $L_{inf\ (cm)}$ = 12.4 and 12.1; $K_{(1/year)}$ = 0.726 and 1.347; $t_{0\ (year)}$=-0.145 and -0.079 were found.

Finally, the hypothesis was tested that if disparate growth patterns among groups arise due to variations in the seawater properties of their respective spawning environments, then, later, they will instinctively prefer water bodies similar to those water. This propensity persists even if the intermixing occurs after experiencing a winter. Moreover, it was also tested that these different water masses will eventually influence the growth of the young-of-year. For this purpose, the temperature, salinity, and fluorescence values were measured at a total of 363 different stations in the years 2013, 2015 and 2018 as part of the summer season (July-August). Subsequently, the seawaters of 75 different stations where anchovy samples were caught were characterized. Accordingly, separate water masses with different characteristics and covering different regions were identified for each year. The otoliths collected from the summer season were grouped, and two different groups were identified that grew differently according to their growth in the first years of their lives. In addition, the otolith radii of the young-of-year, who were only one and a half months old at the time of sampling, corresponding to the estimated birth date of June 1, were used as growth markers. According to the results of the study, contrary to expectations, it was found that adult individuals who experienced a winter were intermixed in the basin and the detected different groups did not prefer a particular water mass ($ANOVA_{2013}$; F (1, 75) = [2.0812], p-value = 0.1533; $ANOVA_{2015}$; F (1, 112) = [3.374], p-value = 0.06888.; $ANOVA_{2018}$; F (2, 84) = [0.5873], p-value = 0.5581). However, the results show that even though the water mass characteristics they are in do not matter for adults, the situation is different for the young-of-years. A statistically significant relationship was found between the growth of the offspring of the sampled year and the water masses in which they were caught (ANOVA; F (2, 515) = [3.863], p-value = 0.022). In addition, when examining the average growth of these young-of-years, in 2015, which is characterized by the high amount of fluorescence, the highest mean growth was noted (M=0.818 mm, SD=0.184). Yet, the lowest average young growth was recorded in 2018, which was the hottest among sampling years (M = 0.767 mm, SD = 0.159).

As a result, it will be possible to track the cohorts within the model with the age-length key for each sampling year generated by the age reading protocol prepared within the scope of this thesis study, which was prepared with a stock assessment perspective. Thus, a more accurate model result reflecting the stock will be achieved. Growth estimates obtained using the otolith method can be used to estimate fishery-dependent and independent parameters with minimal uncertainty. This study identified different growing anchovy groups from otolith samples collected as part of fishery-independent scientific research surveys. The different growing group compositions in the catch can also be determined using the same method, that is, with the first-year growth information obtained from otoliths collected for age reading. In this way, the ratios of different stocks in the catch can be found and the stocks may be managed accordingly. As a result of this study, it is recommended to reconsider the unit stock assumptions made and the size-selective fishery regulation for the Black Sea anchovy. It is further recommended to take into consideration that juvenile anchovy populations, particularly those in their early stages of development, exhibit strong sensitivity to environmental factors. This consideration bears significance in the establishment of stock management plans. Furthermore, it is anticipated that these results will provide a representative framework for future studies examining the effects of climate change on anchovy growth.

Keywords: Black Sea anchovy, Stock Assessment, Otolith, Age, Growth, Environment

ÖZ

ÇEVRENİN KARADENİZ HAMSİSİ ÜZERİNE ETKİLERİ - BÜYÜME SİNYALLERİ ÜZERİNE

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Karadeniz hamsisi, *Engraulis encrasicolus* (Linnaeus, 1758), hızlı büyüyen, kısa ömürlü küçük pelajik bir balık türü olarak ekonomik açıdan öne çıkmakta ve tek başına Karadeniz'den yakalanan toplam balığın %60'ından fazlasını oluşturmaktadır. Karadeniz'deki ticari balıkçılıkta en çok hedeflenen türlerden olan hamsinin sürdürülebilir kullanımı amaçlanmakta ve bu nedenle son yıllarda yaşa dayalı bilimsel stok değerlendirme modelleri sonucu verilen tavsiyeler doğrultusunda yönetilmektedir. Yaşa dayalı bu modeller için popülasyonu temsil eden yaş verisi büyük önem taşımaktadır. Yapılan ilk değerlendirme çalışmalarından günümüze kadar yaş tayinlerinin doğruluğu ve bölgedeki ülkeler arasındaki tutarlılığı her zaman endişe kaynağı olmuştur. Bunun dışında balığın zaman içinde büyümesinin stoku yansıtır bir şekilde parametrize edilmesi de hem stokun genel durumu ve dayanıklılığı hem de modellerin daha güvenilir sonuçlar vermesi açısından önemli bir etken olagelmektedir. Balığın biyolojik yaşam öyküsünün çıkarımsandığı bu verilerin tutarlı ve hata payı en aza indirilmiş şekilde tahmin edilmesi modeldeki belirsizliklerin azaltılmasını da olanaklı kılmaktadır. Çünkü model zaten bu verilerin tamamen doğru olduğunu varsayarak işlem yapmaktadır.

Bunların yanı sıra, 'Birim Stok' bilgisi değerlendirme modellerinin yüzde yüz doğru olduğu varsayılan diğer bir ön kabulüdür. Bu nedenle yönetilecek stokun iyi tanımlanması bu ön kabulü de doğruya yaklaştırmaktadır. Karadeniz hamsisi stok yönetiminde süregelen ön kabul, Karadeniz hamsisi ile Azak hamsisinin ayrı stoklar

olduğu ve asla karışmadıklarıdır. Ancak Karadeniz'deki hamsi göz önüne alındığında 20yy'nin başından bari süregelen Karadeniz ve Azak hamsisi stok ayrımı henüz net bir sonuca ulaşamamıştır. Yine de literatüre göre Karadeniz'de varlığı vurgulanan en az iki ayrı stokun bulunduğu söylenebilir. Fakat son genetik çalışmalar bu iki grup arasında genetik bir fark olmadığını vurgularken, farklılaşmalarını çevresel faktörlerin etkilerine bağlamaktadırlar. Bu da bölgesel stok ayrımlarını ön plana çıkarmıştır. Ancak, özellikle insan ve iklim kaynaklı değişimler göz önüne alındığında, bahsi geçen çevresel bariyerlerin ortadan kalkmasıyla bölgesel ayrımları iyice zorlaşmıştır. Bunun yanı sıra, bilinen iki hamsi grubunun farklı büyüme özelliklerine sahip olduğu literatürde kabul görmektedir. Ancak bu konuda yapılan kapsamlı bir çalışmaya ulaşılamamaktadır.

Bu tez çalışmasının nihai amacı Karadeniz'deki hamsinin sürdürülebilir yönetimine katkı sağlamak için yaş ve büyüme gibi parametrelerden kaynaklı belirsizliklerin en aza indirilerek stoku yansıtan verilere ulaşmaktır. Dahası Karadeniz'deki bahsi geçen farklı stokların büyüme bilgisi kullanılarak ayrımı yöntemi sorgulanmıştır. Bunun için balıkların iç kulaklarında bulunan, işitme ve denge organı olan küçük kalsiyum karbonat kristaller, otolitler, kullanılmıştır. Balıkçılık biliminde önemli bir araç olan otolit, balık yaşını, büyüme hızını, balığın çevresel koşullara verdiği tepkiyi ve en genel olarak popülasyon dinamiklerini anlamak için kullanılmaktadır. Elde edilen bu bilgiler, sürdürülebilir balıkçılık yönetimi için büyük önem arz etmektedir. Bu çalışmada hem yaş hem de büyüme bilgisi sağlamak amacıyla tüm Türkiye Münhasır Ekonomik Bölge'sini kapsayan 2012, 2013, 2014, 2015, 2016, 2018 ve 2020 yıllarında yapılan bilimsel deniz seferlerinde toplanan 3150 hamsi örneğinin sagittal otolitleri kullanılmıştır. Otolit yarıçapı ile toplam balık boyu arasında istatistiksel olarak anlamlı bir ilişki olduğu ortaya koyulmuştur (R^2=0.92; p-değeri<.001). Sırasıyla, hızlı ve yavaş büyüme dönemlerinin izleri olan opak ve hiyalin halkalar yaş tayininde kullanılırken, bu halkaların otolit merkezinden uzaklıkları, yarıçapları, ölçülerek yaşa karşılık büyüme miktarları tespit edilmiştir. Bunun için toplam 3960 birikim yarıçap verisi ölçülmüştür.

Bu çalışma sonucunda model sonuçlarına doğrudan etki edecek iki ayrı çıktı üretilmiştir. Bunlardan ilki "Karadeniz hamsisi yaş okuma protokolü" dür. Bu protokol şu an tüm Karadeniz'de kullanılmaktadır ve bu sayede, standart ve karşılaştırıla bilinir yaş boy anahtarları üretilebilmektedir. Bir diğeri ise, stoku yansıtan daha doğru büyüme parametreleridir. Şimdiye kadar Karadeniz'de hamsi için yaşa karşılık ortalama balık boyu yöntemi ile elde edilen büyüme parametreleri genellikle yılın farklı zamanlarında toplanan örnekler kullanılarak tahmin edilmiştir. Bu yöntem, örneğin yılın hangi zamanında toplandığına bağlı olarak yaşa karşılık gelen boy bilgisinde kaymaya, dolayısıyla yapılan tahminlerde hataya neden olmaktadır. Ayrıca, stoka katılım boyundan daha küçük balıklar yakalanamadığı için büyüme eğrisi oluşturulurken gerçek olamayacak kadar küçük değerde t0 değerleri tahmin edilmekte, bu da büyüme tahminin güvenilirliğini azaltmaktadır. Tüm bu hata kaynaklarının önüne geçmek için bu çalışmada otolit üzerindeki tam yaşa karşılık gelen boy bilgisi ve sıfır yaşındaki boyu temsilen otolit merkez halkası birikim genişliği kullanılarak von Bertalanffy yöntemi ile büyüme parametreleri tahmini yapılmıştır. Bu yöntem ile güney Karadeniz'deki hamsi için L_{inf}=12.9 cm, K=0.765, ve t_0=-0.134 olarak bulunmuştur. Önerilen bu yöntem, hem zaten yaş bilgisi elde etmek için toplanan otolitlerden büyüme bilgisinin de elde edilebilmesini hem geçmişe dönük büyüme parametreleri tahmini mümkün kılmaktadır.

Geçtiğimiz son on yıllarda, Karadeniz çok önemli ekolojik değişimlere sahne olmuş ve bu değişimler pek çok canlı üzerinde önemli etkiler yaratmıştır. Hamsi de bu değişimlerden oldukça fazla etkilendiği bilinen bir türdür. Özellikle "birim stok" tanımını zorlaştıran Karadeniz'deki hamsi alt gruplarının varlığı da bu değişimlerin bir parçası olarak tartışılmaktadır. Bu çalışmada da birim stok ön kabulü tartışması için bugüne kadar yapılan, hala ulaşılabilir olan, çalışmalar derlenmiş ve Bölüm 2'de sunulmuştur. Bu derlemeden çıkarılan sonuca göre, Karadeniz'deki hamsi grupları çevresel ekotipler olarak tanımlanmıştır. Bu tez çalışmasında da farklı formların farklı büyüdüğü bilgisi ve büyümenin balığın çevresine verdiği bir tepki olduğu referansı ile hayatlarının ilk yıllarındaki büyüme oranlarına göre, ki bu toplam asimptotik büyümelerinin ~%72'sine karşılık gelmektedir, farklı grupların varlığı araştırılmıştır. İlk yıl büyüme bilgisi otolit üzerinde ilk yaşa karşılık gelen birikimin merkezden uzaklığı ölçülerek elde edilmiştir. Buna göre güney Karadeniz'de farklı büyüyen ($\phi 1$=2.05, $\phi 2$=2.29) iki ayrı hamsi grubu tespit edilmiş ve "Grup1" ve "Grup2" olarak isimlendirilmiştir (olabilirlik oranı testi; ki-kare-istatistik: p-değeri < .001, df=3). Buna göre, Bölüm3'te sunulan yönteme göre tahmin edilen büyüme parametreleri Grup 1 ve Grup 2 için sırasıyla $L_{inf\ (cm)}$ = 12.4 ve 12.1; $K_{(1/yıl)}$ =0.726 ve 1.347; $t_{0\ (yıl)}$= -0.145 ve-0.079 olarak bulunmuştur.

Son olarak, eğer balığın içine doğduğu ve geliştiği su farklı büyüyen grupları ortaya çıkaran faktör ise, hayatlarının ilerleyen dönemlerinde, bir kış deneyimledikten sonra da içgüdüsel olarak bu sulara benzer su kütlelerini tercih edecekleri hipotezi test edilmiştir. Bunun yanı sıra, bu çalışma kapsamında test edilen bir diğer hipotez ise tespit edilen farklı su kütlelerinin yılın yavru hamsilerinin büyümelerini de etkileyeceği idi. Bunun için yaz mevsimi (Temmuz-Ağustos) örnekleminin yapıldığı 2013, 2015 ve 2018 yıllarında toplam 363 ayrı istasyonda ölçülmüş sıcaklık, tuzluluk ve floresan değerleri ile hamsi örneklerinin yakalandığı 75 ayrı istasyonun çevresel su kütleleri karakterize edilmiştir. Buna göre her yıl için farklı karakterde ve faklı bölgeleri kapsayan ayrı su kütleleri tespit edilmiştir. Yaz mevsiminde toplanan otolitler, Bölüm 4'teki yöntemle gruplara ayrılmış ve hayatlarının ilk yıllarındaki büyümelerine göre farklı büyüyen iki ayrı grup tespit edilmiştir. Bunun yanında, örneklendiği anda tahmini doğum tarihi olan 1 Haziran'a göre henüz bir buçuk aylık civarında olan o yılın yavrularının otolit yarıçapları büyüme işareti olarak kullanılmıştır. Araştırma sonuçlarına göre, beklenenin aksine bir kış deneyimledikten sonra farklı büyüme özelliği gösteren ergin bireylerin havzada birbirine karıştığı ve tespit edilen farklı grupların belirli bir su kütlesini tercih etmedikleri saptanmıştır (ANOVA; p-değeri>0.05). Ancak sonuçlar göstermektedir ki her ne kadar ergin birey için içinde bulunduğu su kütlesinin bir önemi olmasa da yavrular için durum farklıdır. Örneklenen yılın yavrularının büyümesi ile içinde bulundukları su kütleleri arasında istatistiksel olarak anlamlı bir ilişki bulunmuştur (ANOVA; F (2, 515) = [3.863], p-değeri = 0.022). Ayrıca bu yavrular için yıl bazında ortalama büyümeye bakıldığında besin miktarındaki farkla öne çıkan 2015 yılında en yüksek ortalama yavru büyümesi tespit edilirken (M=0.818mm, SD= 0.184), en sıcak yıl olan 2018 yılında en düşük ortalama yavru büyümesi (M= 0.767mm, SD= 0.159) ölçülmüştür.

Sonuç olarak, stok yönetim perspektifi ile yapılan bu tez çalışmasında üretilen yaş okuma protokolü ile elde edilen/edilecek ve her yıl için oluşturulan yaş boy anahtarları ile model içi tertip takipleri mümkün olacak ve stoku yansıtan daha doğru bir model sonucu üretilebilecek. Otolit yöntemi ile elde edilen, belirsizliğin minimize edildiği büyüme tahminleri balıkçılık bağımlı ve bağımsız parametre tahmininde kullanılabilecek. Bu çalışmada balıkçılıktan bağımsız bilimsel araştırma otolit örnekleri ile farklı büyüyen hamsi grupları tespit edilmiştir. Av içindeki gruplar da yine aynı yöntemle yani zaten yaş okumak için toplanan otolitlerden elde edilen ilk yıl büyüme bilgileri ile tespit edilebilir. Böylelikle av içindeki farklı stokların oranları bulunabilecek ve buna göre stok yönetimi yapılabilecektir. Ayrıca, bu çalışma sonucunda Karadeniz hamsisi için yapılan birim stok varsayımlarının yeniden gözden geçirilmesi önerilmektedir. Ayrıca, stok yönetim planlarının oluşturulması sırasında, yavru hamsi popülasyonlarının, özellikle gelişiminin ilk aşamalarında olanların, kısa vadeli çevresel faktörlere karşı güçlü bir hassasiyet sergilediklerinin dikkate alınması tavsiye edilmektedir. Ayrıca, bu sonuçların daha sonra yapılacak olan iklim değişikliğinin hamsi büyümesi üzerine etkilerini inceleyen çalışmalar için, uzun vadede, temsili bir çerçeve çizdiği öngörülmektedir.

Anahtar Kelimeler: Karadeniz Hamsisi, Stok Yönetimi, Otolit, Yaş, Büyüme, Çevre

To my Eros

&

To the ones who make me smile 😊

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GENERAL INTRODUCTION

The Black Sea

The Black Sea lies in Eurasia between latitude 40^{o}55'N - 46^{o}37'N and longitude 27^{o}27'E - 41^{o}47'E, surrounded by Europe, Caucasus, and Anatolia (Figure 1). Six countries Bulgaria, Georgia, Romania, Russia, Türkiye, and Ukraine have coastlines on this sea. In the south, it is connected to the Sea of Marmara by the Bosphorus Strait and the Mediterranean Sea by the Dardanelles Strait. In the north, on the other hand, it is connected to the Sea of Azov by the Kerch Strait. It is one of the largest semi-enclosed basins with its 436,402 km^2 surface area, one-fifth that of the Mediterranean surface area, and 4869 km of coastline (Zaitsev & Mamaev, 1997). Its maximum depth is ~2212m and a volume of 547,000 km^3.

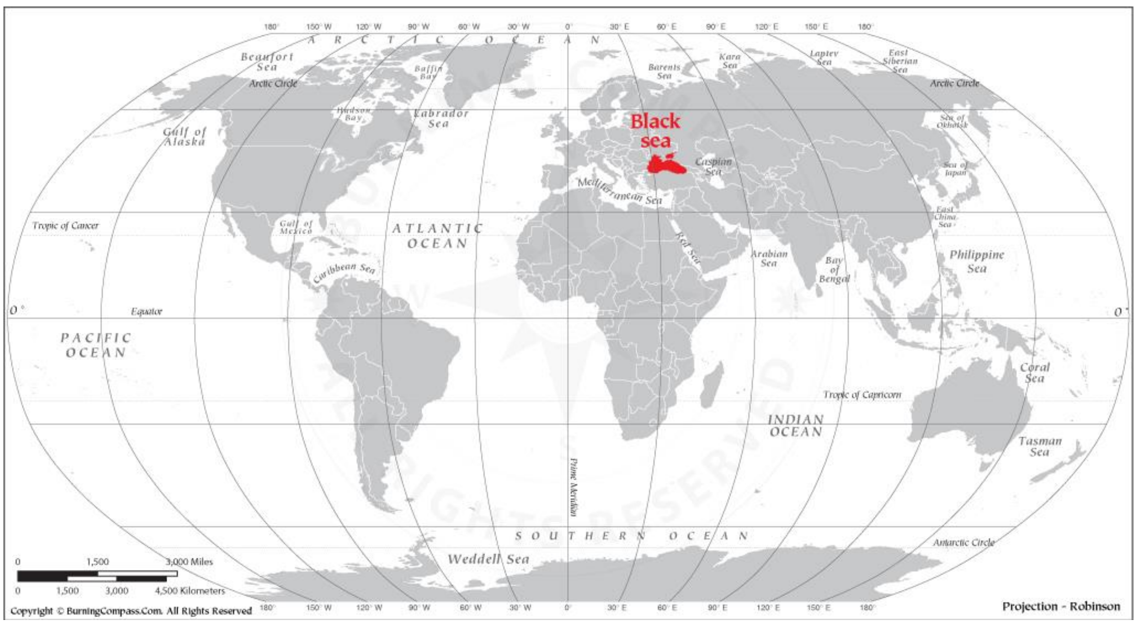

Figure 1: Location of the Black Sea on the Earth (The figure was retrieved on May 18, 2023, from https://www.burningcompass.com/on-world-map/black-sea-on-world-map.html)

The Black Sea is a unique marine environment with its permanent anoxic deep-water mass, occupying almost 87% of the basin (Oğuz et al., 1998). The marine life inhabits ventilated upper ~100 m depth. Contrary to the anoxic deep layer, which is mainly separated from the surface layer by the vertical pycnocline, the water masses at the surface are very dynamic with the presence of turbulent eddies in its two-gyre system surrounded by a seasonally modified cyclonic Rim Current (Oğuz et al., 1993). Intense

Rim Current forms after the permanent thermocline breaks up at the beginning of winter. (Murray et al., 2007; Oğuz et al., 1993). Together with dynamic eddies formations, upwellings, and downwellings, all these current systems play significant roles in mainly upper water circulation and mixing.

The Black Sea has a very large catchment area with a positive water budget (Figure 2). Due to the higher river input, the surface salinity is significantly lower (~18 ppt; 100–150 m) than in deep water (~22 ppt; 1500–2000 m) (Slastenenko, 1955). It is fed by the major rivers of Danube (50% of all annual water), Dnieper (16%), Dniester (2%), Don (10%), Kelkit (< 2%), Kizilirmak (< 2%), Kuban (4.6%). Being Europe's widest and most complex, its catchment area is 1,874,904 km^2 in total (Fasel et al., 2016; Vespremeanu et al., 2018). The largest freshwater input is in the northwestern shelf, occupying ~25% of the total area (Salihoğlu, 2000). This shelf region is also known as the most productive area in the sea (Zaitsev, 2006; Oğuz and Velikova, 2010). Therefore, it is a very important nursery area for many marine fish inhabitants in the Black Sea (Bănăduc et al., 2016).

Figure 2: The catchment area of the Black Sea (Fasel et al., 2016)

All the oxygen-driven biological life harmonized with these physical and chemical patterns in the upper layer of the Black Sea. In this way, the internal resilience of the ecosystem is established. However, some natural, climatic, and anthropogenic factors may weaken this resilience. Some sudden and large-scale transitions may have appeared in the structure and functioning of an ecosystem as a response to these interacting external disturbances. It is called “regime shift- reorganization” (Oğuz and Gilbert, 2007 and the references therein). The Black Sea experienced this fundamental alteration in the structure and functioning of its ecosystem in the second half of the 20th century due to anthropogenic stressors (Oğuz and Gilbert, 2007; Murray et al., 1989).

Before the 1960s, the internal ecosystem was in equilibrium with relatively low phytoplankton and small pelagic stocks and high zooplankton and large pelagic stocks. It is accepted that this balance reflects the pristine ecosystem (Yunev et al., 2017; Zaitsev, 1992). The gelatinous species’ low biomass was another characteristic of the pre-eutrophic period (Yunev et al., 2017). Regarding commercial fishing, the early 1960s can be defined as the period when fishing pressure was hardly felt. Accordingly, small pelagic species accounted for 68% of the catch in those years, while large pelagic species accounted for 20% (Gücü, 2002 and the references therein). However, in the late 1960s, predator control decreased on small pelagic fish (Daskalov, 2002 and the references therein).

The first signs of regime shifts became observable in the late 1960s, mainly associated with nutrient overloading (high nitrogen and phosphorus) from the rivers due to the higher rate of industry and agriculture along the catchment area (Alexandrov and Zaitsev, 1998; Strokal and Kroeze, 2013; Yunev et al., 2017). With this effect, the oligotrophic characteristic of the Black Sea started to reflect more eutrophic properties.

The 1970s-1980s was known as the eutrophic period for the Black Sea. Only the measured limiting nutrients amount of phosphorus and nitrogen in the mouth of the Danube increased 21 and 8.5-fold, respectively (Kideys, 2002). The nutrient pollution was increasing so uncontrollably that in 1975 according to Zaitsev and Mamaev (1997), the average phytoplankton biomass had increased 1.2 times just in the region between the Dniester and the Danube. In the North Western Shelf, the biomass of the phytoplankton had increased 30 times (Zaitsev and Alexandrov, 1997). Already by 1973, the first mass mortalities of the Black Sea benthos and bottom-living fish in the North-Western Shelf were recorded due to increased eutrophication-driven hypoxia and anoxia (Zaitsev and Mamaev, 1997). This place is also the main spawning and nursery area for the fish species. The loss of suitable habitats was one of the reasons that limit fish recruitment. Moreover, oxygen deficiency had another direct impact on fish survival (Kideys, 2002 and the references therein).

In summary, eutrophication over this period changed the amount and composition of phytoplankton within the system (Zaitsev, 1992; Yunev et al., 2017). Within the same period, the predator pressure on small pelagics decreased by the perishment of large pelagics with the increasing fishing pressure. It was a sign of the trophic cascade (Gücü, 2002; Daskalov, 2002; 2007; Akoğlu, 2013). Small pelagic biomass also increased in direct proportion to the increased carrying capacity of the eutrophic system and decreased predator pressure (Llope et al., 2011). Meanwhile, fishing pressure, now targeting small pelagic species, continued to increase. As this increase was reflected in catches, small pelagic fish accounted for more than 90% of all fisheries in the 1980s (Zaitsev, 1992; GFCM, 1993, as cited by Gücü, 2002). So, together these top-down and bottom-up effects combined with the climate significantly altered the stability and resilience of the Black Sea ecosystem (Gücü, 2002; Llope et al., 2011; Oğuz, 2017; Möllmann and Diekmann, 2012)

On the other hand, the composition and abundance change of phytoplankton in the 1980s caused the system also work in favor of the jelly food chain. The total biomass of jelly bloomed considerably during these times (Mutlu et al., 1994). Moreover, in November 1982, the intrusion of a new nonindigenous gelatinous comb jelly, ctenophore *Mnemiopsis leidyi*, into the Black Sea was first reported (Pereladov, 1988 as cited in Shiganova et al., 2001). So much so that by 1989, the biomass of this intruder reached its highest wet weight of 4.6 kg per m^2 (Vinogradov et al., 1989, as cited in Shiganova et al., 2001). This creature competed for food with species like the anchovy, the most economically important small pelagic species, which lives near the surface. Also, by consuming the eggs of these fish for food, they have damaged the fish population (Travis, 1993; Kideys, 1994; Shiganova and Bulgakova, 2000). These ctenophores did not only benefit from the high nutritional advantage. One of the most important factors in the increase of gelatinous comb jelly biomass was the occupation of the niches emptied by the small pelagic stocks, which decreased with the high fishing pressure. Thus, the view has emerged that this situation triggered the collapse of small pelagic species (Gücü, 1997; Gücü, 2002; Daskalov, 2002; Llope et al., 2011; Eremeev and Zuyev, 2007). For instance, the 1989/90 fishing season saw the anchovy catch fall almost seven-fold due to these fishing pressure and competition (STECF, 2015). For Türkiye, which accounts for the largest share of anchovy catches, the cost of this decline in anchovy catches from the second half of the 1980s to 1989 was estimated at US$309 million. Furthermore, the total cost to the Black Sea is estimated at $1 billion per year. (Campbell,1993; Caddy,1992, as cited in Knowler, 2005).

As a result of the reorganization of the ecosystem, the estimated oxygen content of the upper layer of the Black Sea has already decreased by 44% over the past 65 years (Capet et al., 2020). Furthermore, the Black Sea is now nominated as one of the regions most likely to be affected by global warming (Belkin, 2009; Todorova et al., 2019). It is therefore very likely that there will be novel climate-related changes. Therefore, protecting marine food sources has increasingly become a priority (FAO, 2021; UNESCO-IOC – Sustainable Development Goal 14, 2021). Hereby, it is crucial to learn from the past in order to understand the current situation and predict the future.

Arguably, the most important lessons learned from this long and difficult ecosystem restructuring period that the Black Sea passes through is the importance of understanding how organisms respond to environmental change and implementing scientifically advised sustainable management plans. We can look for the reason for the collapse of Black Sea fish stocks in the absence of a stock assessment made with scientific methods and in uncontrolled fishing without considering environmental factors.

The Anchovy in the Black Sea

The European anchovy, *Engraulis encrasicolus* (Linnaeus, 1758) (Figure 3) has a wide distribution in the world from Norway to Southern Africa, and throughout the Mediterranean, Black and Sea of Azov (Whitehead et al., 1988). The taxonomic classification of the European anchovy inhabiting the Black Sea has long been debated. Yet, at the beginning of the 20th century, Alexandrov (1927) and Pousanov (1923; 1936) had classified two coexisting subspecies of *E. encrasicolus ponticus* as the Black Sea anchovy and *E. encrasicolus maeticus* as the Azov anchovy by their reproductive area and the temporal and spatial overwintering migration (For more information, see Chapter 2).

Figure 3: European Anchovy (*Engraulis encrasicolus*)
(https://www.wikiwand.com/en/Anchovy)

European anchovy migration is mainly associated with spawning and feeding behavior (Silva, 2014). However, the anchovy in the Black Sea migration is triggered and driven by the temperature decreases in winter (Chashchin, 1996; Chashchin et al., 2015). Anchovy, by following the routes in Figure 4, overwinters in the southeastern Black Sea and the Southern Crimean regions. In their juvenile and adult stages, they move actively, yet during eggs and larval stages, they are dispersed passively by currents (Silva, 2014).

Anchovy is a fast-growing, small, pelagic, foraging fish. It has a short generation time with large reproductive potential (Lisovenko and Andrianov, 1996). In the Black Sea maximum recorded age is five (ICES, 2010, as cited in Frose and Pauly, 2023), even though it is very rare nowadays. It becomes fully mature at age 1, but some portion of age-zero anchovy spawns two-three months after they hatch (Lisovenko and Andrianov, 1996). The species, in general, is highly dependent on environmental conditions and exhibits plastic reproductive tactics (Millán, 1999). In the Black Sea, it spawns in batches. The annual fecundity can reach 200000 eggs laid in around 50 batches within a spawning season (Lisovenko and Andrianov, 1996). They spawn in the warm layer above the thermocline (0-25 m) from mid-May (water temperature 15-16 °C) to mid or late August (25-26 °C) (Lisovenko and Andrianov, 1996).

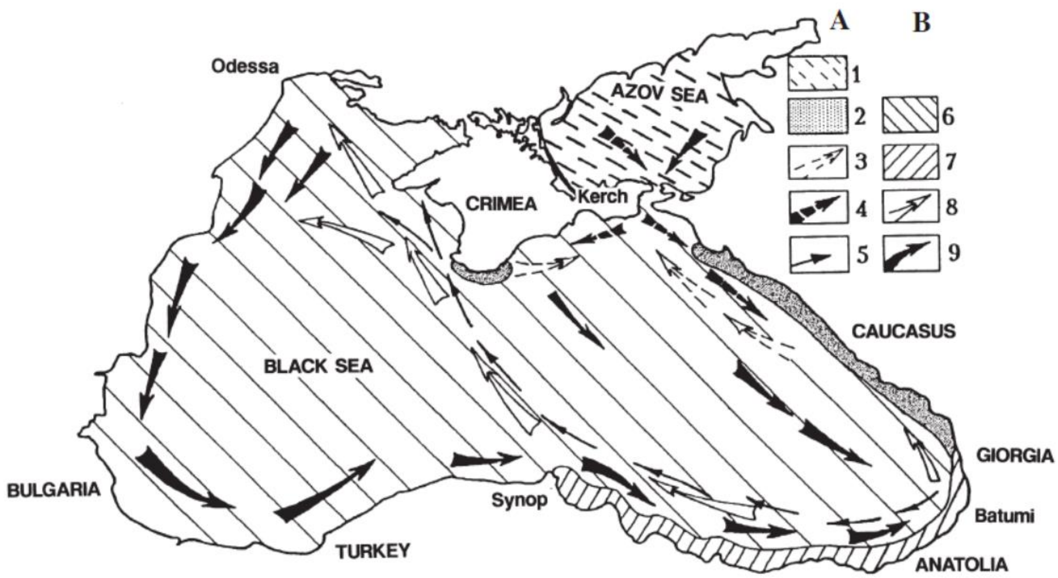

Figure 4: Anchovy overwintering migration routes demonstration. the Azov anchovy: 1_spawning and foraging region; 2_wintering region; 3_spring migrations; 4_autumnal migrations; 5_periodical migrations of a mixed population. The Black Sea anchovy: 6_spawning and foraging region; 7_wintering region; 8_spring migrations; 9_autumnal migrations (Chashchin,1996).

According to the known information on anchovy development in the Black Sea, provided by Lisovenko and Andrianov (1996), the embryonic period lasts about 24 hours (at a temperature of 22.0–23.4 °C). On the other hand, according to the studies performed for the European anchovy, during the first two days of hatching, larvae feed on yolk sacs (highest growth due to endogenous nutrition), thereby preventing starvation (Durovic et al., 2012). They feed opportunistically mainly by zooplankton and phytoplankton. Depending on food availability, small larvae can eat more than their body weight per day (Blaxer and Hunter, 1982), and according to a study conducted in the western Mediterranean Sea, development from the egg phase to the juvenile phase can take around 60 days (Palomera et al., 1988). Hence, depending on the environmental conditions, the maximum growth rate is achieved in their first year of life (Freon et al., 2005; Bacha et al., 2010). Growth efficiency is inversely proportional to age. So, further condition and survival of fish mainly depend on this first-year growth, the "carry-over effect" (Garrido et al., 2016; Moore and Martin, 2019). Adults, on the other hand, show intense feeding behavior to store energy in early winter for winter migration (Shulman et al., 2007; Shulman 2002) and late winter/early spring for spawning (gonad maturation), respectively (Silva, 2014). Furthermore, temperature has an important impact on the anchovy's feeding behavior. Feeding rate increase with the increasing temperature. For instance, laboratory experiments of Sirotenko and Danilevskiy (1977) showed that when the temperature increased from 15.4°C to 29°C, the daily food requirement of Black Sea anchovy for satiation increased from 7% of the wet weight/day to 15.6%. From this, it can be concluded that environmental conditions could eventually affect the growth of anchovies. However, in the Black Sea, no study has yet been conducted on the direct effects of environmental conditions on the growth of individual Black Sea anchovies.

Regarding the anchovy fishery, in retrospect, we can enumerate the leading and triggering causes of this ecosystem change: eutrophication (Zaitsev and Mamaev, 1997), invasion of Ctenophore, *Mnemiopsis leidyi* (Kıdeyş, 2002; Oğuz and Gilbert, 2007; Oğuz et al., 2008), overfishing of pelagic fish stocks (Gücü, 1997; 2002; Daskalov, 2002; Llope et al., 2011), and climate change (Oğuz et al, 2003). With the advent of fishing technology and the depletion of the large pelagic fish total catch of anchovy increased dramatically in the early 1980s. The total catch, which reached over 500000 tons during these times, experienced a great collapse in the late 1980s and early 1990s (Gücü, 1997; Oğuz, 2007; Oğuz et al., 2012). After all these effects and losses, the period starting in the first half of the 1990s and covering the 2000s is called the recovery period (Oğuz, 2017). Although the system appears to have survived the crisis, at least for small pelagics like anchovy, it can be said that the effects of eutrophication and the trophic cascade have created new ecological conditions of today. The system no longer produces as much profitable catch as it did in the mid-1980s. However, since then there has been an increase in average yields, with large fluctuations, compared to the time of the collapse (Figure 5; GFCM, 2018). However, the latest accepted stock assessment results showed that the stock is still subject to overexploitation (GFCM-SAF, 2018).

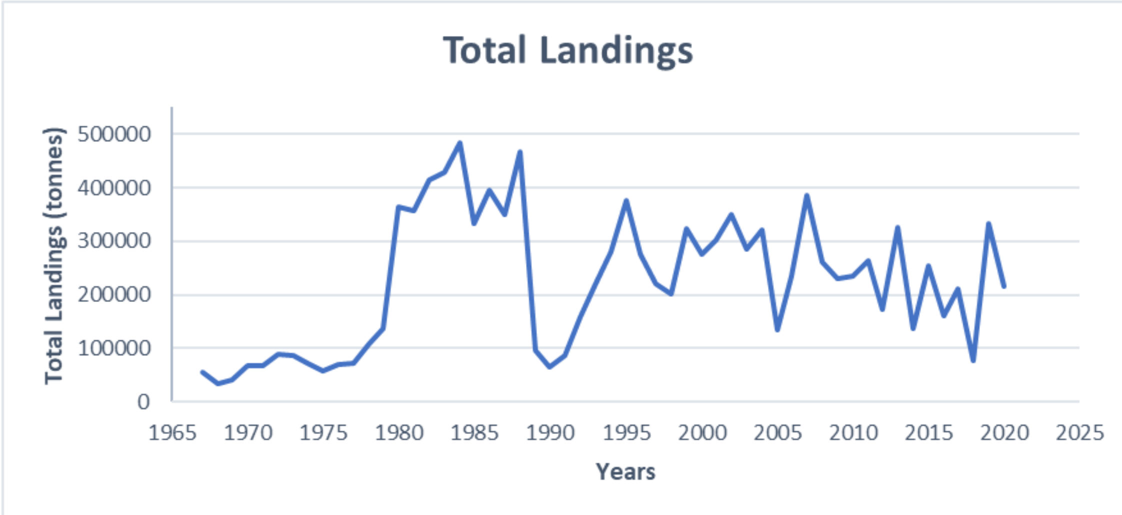

Figure 5: Total anchovy landings in the Black Sea (GFCM-SAF, 2020)

What is Fish Otolith and How Is It Used in Fishery Sciences?

Oto- + -lith, from Ancient Greek ὠτο- (ōto-), stem of οὖς (oûs, "ear"), and λίθος (líthos, "stone"). They are ear stones. Technically, the otoliths, are calcified structures in the inner ear of teleost fish.

The hearing structures of all vertebrates share some common features, such as three semicircular canals, and three otolithic end organs. Each otolithic end organ has calcium carbonate crystals in the form of otoconial masses (Popper et al. 2005). However, in teleost fishes, each otolithic end organ has its own solidified single mass on each side called otolith pairs named sagittae, lapilli, and asteriscii (Popper et al. 2005; Campana and Jones, 1992). The largest is the sagitta which is located in the sacculus. The second otolith, asteriscus, can be found in the caudal part of the sacculus, while the third otolith pair, called lapillus, is located in the utriculus (Figure 6). The otolithic membrane surrounds the otolith and mediates the connection between the sensory epithelia and otoliths (D'Iglio et al., 2021).

Otoliths, ear stones-three times denser than the fish body (Popper et al., 2005), are calcified structures composed of calcium carbonate ($CaCO_3$; 97-99%), other minor amounts of trace elements (Na, Sr, K, S, N, Cl and P) and organic matter (otoline) in the inner ear of the teleost fish that function as hearing, sense of gravity, and directionality

(Campana and Thorrold, 2001; Popper et al., 2005; Rodríguez Mendoza, 2006; Melancon et al., 2008). Sagitta (Figure 6), the larger one, has been used most frequently in age reading and growth studies since the late 1800s (Isely and Grabowski, 2007; Campana, 2005). There is a wide range of diversity in the shape and size of the otoliths over the fish species and it is thought that it is due to the specialization in the hearing and fish growth (Popper et al., 2005).

Otoliths are stones, not bones. This characteristic gives them two features; (i) biologically inert, once the material is deposited, cannot be resorbed again by the organism (Rodríguez Mendoza, R. P., 2006) (ii) physiological structure that grows throughout the life of the fish with precipitation of the organic and inorganic materials in the endolymph fluid (Campana and Neilson, 1985; Payan et al., 1997; Popper et al., 2005; Popper and Lu, 2000). Therefore, otoliths are also named as the “black box” of a fish. It records not only the growth of fish (Thomas and Sweearer, 2019; Campana, 2005) but also the chemical composition of the environment where fish live (Campana, 1999; Melancon et al., 2008). Climate and temperature information can also be extracted from otolith isotope analysis (Sengupta et al., 2022; Leppi, 2021). Moreover, the fish migration routes can be interpreted from the microchemical analysis (Elsdon and Gillanders, 2003).

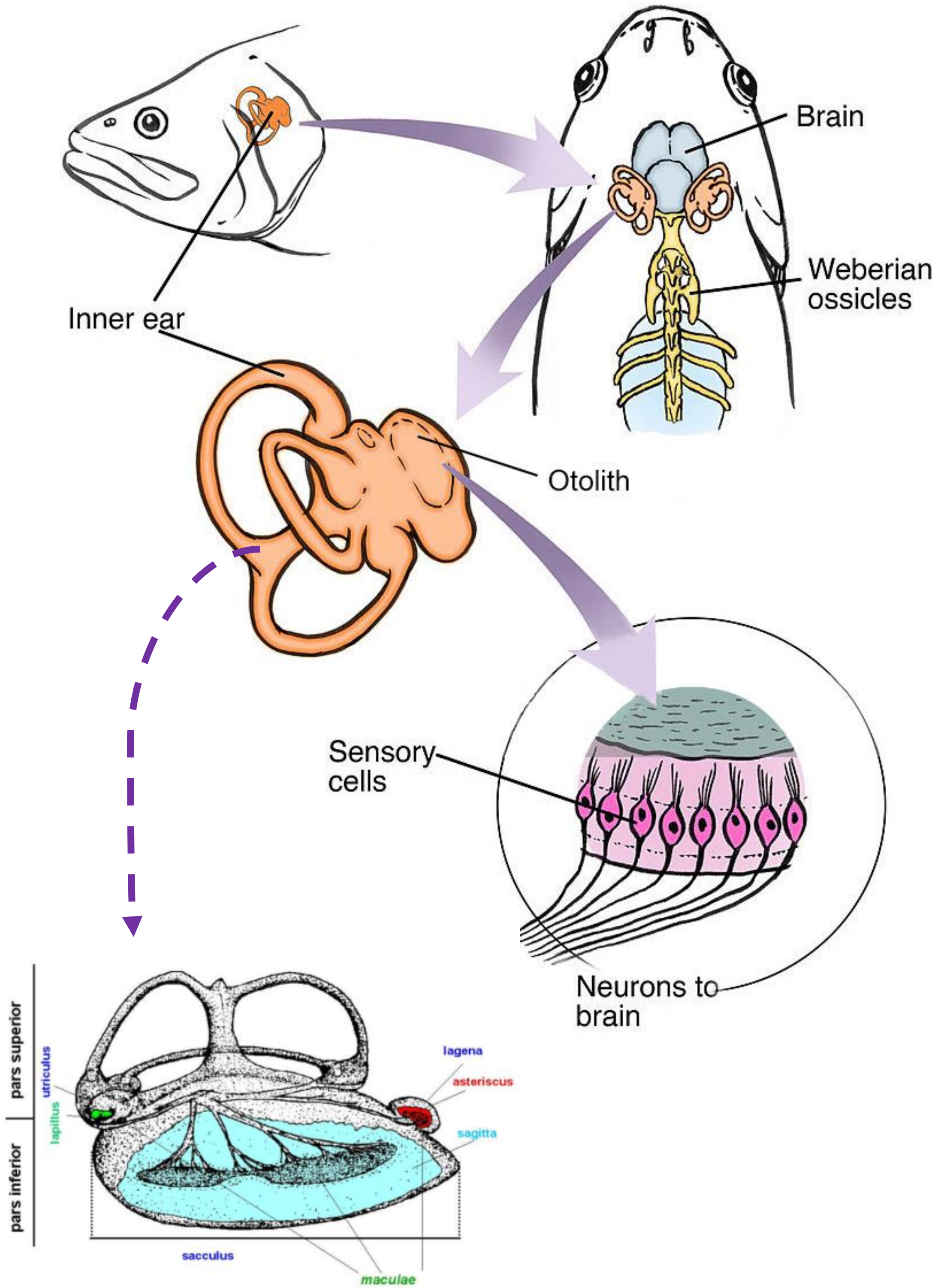

Figure 6: Fish inner ear structure (Lombarte, 1990; the largest scheme retrieved from https://www.offthescaleangling.ie/the-science-bit/fish-hearing/)

The uneven and cyclic growth rate (endogenous circadian rhythm) of fish creates band-like structures on the otolith (Figure 7). When fish grow slowly, the hyaline, thin, band formation occurs. This zone, composed largely of organic material, is observed translucent/dark-in-color under the reflected light. Fast growing season, on the other hand, opaque, wider, band formation is observed. This zone, composed mostly of inorganic material, reflects the light and appears light-in-color (Pannella, 1971; Wright et al., 2002). One hyaline and one opaque formation called an annulus represent one year of fish life. Accumulation of these annual growth increments (annuli) is used as a chronological sign while aging the fish (Rodríguez Mendoza, 2006). However, it should be noted that as a fish ages, its growth rate decreases and, as a consequence, the width of the increments per unit of time on the otolith narrows.

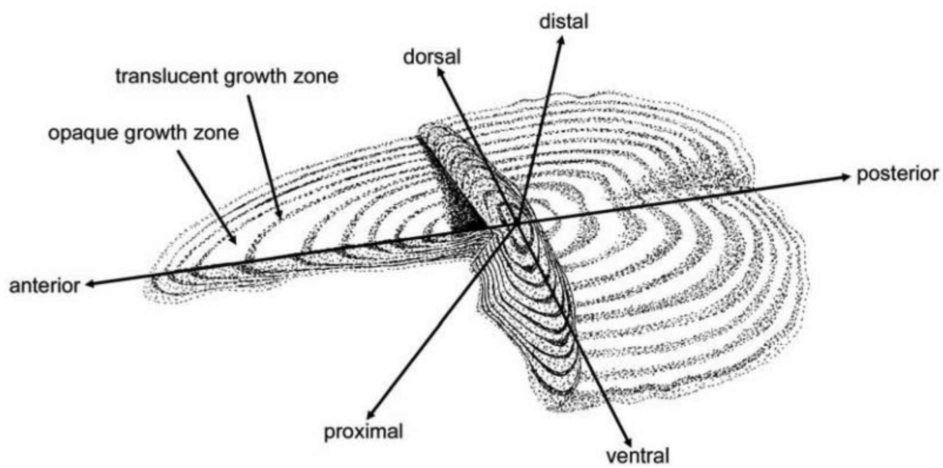

Figure 7: Cross-section of sagittal otolith with concentric annual growth zones and general otolith morphology (Matta and Kimura, 2012).

The otolith starts to develop around the primordia, the nuclear area where the growth increments prime, in the late-egg stage of fish (Furlani et al., 2007). Depending on the species, the obvious incremental accretion becomes obvious after some time of formation (Wright et al., 2002). After that, the accretion of the materials in concentric layers around the primordia of the otolith continues throughout the fish life (Campana and Thorrold, 2001). However, there is a large gap in the literature to explain the exact chemical and physiological processes of otolith nucleation and growth (Melancon et al., 2008). However, it is known that the chemical composition of the otolith core is different from the other parts (Melancon et al., 2008 and the references therein). The core is part of the otolith that accumulates very early in the fish's first year of life (Matta and Kimura, 2012). Therefore, the darker/translucent core region is mostly associated with the maternal effect (Melancon et al., 2008) and the end of the yolk-sac phase (Palomera et al., 1988). Because this region represents the natal environment, it is important to take this region of the otolith into particular consideration in studies that examine the influence of the water quality in which the fish live on the growth of the fish.

Otolith is a tool to understand the biochronological life history of an individual fish. Compared to other body structures, otolith increments are the most commonly used ones for interpreting the age of fish. However, it is expensive in terms of expertise and experience. The time required and the sacrifice of fish to extract the otolith are the main disadvantages of this method of dating (Isely and Grabowski, 2007). Nevertheless, the amount of information that a single otolith can provide about the life history of the individual fish is one of the highest compared to other data collected by fisheries methods. Therefore, using biochronological back-measurements of the otolith increments in growth studies is a substantial element that will contribute to effective fisheries management (Thomas and Swearer, 2019).

Age Determination

In the new decade of 2020-2030, the resilience and sustainable usage of marine food sources, especially fish, are becoming more and more substantial (FAO, 2021; UNESCO-IOC- Sustainable Development Goal 14, 2021). In this context, the Black Sea demonstrates its importance by accounting for about 8% of the total marine fisheries revenue in the General Fisheries Commission for the Mediterranean (GFCM) areas (FAO, 2022). In this unique basin, ~90% of this marine capture is represented by eight species which are priority managed under the supervision of GFCM. Among them, anchovy inhabited the Black Sea is economically prominent by accounting for 64.7% of the total landings in 2018-2020 alone (Figure 8). Being a top-target species for commercial fisheries in the region, anchovy stock has been managed by reference to the results of age-based, advanced, scientific statistical stock evaluation models.

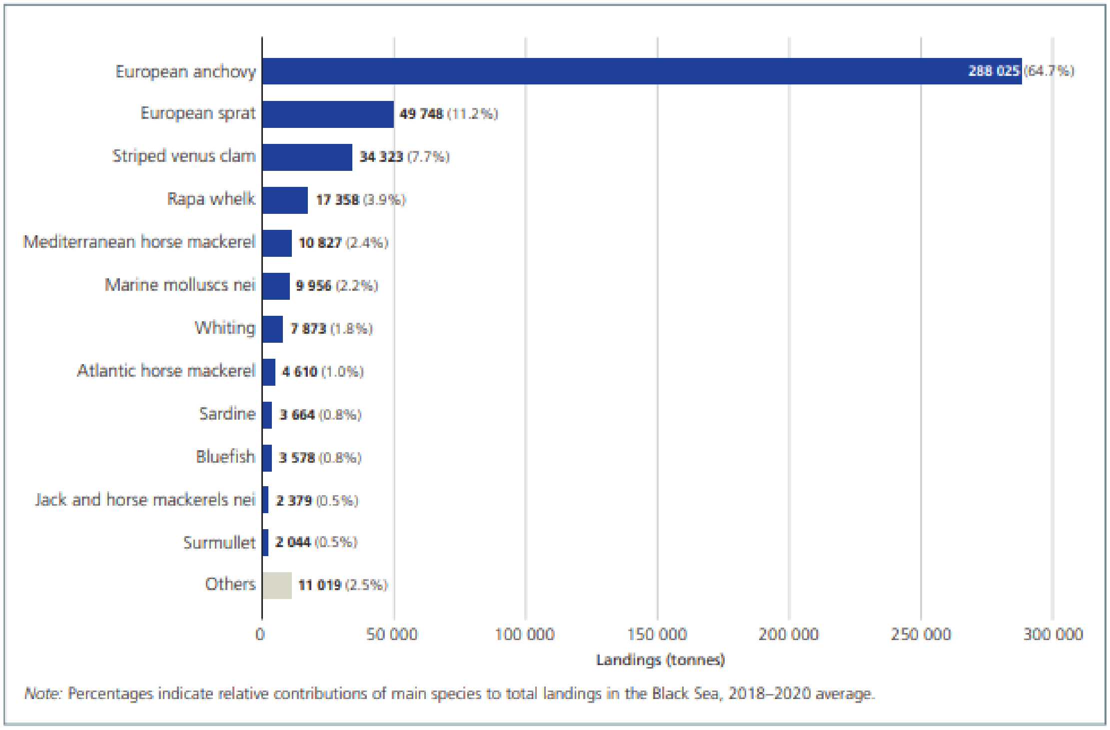

Figure 8: Total landings by main species contributing at least 0.5 percent of the total catch in the Black Sea, 2018–2020 average (GFCM, 2022)

According to the reachable literature, although the hydroacoustic surveys and the fish research were started in the 1950-the 60s (Mayorova, 1954; Pektas, 1954; 1957), the first total anchovy biomass estimates off the Turkish coast was conducted by Osrodek (1975) using the hydroacoustic survey data collected on March 26-31, 1972 (Prodanov and Stoyanova, 2001). After that, the total anchovy biomass was also estimated by Ivanov and Beverton (1985) and Ivanov and Mikhailov (1991). However, the application of age-based stock assessment models for Black Sea anchovies began in 1997 with the age-structured Virtual Population Analysis (VPA) models of Prodanov et al. (1997) for the period 1967-1993. Then, for the period from 1979 to 1993, the VPA Model was again applied by Prodanov and Stoyanova (2001). With the onset of the fisheries collapses, a NATO-Science for peace, Science for stability TU-Fisheries Project was launched throughout the Turkish Exclusive Economic Zone (EEZ) for the period 1988-1993, aimed at acoustic surveys. With this study, scientific stock management studies have gained momentum in Türkiye, the country where the most anchovies have been caught after the collapse. (Daskalov and Rätz, 2011; Tutar, 2014).

Furthermore, since last decade, stock assessment studies have been carried out by the riparian countries under the umbrella of the Scientific, Technical, and Economic Committee for Fisheries (STECF) (2010-2017) and General Fisheries Commission for the Mediterranean (GFCM) (2012-still). Since then, this precious stock has been assessed more intensively and extensively with age-based stock assessment models. Until recently, Black Sea anchovy stock assessments were performed using the eXtended Survival Analysis (XSA) (STECF 13-20; STECF-14-14; STECF-15-16; STECF-17-14; GFCM, 2014; GFCM, 2015; GFCM, 2017; GFCM, 2018). Since 2021, the age-based State Space Model (SAM) has been used (GFCM, 2021).

These models are mathematical and statistical tools to estimate the quantitative predictions about the status of the stock and its response to certain management strategy implementations (Hare and Richardson, 2014). They require detailed biological information about the stock. The stock-specific biological life history parameters are size/age structure, growth rate, natural mortality, age and length at first maturity, and fecundity estimations. They give a general picture of the targeted population and are therefore used as a base in these assessment models. Thus, the best possible measure of these stock-specific biological parameters gives more realistic model results and, consequently, an accurate stock management plan.

The age information is the backbone of the age-based stock assessment models (Cotter et al., 2004). In the case of the Black Sea anchovy, the age information was problematic. The first discussion and therefore notification on the importance of accurate age information appeared in the 2013 assessment report (STECF 13-20) stating that "The assessment results are very sensitive to the age-length keys (ALKs) used to estimate the catch at age matrix and there are noticeable differences in the ALKs used by the countries". Since then (since STECF 13-20; since GFCM, 2014), age determination has always been a concern. The potential inconsistencies in age data were attributed to the methods used by each riparian country (GFCM, 2014). As a result, adequate stock assessment results could not been achieved so far, as age inconsistencies in the combined data make cohort tracking for the model very difficult. At this point, the development of a common-age reading protocol has emerged as an inevitable need.

Unit Stock Issue and the Mixing of Anchovy Stocks in the Black Sea

The Black Sea anchovy and Azov anchovy feed and spawn in the entire Black Sea and the Sea of Azov, respectively (Aleksandrov, 1927; Pusanov, 1936). However, it is difficult to spatially separate these stocks when migrate to the Southeast Black Sea region for wintering, where they are exposed to fishing during this migration, and the physical seawater barriers disappear. Therefore, it has been always hard to distinguish the catch composition during this temporally mixing period (Chashchin et al., 2015).

Identifying populations of a species for management purposes is an important issue for fisheries management (Ihssen et al., 1981). In fisheries management, the stock is a unit used to define biological and management boundaries. In fact, a unit stock is usually viewed as a group of fish fished in a specific area or using a specific method (Secor, 2014). The subset of a species inhabiting in particular boundaries has different growth and mortality parameters (Sparre, 1998). Moreover, they have their own demographic structure, stock-recruitment relationships, and production (Secor, 2014). Therefore, it requires stock-specific fishery management implementations. Indeed, the biology of the stocks plays a critical role in this concept. According to Hare and Richardson (2014), there are two kinds of biological stock definitions: genotypic and phenotypic. The former, synonymous with the ecological population, forms randomly interbreeding individuals of a species whose genetic integrity maintains no matter whether they are temporally and spatially isolated (Futuyma, 1986). The latter is known as intraspecific groups separated over the period during which phenotypic differences can evolve. They vary in certain traits due to genetic responses to certain environmental factors (Ihssen et al., 1981).

In the Black Sea, the intraspecies structure of the known anchovies has been assumed to have two hierarchically upper forms, defined according to their morphological and behavioral characteristics. One of them is the Black Sea anchovy and the other is the Azov anchovy. Different groups are known to exist, but their nomenclature, how to be distinguished, and which stock definition they have are still a matter of debate. There are many studies on this subject and most of them show divergency and sometimes conflicts in their outcomes (see Chapter 2).

Growth Estimation in Fishery Management

The other crucial issue for stock management is to understand the population dynamics of the targeted stock as accurately as possible. In marine life, scientists do not have the opportunity to monitor every individual fish in the ecosystem. Attempting to obtain comprehensive and accurate estimates using information from the population sampled from a small fraction of it is already a limiting factor. Indeed, the stock assessment of fish stock is an exercise in simplification of complex population systems (Cadrin and Secor, 2009; Hilborn and Walters, 1992). Therefore, the abstracted wide-spectrum Russel (1931)'s production components of growth, recruitment, and mortality parameters have been used to define the population and its dynamics (Pauly, 1986). As a result of this life history knowledge, one can develop fishery management plans based on the population's reality and determine whether or not these plans have been successful (Debora and Schueller, 2013; Mangel et al., 2013; Lorenzen, 2016).

Individual growth is the increase in length and weight of fish over a period of time, generally as a function of age (Isely and Grabowski, 2007; Hopkins, 1992). Indeed, fish growth characterization is one of the first steps in developing the fisheries management plan (Allen and Hightower, 2010) and is essential for the sustainable assessment of harvested populations (Maunder and Piner, 2015). Combination with reproduction, mortality, and growth information are used to determine the productivity of populations which is the targeted information to be obtained from an assessment (Morgan et al., 2009).

The demography is needed to be known to estimate first maturity age/size (Udupe, 1986), longevity (Das, 1994), mortality (natural and fishing mortality), year-class strength (Campana, 1996), and size/age composition of stock and productivity (Ohlberger et al., 2022). All these parameters are statistical arguments that help to describe and evaluate the managed stock's life history, present status, and future projections. Therefore, accurate age determination is the first step in obtaining a growth parameter that reflects stock (Francis, 1990). However, age is not the only source of error in growth parameterization.

In the age-based stock assessment models stock sizes and exploitation history are obtained from the summation of catches over years on a cohort-by-cohort basis. To do so the catch-at-age data is needed. From the fishery, every year the length frequency data has been collected. However, for most stocks assessed, it is not easy to find the Age-Length Key (ALK) for each fishing year due to the age determination requiring expertise, labor-intensive, and time involved. Therefore, if experts have an ALK, they can convert catch-at-length data to catch-at-age data by grouping length classes into known age groups. This method is called cohort slicing. In this method, length intervals, in other words, the bins, are defined for each age. The major assumption of this method is that there is no overlap in length between cohorts. However, this assumption does not reflect reality. Length-at-age can be very variable over the years, especially in fish whose growth is highly dependent on environmental factors, such as anchovy. Moreover, cohort overlap is very likely in older fish, as growth slows with age. Ailloud et al. (2015) and the references therein indicated that the use of cohort slicing leads to an overestimation of the abundance of weaker year classes and an underestimation of the stronger year classes. Therefore, this method is not recommended but, despite the bias that it can lead to, due to the lack of yearly information about the stock it is used frequently (Pauly and Morgan, 1987; Kell and Kell, 2011; Ailloud et al., 2015).

Even if age data is collected for each year so that there is no need to use the slicing method, there is still another source of error. That is, fish that are caught or sampled to obtain catch-at-age data or growth estimation are generally sampled at different times of the year. Namely, an age covers the entire year. For instance, fish caught at the beginning and end of this year, based on the date of birth, are still considered 1 year old. However, fish size is not the same at the beginning and end of the year as growth continues. The way to eliminate this error is to sample at exactly the same time each year, but this is not always possible.

The otolith index/angle methods actively used in the Black Sea to separate anchovy stocks and why they cannot be used in the southern Black Sea

After addressing the age problem and sources of error in growth estimation, the debate on the separation of the aforementioned two distinct Black Sea anchovy groups is another topic that this thesis work focuses on. The whole story has been documented in Chapter 2, but, unfortunately, there is still no easy-to-use tool to separate these groups annually (for annual stock assessment studies). It seems that looking for an answer via the otolith, which is already used in age determinations every year, is the most plausible method.

The otolith-based distinction has been used to distinguish groups of anchovies in the Black Sea. This method uses the length-to-width ratio of the sagittal otolith, called the otolith index. It is one of the methods commonly used to distinguish the Azov and Black Sea anchovies found in the Black Sea. As it was cited in the literature, the use of otoliths to distinguish anchovies began with Skazkina (1965), but the manuscript of this study cannot be reached today (Vodyasova and Soldatov, 2017; Zuyev and Skuratovskaya, 2023). As evidenced by the studies using this method, Skazkina proposed specific otolith indexes for the Black Sea and Azov anchovy. In these studies, the otolith index for Azov anchovy was cited as 1.96 by Chaschin (1996) and 1.5-2.2 by Vodyasova and Soldatov (2017). The cited ratio for the Black Sea anchovy was 2.15 and 1.9-2.6, respectively. It is questionable whether this determined value changes from year to year. Indeed, it has already been emphasized by Chashchin (1996) that the indices do not remain the same every year and change from year to year and that the reason for this change is the high rate of hybridization (Vodyasova, 2013). He claimed that the hybrid level reached a point in some years where the statistical differences between Azov and Black Sea anchovy otolith index dropped from 0.19 to 0.064 (to see possible reasons for this hybridization, see Chapter 2).

As can be seen in Zuyev and Skuratovskaya (2023), where the results of the study by Danilevsii and Mayorova in 1979 are shared, the otolith indices of the fish collected in 1960 and 1968 were calculated according to the regions where they were collected. It is estimated that the indexes are determined by the region where the fish are caught or by morphological etc. parameters. This is because the articles that can be accessed do not clearly state how the indexes are determined. The referenced articles could not be checked separately due to the language barrier and online accessibility.

To check whether this method can also be applicable, this method was adapted to the 3150 otolith index data from the anchovy caught in the Southern Black Sea region during the summers of 2013, 2015, and 2018. The trial results are presented in Figure 9. As can be seen, the overlapping area accounts for approximately 69% of the total sample. This shows that the uncertainty is very high. This result is enough to say that this method is not applicable, at least for the southern Black Sea region.

The other approach is to use the otolith angle. Vodyasova and Soldatov (2017), and Vodyasova (2013) also emphasized the errors in the distinctions made with only the otolith index and suggested using angles to solve this problem. The angle measured is the area between the rostrum and anti-rostrum of the otolith. According to Vodyasova and Soldatov (2017), the otolith index for Azov anchovy is from 1.8 to 2.2, and the angle is from 92^{o} to 148^{o}. For Black Sea anchovy, the otolith index is 2.0-2.4, and the angle is from 55^{o}-95^{o}. According to these values, when testing the southern Black Sea samples, 4.08% of the data was represented by the Azov anchovy, and 21.7% by the Black Sea anchovy. While 2.8% of the samples remained in the overlap region, 71% were completely outside the specified regions.

However, apart from the index and angle, during the laboratory analyses, high variations in the distance from the center of the otolith to the outer hyaline edge of the first age ring were observed on the otolith. This noticeable difference has been taken into account. Because it is known that anchovy, as a short-lived and fast-growing fish, exhibits its maximum growth rate in the first years of its life (Freon et al., 2005; Bacha et al., 2010). Moreover, it has been underlined on every occasion that Black Sea anchovy (faster) and Azov anchovy (slower) have different growth rates (Chashchin, 1995; 1996; 2015). However, a study in which the growth rates were compared comprehensively could not be found.

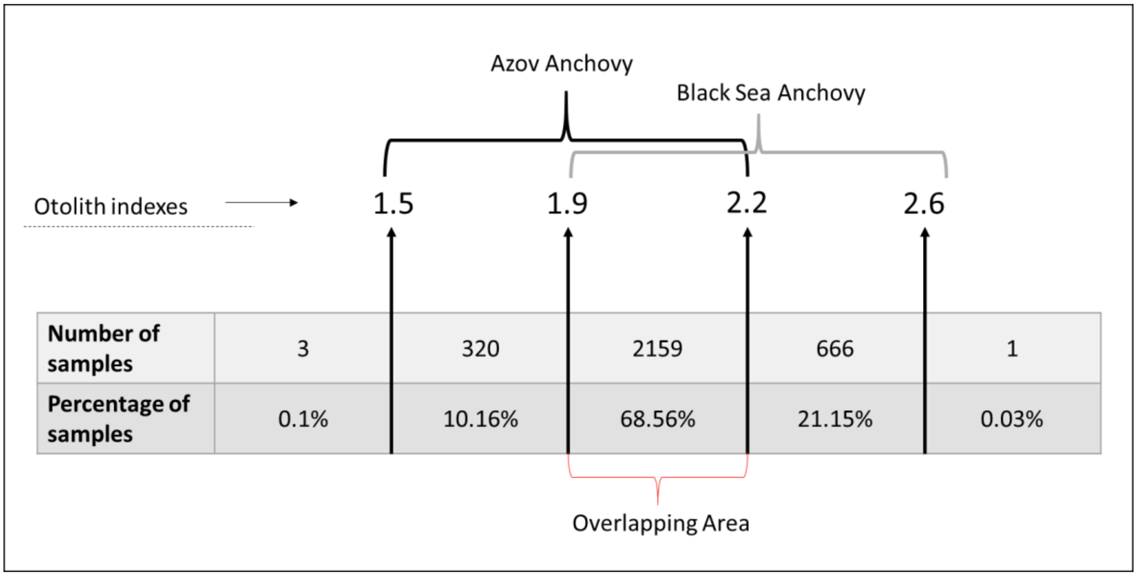

Figure 9: The results of the otolith index values applied to the data of the current study

The effects of environmental factors on growth

The growth of fish is the measurable response that is affected by intrinsic and extrinsic factors and their synergistically complex dynamics (Denechaud et al., 2020; Morrongiello and Thresher, 2015). Although to the literature, genetics has no impact on the Black Sea and Azov anchovy separation (see Chapter 2), the other bioenergetic internal factors affecting growth are difficult to identify and measure on a continuous scale due to their complex nature (Warren and Davis, 1967). On the other hand, we can measure some of the external factors that are thought to have a significant impact on marine fish growth. Among them, the most commonly used and measured of these exogenous factors are seawater temperature which triggers the winter migration and reproduction (Chashchin, 1996; Lisovenko and Andrianov, 1996) salinity which plays a role in the survival of the eggs and larvae (Mayorova, 1950; Zuyev, 2019; Yuneva et al., 2020), and fluorescence as a food source (Fernández-Corredor et al., 2021; Yamamoto et al., 2018).

It was aimed to determine the water masses in the Southern Black Sea characterized according to these three environmental factors. Starting from here, the relationships between these possible water bodies with different characteristics and the growth of different growing adult anchovy and the growth of the young-of-year were investigated.

Because it is known that global warming will eventually affect the fish population's growth (Daufresne et al., 2009; Inouye, 2022). Thus, finding a way to understand the possible impacts of climate change on the anchovy population in the Black Sea and the population’s response to these effects has already gained importance. Furthermore, measuring this effect with growth parameters is predicted as a sustainable method for both comparisons with past data and future studies.

The Statement of Purpose of the Study

The main objective of this thesis is to contribute to the improvement of the stock assessment of the Black Sea anchovy. Age determination is one of the main issues that need to be solved to get more accurate stock management advice. The solution to this is the age length key, which each country creates in a way that can be compared and combined using a common method for each year. Thus, as a part of this thesis, a study was started to compile the age reading information of anchovy in the Mediterranean and Black Seas. Existing information has been combined and advanced by considering the biological characteristics of anchovy in the Black Sea. As a result, the anchovy age reading protocol, which was prepared with the common consent of the experts in the Black Sea under the roof of GFCM and is now applied in the whole Black Sea, constitutes Chapter 1 part of this thesis.

The main assumption of stock assessment models is that the unit stock to be managed is correctly defined (Begg et al., 1999). However, for the case of the Black Sea anchovy, defining the stock and its boundaries in terms of phenotypic, behavioral, and genetic characteristics and the fishery they exposed have been debated since the early 20th century. On this subject, which has not yet come to a definitive conclusion, it was crucial to combine all of this information in order for this current thesis to fulfill its purpose of improving anchovy management. To achieve this, all the studies that could be reached despite the language barrier were collected in a literature review, which became Chapter 2 of this thesis and entitled "Learning from the Past: A Review of Anchovy Classifications in the Black Sea".

Individual growth information is another important parameter used within the model to reconstruct the stock virtually (Maunder and Piner, 2015; Morgan et al.,2009). In the Black Sea anchovy case, it is estimated outside the age-based assessment models (XSA, SAM). The von Bertalanffy growth model, most commonly used in fisheries science, is used to parameterize the increase in length and weight of anchovy over a period of time (Isely and Grabowski, 2007; Beverton and Holt, 1957). In this growth estimation, the unit of time is usually expressed in terms of age. A correct age estimation, therefore, eliminates an important source of error. However, the collection of samples at different times of the year and the use of slicing to group length classes into known age groups can also be sources of error in the growth calculation. Therefore, this study aims to develop an otolith-based method to eliminate these two separate error sources and calculate a more accurate growth according to the full length corresponding to all ages. It is believed that this otolith-based method will also allow accurate historical growth estimates to be made. This proposed method is explained in the manuscript "An Otolith-Based Approach to Estimate the Growth Parameters: A Case Study for the Anchovy in the Black Sea," which is Chapter 3 of the thesis.

It has been known that Black Sea anchovies and Azov anchovies grow at different rates (Chashchin, 1995; 1996; 2015). However, no comprehensive study on the subject was cited in the same sources. Furthermore, according to the latest genetic analysis using new techniques, it has been underlined that there may be no other anchovy population in the Black Sea (Vodyasova and Abramson; 2017; Nebesikhina and Lebedev, 2019; Nebesikhina et al., 2019). The phenotypic differences between groups may be epigenetic in nature (Nebesikhina and Lebedev, 2019). Exposure to certain environmental conditions can lead to different anchovy stocks in the Black Sea (Nebesikhina et al., 2019). On the other hand, discriminating the stocks using the growth pattern recorded from the hard body part, otolith, of fish is a method that has been practiced since the early 1900s. For the purpose of defining the unit stock, it has been applied to many stocks before (Hjort, 1914; Gilbert, 1914; Mapp et al., 2017; Begg et al., 2001; Ventero et al., 2017; Carbonara et al., 2023). A similar approach was applied to the Black Sea anchovy in this study. Based on this information, it was hypothesized that anchovy groups with different growth traits in the southern Black Sea region could be separated using their growth rate signals in the first year of their life on the otolith. The manuscript titled "Detection of anchovy (*Engraulis encrasicolus*) groups with different growth characteristics in the southern Black Sea using otolith" presented in Chapter 4 has emerged from the test results of this hypothesis. The growth parameters for each group were also determined according to the otolith method suggested in Chapter 3.

If there were different growing groups of anchovies in the Black Sea, to what extent would different water masses have an influence on these growth differences and the seawater preferences of these fish? This is a question that needs to be answered in order to understand the dynamics of different groups and evaluate the unit stock assumption. The last part of the study was dedicated to seeking an answer to this question. The results were presented in Chapter 5, the last chapter of the thesis, entitled “Impact of Environment on the Anchovy Growth: A Hydrographic Perspective”. It is believed that the results of this last study while testing the accuracy of the unit stock assumption, will also help to understand the effect of environmental factors on anchovy growth. Thence, environmental effects will be another factor to be taken into account while making stock management planning. Moreover, it has been thought that the outcomes of Chapter 5 could be used as proxy information in climate change studies in the Black Sea.

Overall, the main findings of this thesis aimed to contribute to the stock management of anchovy in the Black Sea. Answers were sought to questions on precise age determination, a literature review about the anchovy groups in the Black Sea, estimating the growth parameters from the anchovy otoliths, stock separation according to growth information collected from the otolith, and the relationship between seawater characteristics, where the samples were caught, and the growth of different adult groups and juveniles. All investigations were systematically elucidated in the following Chapters, adhering to the format of an article manuscript.

CHAPTER 1

A Guide for The Black Sea Anchovy Age Reading

Determining the age of a fish is, by definition, a subjective issue (ICES, 2020). For this reason, a compiled protocol of the rules presented through common decisions is an element that minimizes inconsistencies between age readers. The aim of this thesis was therefore to create such a protocol for anchovy in the Black Sea. For this purpose, with the support of the GFCM BlackSea4fish Project, the laboratory of Alexander Chacschin, the most experienced in this field in the region, was visited in YugNiro, Odessa, Ukraine, to understand the rules by which age is traditionally determined in the Black Sea reading. In addition, with the Erasmus+ internship scholarship at Ifremer, Laboratory of Fisheries - Sclerochronology Centre, Boulogne-sur-Mer, France, how to determine the age of anchovies in the Mediterranean was learned. Considering the traditional approaches in the Black Sea and modern approaches in the Mediterranean, Carbonara and Follesa eds. (2019), and Bellodi et al. (2020) protocols as well as the biological and behavioral characteristics of anchovy in the Black Sea, the protocol presented below was prepared. This protocol took its final form at the Anchovy Age Reading Workshops (GFCM, 2019; 2023) catalyzed by GFCM, with the participation and contributions of experts from all Black Sea countries.

Age determination is one of the main issues that need to be solved to get more accurate stock management advice. With this protocol, the age keys produced by each Black Sea country have become more compatible, comparable, and combinable. This can be described as an important step for the sustainable management of anchovy.

Determining the age, on the other hand, is based on the information gathered from the seasonal changes in the growth of individual fish are recorded in different hard structures of the fish (Thomas and Sweearer, 2019; Campana, 2005). Otoliths are calcified and biologically inert structures responsible for hearing and balance (Campana and Thorrold, 2001; Popper et al., 2005; Rodríguez Mendoza, 2006; Melancon et al., 2008). Seasonal growth changes in otoliths are observed as alternating opaque and translucent rings. The number of these rings is taken as the age of the fish, knowing that paired opaque and translucent rings represent a year. Typically, the method of counting the rings from the nucleus to the edge of the otolith is used to obtain an estimate of the age of each fish (Carbonara et al., 2019).

The cross-section of the otolith is a way to see annual rings more clearly. However, because the anchovy is a short-lived species (in recent years a maximum age of 5 years has been reported: GFCM, 2018), it is not common for the older age rings to accumulate in such a way that the reader cannot recognize it. Therefore, without a cross-section, the anchovy otolith can be read under the microscope with a reflected light source.

This chapter summarizes age determination from the unsanctioned anchovy sagittal otolith.

Otolith Storage

Carefully (undamaged) extracted sagittal otolith pairs should be well-cleaned and dried. During the study, it was observed that the remaining organic particles on the otolith destroyed the otolith in the long term. Therefore, cleaning is an important step for further investigation. Properly removed otoliths can be kept dry. For storage, paper envelopes or small vials with strong lids (so that they don't open on their own later) can be used. It is important to label the otolith storage equipment as follows:

- Fish code or number
- Length of the fish
- Catch date (*day/month/year*)
- Catch location

Preparation of otoliths

Otolith pairs are immersed in alcohol (70% of ethyl alcohol), glycerin (70% glycerin), or glycerin solution (~70% of pure glycerin, ~20% of alcohol, and ~10% of water) to make rings more visible under the reflected light (Figure 1.1) (Morales-Nin, 1992). Alcohol is recommended if otoliths are planned to be stored after reading. The otolith should be removed from the alcohol and dried immediately after the age reading in order to minimize the possible damage that may be caused by alcohol. On the other hand, the use of glycerin is not recommended by the Black Sea experts if it is aimed to preserve the otolith. Since the damage it causes will prevent the otolith from being stored and read again (A. Chashchin, personal communication, December 2019).

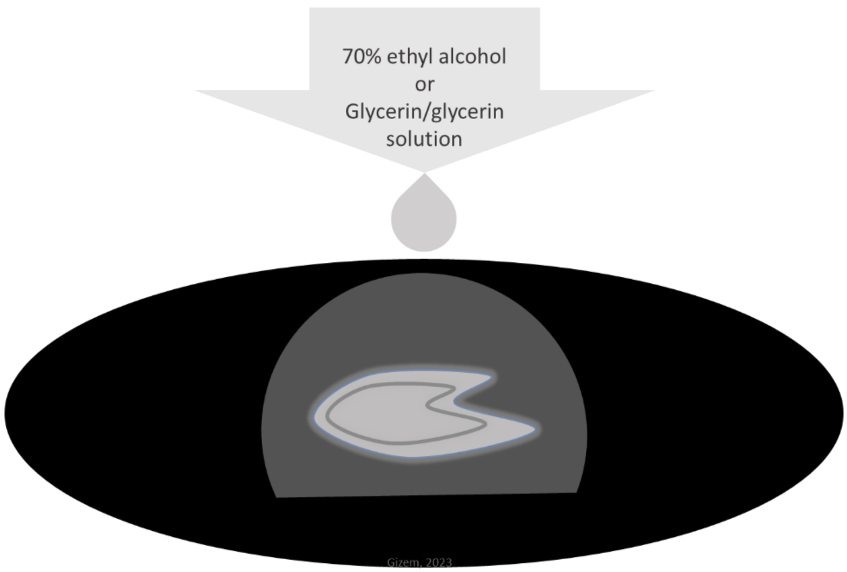

Figure 1.1: Immersion media solution for otolith examination under the binocular microscope

The otoliths should be observed under the reflected light against a black background by using a binocular microscope with a magnification of 25X. Magnification can be increased/decreased to improve the visibility of the edges and some ring formations. The aim is to distinguish the closest ring formations; therefore, magnification can be set according to the resolution and hardware capacity of the used microscope.

Light sources should be directed from the sides, not from the top (Figure 1.2). The light intensity can also be increased or decreased to sufficiently track the ring formations and edge structure.

It is important to observe the otolith by moving it at various angles to detect possible ring formations. In order not to damage the otolith, a needle-tipped apparatus or forceps can be used while moving it.

Some rings can be more visible while rotating and moving the otoliths under the microscope. Therefore, the use of otolith photography is not recommended during the age-determination process. However, they can be used for shape analysis, increment measurements, and some morphological studies.

Before starting the age reading, otoliths should be oriented as the distal surface (convex side) up and the proximal surface (concave side) down (Figure 1.3).

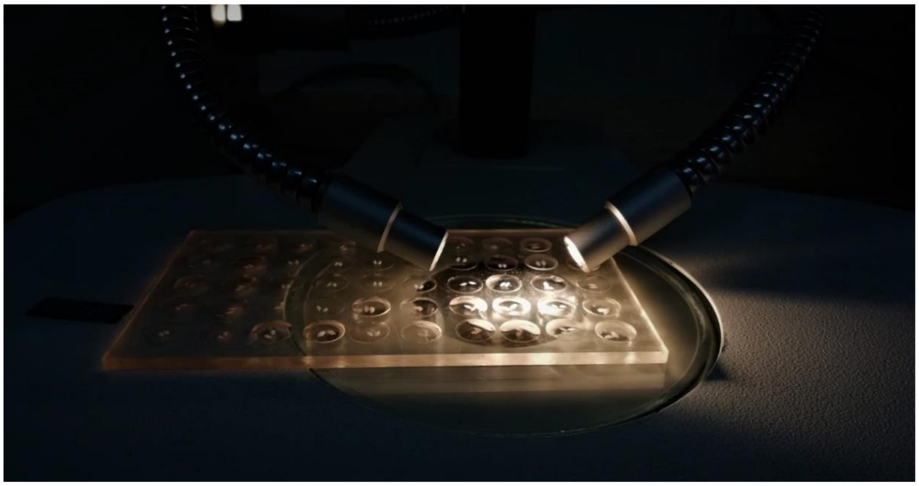

Figure 1.2: Light sources should be directed from the sides

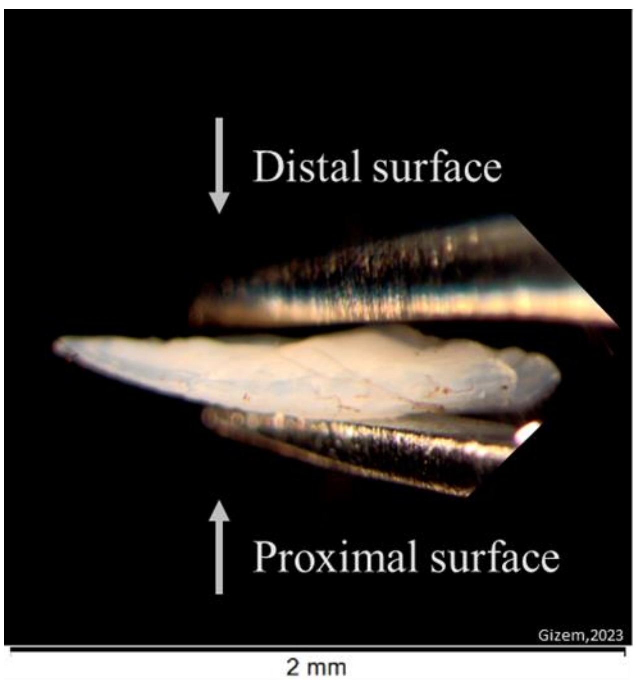

Figure 1.3: Demonstration of the distal and proximal surface of the anchovy.

Aging

Anchovy experiences one slow growth (winter) and one fast growth (summer) period throughout the year (Huret et al., 2019). These growth differences influenced the material accumulation on the otolith and created two different zones as:

- hyaline which can be observed as a dark zone under the reflected light and represents the slow-growing period
- opaque which can be observed as a light (white) zone under the reflected light and represents the fast-growing period.

Each opaque-hyaline pair is interpreted as a year in age determination. In the Black Sea region, experts prefer to count the hyaline zone while aging the anchovy (Table 1.1).

Rule of Thumb Assumptions

Theoretical birth date: 1st of June

First growth increment (1stAge Ring): A ring completed without deformation is counted as a first-age ring if it is clearly visible throughout the otolith, regardless of its distance from the center.

Other annual growth increments: Except for the first hyaline ring, which should be observed completely all around the otolith, subsequent age rings are not expected to be observed in the entire otolith. But the ring formation, a true age ring candidate (age two and older), should be observed in at least two different regions of the otolith (A. Chashchin, personal communication, December 2019).

The rostrum, post-rostrum, and anti-rostrum are the best areas where the age ring increments and formation of the new zone are distinguished (Figure 1.4). This is particularly important for older fish. As the fish get older rings are barely recognized in the dorsal and ventral zones and they might not be distinguishable around the otolith. Therefore, focusing on the anterior and posterior parts of the otolith will be more helpful to determine the age.

The "+" symbol is traditionally used in all Black Sea countries, to indicate that the fish passed its birthday (Figure 1.5). For instance, if the anchovy was caught in September and two completed hyaline rings were observed this fish would be aged as 2+. It means that the fish is older than two years old by 4 months.

Table 1.1: Common rules for aging the anchovy in the Black Sea

Catch Date: before the 1st of June	**Catch Date: after the 1st of June**
The outer hyaline ring should NOT be considered an age ring unless it is followed by an opaque formation If the opaque formation begins at the edge of the otolith (this means that the hyaline ring is complete), then the number of hyaline rings corresponds to the age of the fish.	The number of completed hyaline rings corresponds to the age of the fish. The new opaque region observed at the edge corresponds to summer growth (indicated by +). ***If the hyaline formation is observed at the edge of the otolith, it should not be considered an age ring as it is an incomplete increment.*

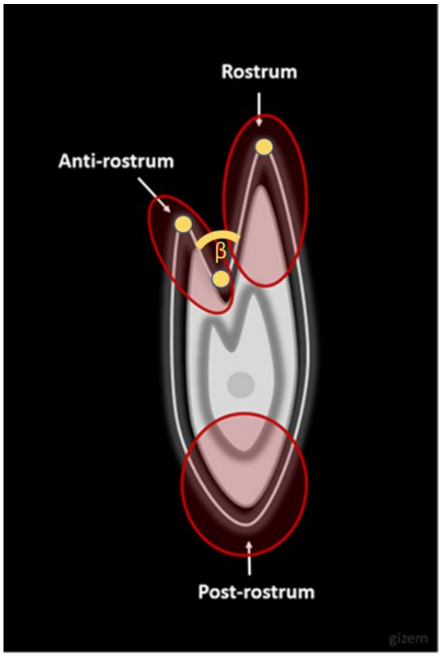

Figure 1.4: Recommended areas on the otolith where age rings become most visible

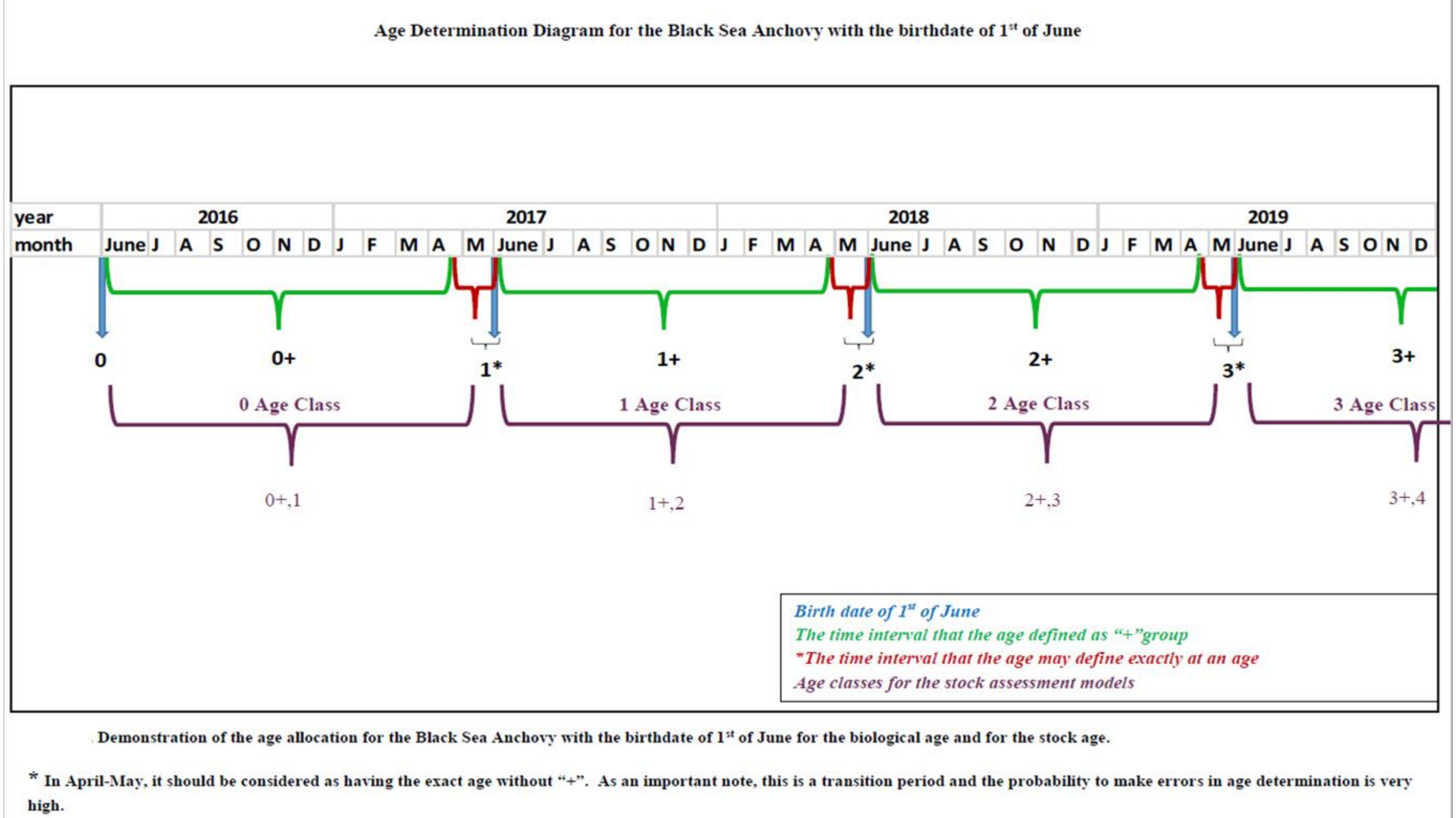

Figure 1.5: Schematic representation of the anchovy age determination

The hypothetical growth of the anchovy never stops. However, the growth rate decreases as the fish get older. Therefore, the distance between the consecutive age rings is expected to be narrowed as the fish get older. For instance, if the width of the third year's annuli is larger than the second year's, it may be interpreted as a biological abnormality in the growth of the fish. Therefore, while deciding the age ring, this information should also be considered together with the other parameters such as the otolith thickness, the angle fulness[1], dorsal outgrowth[2], etc. as a sign of the older fish otolith (Figure 1.6, 1.7). Because, given that the growth performance of Black Sea anchovy is also determined by food availability, which varies greatly from one year to the next (e.g., the negative effect of the ctenophore *Mnemiopsis leidy*), there may also be cases where the distance between successive age rings does not decrease as the fish ages (although it is very rare, important to keep in consideration). Therefore, a holistic approach should be followed when deciding on a real-age ring.

If the first hyaline ring is not followed all around the otolith clearly, it should be considered as a FALSE ring. It is also noted that in the case of Azov Anchovy, it is sometimes possible to observe a fully formed but not bright ring on the thin otoliths of the juveniles (0+). This kind of formation should be considered as a FALSE ring.

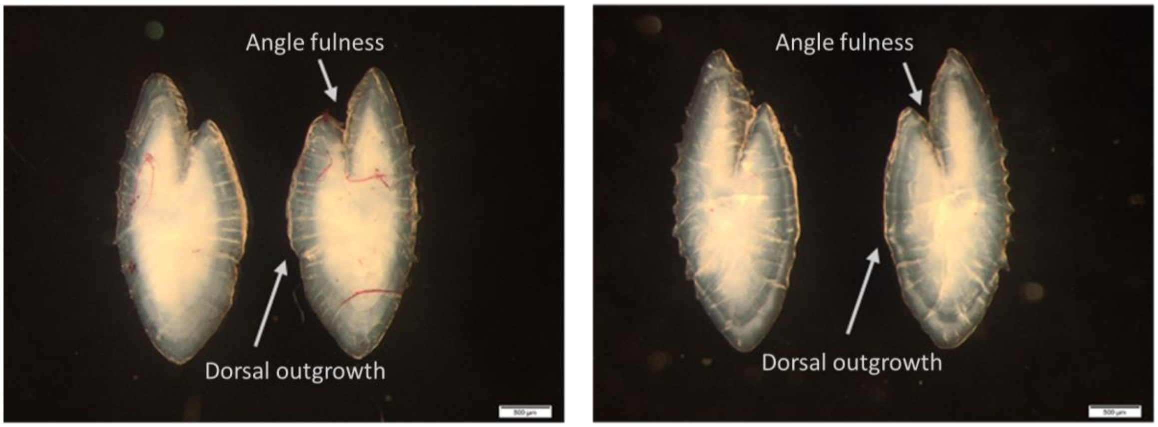

Figure 1.6: Both anchovies are caught in July 2018 and both are 12.1 cm in total length left hand side is age 3+, right is 2+.

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[1] Angle fulness: The angle between the rostrum and the anti-rostrum of the otolith (otolith groove) tends to be filled by material accumulated on the otolith as the anchovy get older.
[2] Dorsal outgrowth: The dorsal part of the otolith is also growth more compare to the post rostrum as the anchovy get older.
Therefore, these signs can serve as an indication of the fish's age.

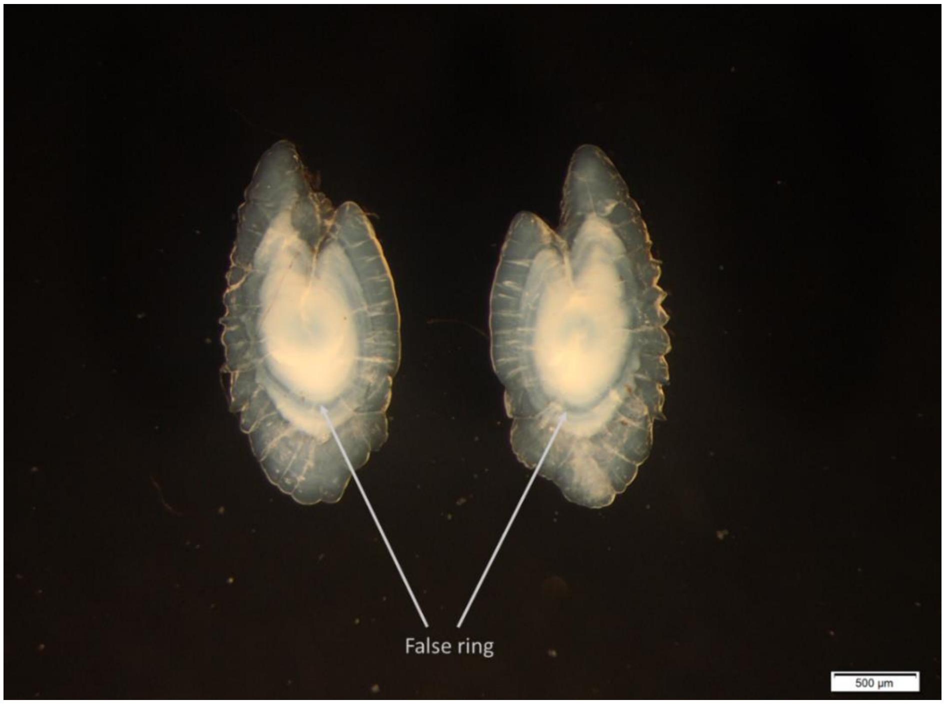

Figure 1.7: Anchovy otolith catch date July 2018, 9.9 cm in TL, 1+.

The first ring formation in Figure 1.7 is a false ring. This decision was discussed and based on two different reasons by the expert group. First, the innermost hyaline formation does not resemble the characteristic shape of the otolith. Another is that, if the first ring were considered as the real age ring, the possible second age ring would be very close to it. If it were, the growth of the fish in its third year would be two or three times greater than in its second year. This is not a generally accepted biological fact (with exceptions). Anchovy growth rate generally decreases as the fish ages. Therefore, it was decided to conduct the age reading according to the rule that the distance between successive age rings decreases as the fish gets older.

In Figure 1.8, the inner ring of the otolith (on the left-hand side) is considered a false ring. The first reason for this is the asymmetrical deformed shape of the ring. Also, the otolith on the right belongs to a 4.6 cm long fish. It has a hyaline formation. Such hyaline increments were observed in a significant part of the samples, which were confirmed as zero years old by both the date of capture and the size of the fish. These samples were used as a reference in the false ring evaluation.

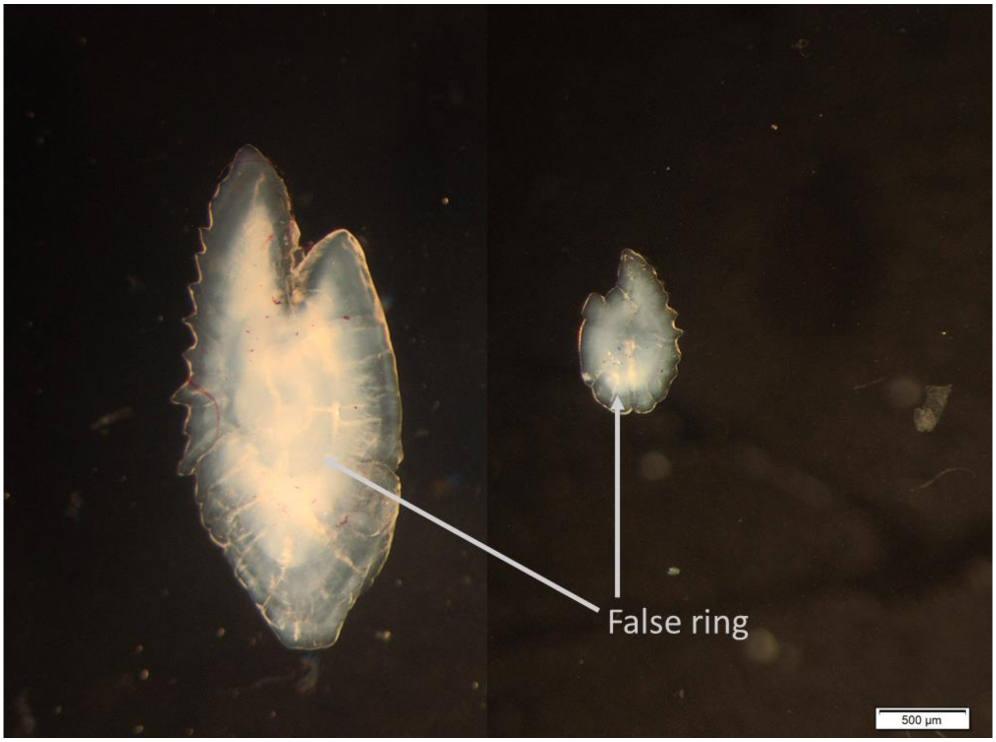

Figure 1.8: left=11.8 cm, 2+; right = 4.6 cm 0+ (catch date for both is July 2018)

Additional recommendations

Since the completion of the last hyaline ring cannot be seen clearly, the probability of age reading error is high in anchovy otolith collected in April, May, and early June.

The otoliths which are not easy to interpret can be discarded. The discarded amounts should not exceed 10% of all samples.

It is recommended to prefer freshly extracted otoliths as much as possible for age determination. For storage, otoliths should be well-cleaned and dried.

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CHAPTER 2

Learning from the Past: A Review of Anchovy Classifications in the Black Sea

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Abstract

Anchovy in the Black Sea and the Sea of Azov are ecologically and economically very important fish species for the basin. Almost all articles on this valuable fish begin with stereotypes of two subspecies/races/populations divided into Black Sea and Azov anchovy. However, there is some uncertainty about this taxonomic difference, and the nomenclatural classification between them is still debatable. The studies conducted to reveal these differences are largely incompatible with each other and contain some contradictions. However, today, it is essential to identify different possible groups for the sustainable use of Anchovy resources. So, this review aimed to comprehensively combine all available information about the intraspecific/ intrapopulation differences of anchovy groups in the Black Sea. According to the results obtained, the subspecies that were accepted to exist in the past and whose morphological, behavioral, and biological differences were revealed are not even genetically different from each other according to the analysis made with modern genetic techniques. It remains an indisputable fact that the precise classifications need further study and discussion. Yet, considering the climate change effects on the Black Sea and consequently on the anchovy in the Black Sea, the environmental ecotypes can be accepted as the best definition that portrays the anchovy

groups in the Black Sea. Understanding under which ecological conditions these ecotypes are formed can be a further question to answer while doing classification research.

Keywords: Black Sea anchovy, Azov anchovy, *Engraulis encrasicolus*, *Engraulis encrasicolus ponticus*, *Engraulis encrasicolus maeticus*

Introduction

It is known that the dramatic depletion of resources, together with the rapidly changing climate and increasing human population, endangers the sustainability of the oceans and the marine basins, which are the primary resources for our ever-increasing needs. The resilience and the sustainable usage of marine food sources, especially fish, are becoming more and more critical (FAO, 2020; UNESCO-IOC- Sustainable Development Goal 14, 2021). The Black Sea represents 7.9 % of the total revenue from marine capture fisheries in the General Fisheries Commission for the Mediterranean (GFCM) areas (FAO, 2022). 90% of this marine capture is represented by eight species, which are priority-managed under the supervision of GFCM. Among these eight, one of the economically and ecologically important priority-managed species is the anchovy, with 64.7 % of total landings (FAO,2022).

European anchovy (*Engraulis encrasicolus*, Linnaeus,1758) is a fast-growing, short-lived, foraging, small pelagic fish species that belongs to the Kingdom: *Animalia*, Phylum: *Chordata*, Class: *Actinopterygii*, Order: *Clupeiformes*, Family: *Engraulidae*, Genus: *Engraulis*, and Species: *Engraulis encrasicolus*. It is a polytypic species, forming different groups (Akkuş et al., 2018). It can be found throughout the Eastern Atlantic from the North Sea to Central Africa and in the entire Mediterranean Sea, including the Black Sea and Sea of Azov (only during their feeding and reproductive periods) (Pusanov, 1936). Being a target species for commercial fisheries, anchovy in the Black Sea and Sea of Azov have ecological, cultural, and economic importance for the basins. From the long-existing literature, it is acknowledged that the intraspecies structure of anchovy in these two seas includes hierarchically upper two subspecies/races/populations/forms (the used classifications in the literature): the Black Sea anchovy *Engraulis encrasicolus ponticus* (Alexandrov, 1927), which mainly feed and reproduce in the entire Black Sea (Chashchin, 1995), and the Azov anchovy *Engraulis encrasicolus maeticus* (Pusanov, 1923;1936) which prefers to forage and spawn in the Sea of Azov (Chashchin, 1995). However, some of the further studies have used additional taxonomic names. For instance, Zuyev (2019) reported that Maiorova (1950;1954) had divided the Black Sea anchovy race into two tribes (natio) as a western (*E. encrasicolus ponticus occidentalis*) and eastern (*E. encrasicolus ponticus orientalis*) based on their morphological, biological, and ecological characteristics.

Portraying the unit stock to be managed is a fundamental presumption in stock management (Ihssen et al., 1981). The spatially and temporally variable existence of different forms of anchovy in the Black Sea complicates a clear stock distinction for this species. Indeed, their scientific classification is still a matter of debate. At the beginning of the 20th century, the morphological differences first noticed by scientists of the former Soviet Union were attributed to different subspecies of anchovy in the Black Sea (Zuyev, 2019). Since then, new observations, arguments, and techniques have been used to explain the intraspecific heterogeneity of the anchovy in the Black Sea. However, a significant part of these works was in Russian and, therefore, could not pass to the other side of the iron curtain due to the language barrier. In the limited number of studies transferred to the scientific world, two subspecies arguments, widely accepted for a long time, were put forward. After the acquisition of technologies that allow automatic access and translation to the articles of Russian authors, it has been observed that different views have not been transferred to open science until now. Yet, these produced pieces of knowledge are sometimes incompatible and not complementary. The answer to the main question about the differences between the so-called Azov anchovy (Aa) and the Black Sea anchovy (BSa) is still not clear. Are there two distinct forms present in the basin? If present, how can we differentiate them? Which different stock boundaries can be established concerning these differences? These fundamental questions need to be answered for future stock management studies. The aim of this study is therefore to review the studies published so far and compare their findings on the existence of possible different subspecies, populations, races, herds, and forms. These terms were used as they were used in the referenced studies. To demonstrate the confusion in the literature, the original has been preserved.

The First Considerations on the Occurrence of different anchovy forms in the Black Sea

The connection between the Black Sea and the Mediterranean Sea was lost and reestablished throughout geological times (Ryan et al., 1997). The sea level rise and fall due to glaciation and melting led to discontinuous connections (Çağatay et al., 2000). Each disengagement of the Black Sea had again developed its own ecosystem and, hence, the evolution of the organisms living in it. On the other hand, each reconnection has led to the extinction of some species and the settlement of others in the Black Sea (Bănăduc et al., 2016). Indeed, the presence of anchovy in the Black Sea is thought to have emerged as a part of this process with the migration of European anchovy from the Mediterranean within consecutive times (Magoulas et al., 1996).

Before the modern Black Sea - Mediterranean Sea connection was established in the late Pleistocene-Holocene periods, the Black Sea had experienced three known glacial periods. The first connection occurred in the interglacial period (100.000-150.000 years ago). With the intrusion of the Mediterranean water, the more saline Karangad Sea formed with ocean-based flora and fauna. Then, with the following Würm Glaciation (18000-20000 years ago), the connection was lost. The consequent deglaciation had caused the desalinization process, and brackish Neoeuxinian Lake-Sea was formed. During this time, although the Pontic relics could survive, biota that was sensitive to salinity had become extinct. Following this extinction and colonization process, the modern connection between the Mediterranean and the Black Sea was re-established (10000-7000 years ago) after the last glacial maximum period. With the gradual salinization, the Black Sea experienced the transition from brackish water to a marine ecosystem. The Atlantic-based Mediterranean species intrusion has taken place since then (Magoulas et al., 1996; Major et al., 2002; Çağatay et al., 2000; Myers et al., 2003; Ryan, 2007). After that time, the halophobic Pontic relics are expected to move less saline river mounts in the basin (Zaitsev and Mamaev, 1997). It is thought that the Black

Sea anchovy, as a latecomer, and the Azov anchovy, as a former-comer, first met after this last connection (Zuyev, 2019). Thus, it is believed that the phylogenetic history of the anchovy in the Black Sea seemed to occur after this meeting and is still an unresolved issue.

This species is well known to local people as it is one of the vital resources for the people living on the Black Sea coast (Zengin, 2019). As a remark, the local people recognized the different anchovies by their morphology, time of migration, and even by their taste. However, according to the literature that could be reached, this information was handled scientifically for the first time by Zernov (1913; cf. Zuyev, 2019). As indicated in this study, by the feedback from the fishermen in the Balaklava-Sevastopol region, Zernov recognized the existence of two anchovy groups with their external features and behaviors. One of them is black in color and larger in size had appeared in Autumn when the water temperature dropped to 14 °C. On the other hand, the other one, lighter in color and smaller in size, started to be observed when the water temperature fell to 9 °C. Moreover, the lighter-colored ones first appear in the southern part of Crimea when the water temperature increases in spring.

Maksimov (1927; cf. Zuyev, 2019) was the first to divide these two groups into the Black Sea anchovy, darker in color and larger in size, and the Azov anchovy, lighter in color and smaller in size. In addition to their physical characteristics, the wintering time of their arrival on the coasts of Crimea was the parameter he used to distinguish them. With no further taxonomic proposition, he defined these two groups as different variants of the anchovy.

At almost the exact time, the taxonomic names defined for these two groups as Black Sea anchovy, *Engraulis encrasicolus ponticus* Aleks., and Azov anchovy, *Engraulis encrasicolus maeoticus* Pusanov, by Aleksandrov (1927) and Puzanov (1936). According to Aleksandrov (1927), the Black Sea anchovy inhabits the western part of the Black Sea during summer. However, due to strong currents, the partial penetration into the Sea of Azov and the intrusion into the eastern Black Sea were also possible. However, Azov anchovy inhabits the eastern Black Sea and the Sea of Azov (Zuyev, 2019). On the other hand, according to Puzanov (1936), the Black Sea anchovy inhabits and reproduces in the entire Black Sea but does not enter the Sea of Azov, where the Azov anchovy feeds and spawns.

These studies were based on knowledge and observation acquired from the fishing grounds at the Crimea peninsula. The parameters used to distinguish these forms are their morphological features and their winter and summer dispersal behaviors. However, in 1950 and 1954, Mayorova shared the results of the Black Sea Scientific and Industrial Expedition of VNIRO conducted in 1948-1951. Unlike the previous studies, they reported two races (as it was in the original article) of anchovies coexisting in the eastern part of the sea. Moreover, they claimed that Aa might have dispersed in the northwestern shelf as well. According to their observations, the BSa could also enter the Sea of Azov for feeding. What was clear is that the BSa was one of the most numerous fish in the Black Sea (Mayorova, 1954). Indeed, they divided this race into eastern (*E. encrasicolus ponticus orientalis*) and western (*E. encrasicolus ponticus occidentalis*). The western inhabitants primarily spawn in the Northwestern region but generally in the west of the Black Sea. Conversely, the eastern tribe inhabits the Black Sea's east part, spawning the same area. They mingle during their overwintering. These two tribes can differ from each other basically by their migration and spawning times. Eastern tribe comes earlier to the overwintering ground. It starts and ends its spawning activity earlier than the western tribe (Zuyev, 2019). Aa was not included in these two tribes.

The Observations on Its Migratory Behavior

While mentioning the differences between the Azov and the Black Sea anchovy, the most pronounced information in the articles is their distinct feeding and spawning areas. Also, their migration behavior is directly related to the harvest of this economically important species (Chashchin et al., 2015).

The Aa feed and spawn in the Sea of Azov from May to August. The Sea of Azov is very shallow; therefore, it is rapidly affected by atmospheric weather conditions. With the cold northern winds, in September, the Sea of Azov cools down rapidly, and the migration of the Aa starts from Azov to the Black Sea through the Kerch Strait, where and while they expose fishery (Chashchin, 1995). The intensity of the migration is inversely proportional to the speed of the temperature change. Its maximum level is observed between mid-October and early November. As the Aa schools leave the Sea of Azov, they enter relatively warmer Black Sea water. Then, the school disperses again to feed in warm water till the temperature declines (Chashchin, 1996). The Caucasus and the Crimea South coast are believed to be the significant overwintering areas of the Aa. According to Chashchin (1995) and the references therein, the Aa moves back to the Sea of Azov in mid-April and late May, depending on the temperature.

On the contrary, the BSa spends its feeding and breeding season all over the Black Sea, with a greater concentration on the Northwest shelf. The water-cooling process in the Black Sea is relatively extended than it is in the Sea of Azov. Thus, the BSa spawning period is more extensive (till September), and its school formation time is later than the Aa (Chaschin, 1996). They reach their wintering region in late November-early December. During their migration and overwintering, they are also exposed to intense fishing pressure, like Aa. Pusanov (1936) is one of the first known records describing the migration route of anchovy. Accordingly, the main overwintering area of the BSa is the Turkish Anatolian and Georgian coasts following the route of Romanian and Bulgarian coastlines. Thereafter, another path suggested by Danilevsky (1964, cf. Chaschin, 1996) is that the anchovy migrates from the Northwestern shelf, which is believed to be the BSa's central feeding and spawning area, to southern Crimea. From there, they migrate to the southeastern Black Sea (Turkish and Georgian coasts). Besides, according to the parasitological examination results, BSa was detected in southern Crimea during their wintering time in December 1967 by Danilevsky and Kamburov (1969). Unlike these routes, Chashchin (1996) added that in some mild winters, they winter on the Caucasian coast by following the Eastern Black Sea Region and return to the shelf by following the same route. Moreover, based on discrimination analysis, he emphasized the lack of BSa in southern Crimea. By referring to the previous study, he further postulated that the BSa seen in Crimea might have been due to misidentification. Later, the 17 years of observations made between 2000 and 2016 indicated that the BSa can overwinter off Crimea temporarily. In 2003, 2011, and 2012, anchovies exiting the Kerch Strait did not migrate further and stayed off the Crimea (Shlyakhov, 2017).

Even though they seem to overwinter in different regions, depending on the temperature, Aa may migrate further south and spend their winter on Georgian (Batumi) and even eastern Turkish coasts (Chashchin, 1995;1996). So, during wintering, the Aa and the BSa mix due to spatial and temporal overlapping. Moreover, the overwintering ground is not the only place where Aa and BSa interfere. According to Chashchin (1996), the Aa was observed on the Northwestern shelf during the summers of 1979, 1981, and 1985. But the peak was witnessed in 1987. It was estimated that the two third of the spawning biomass of anchovy during this year in the western Black Sea belongs to the Aa. Most probably, the colder winters trigger this mixing during summer. In the same article, the reason for this situation has been pointed out as: after they mix in their wintering areas while migrating back to their spawning and feeding areas, Aa may trail the BSa. Furthermore, the Aa have even been spotted off the Bulgarian coast during the summer months (Ivanova et al., 2013).

On the other hand, BSa was also observed in the Sea of Azov during the summer months. Mayorova and Chugunova (1954) observed the mass migration of BSa into the Sea of Azov. Then in 1966, sizeable BSa schools were also witnessed while they entered the Sea of Azov through the Kerch Strait (Altukhov and Salmenkova, 1981). The reason for this migration was explained by Danilevsky (1960) as the unfavorable food conditions occur in the Black Sea, BSa migrates to the Sea of Azov to feed, and in some years, it also spawns there. The same is also true for the Aa for their presence in the Black Sea.

Chashchin (1995) indicated that the ranges of two anchovy races annually vary with respect to their biomass. It affects the dispersion of the fish through the niche of other races and triggers the mixing of the spawning stocks during their reproductive periods. These overlapping zones, especially during the anchovy's summer-reproductive period, strengthen the hybrid hypothesis among the Aa and the BSa.

It was also mentioned that these counter-migrations and mixing can affect the survival of eggs and larvae depending on the salinity of the water and the buoyancy of the egg (Chashchin, 1996). Aa reproduces at 10-17 ‰ (Dementeva, 1958, cf. Yuneva et al., 2020), 10–12 ‰ (Mayorova, 1950), 10-15 ‰ (Danilevsky and Mayorova, 1979, cf. Zuyev, 2019), but for the egg and larvae development, the needed salinity is 10-12‰

(Bokova, 1955, cf. Yuneva et al., 2020). However, the BSa can spawn at a salinity of 17-20 ‰ (Danilevsky and Mayorova, 1979, cf. Zuyev, 2019). In fact, BSa and Aa hybrid forms are mainly seen in the Black Sea, especially on the Northwestern shelf, due to their occasional lower saline property (Chashchin et al., 2015). They underlined that the relation between hybrid increases and the abundance of the Aa in specific years. Moreover, these hybrid young return to the Northwestern shelf after completing their overwintering in the south. But they also support the idea that the offspring of these hybrid forms are eliminated by natural selection in their egg stage. Although it is very controversial, they underlined that "The hybrid spawn has a poor perspective to propagate the inheritable characters, when meeting the Black Sea waters with higher salinity, and is being gradually eliminated from the population."

Besides race's annual range, this phenomenon is also associated with the environmental conditions' suitability (Chashchin, 1996; Melnikova, 2013; Zuyev, 2019). After the cold winter, seawater starts to warm at the mid-end of the spring. As the water temperature began to increase, the wintering schools began to disperse. In the literature, it is especially emphasized that anchovy races that have finished their wintering return to the north in larger groups to their main spawning grounds (Chashchin, 1996; Ivanov and Beverton, 1985). Whereas, in many studies, the first of which was in the 1950s, it was found that eggs were found in the southern Black Sea at least as much as in the north (Mayorava, 1950). But in recent years, more anchovy eggs have been observed in the Southern Black Sea than in the north (Gücü et al., 2016; Şahin and Hacimurtazaoğlu, 2013; Salihoglu, 2000; Niermann et al., 1994). Gücü et al. (2016) revealed that the number of eggs and the survival rates of eggs to larvae have improved in the southern part of the Black Sea. So, as Niermann et al's 1994 study proposed that the emergence of new residents' stock in the southern Black Sea region could explain the increased number of eggs found in the area. This offers a potential explanation regarding the stock to which these eggs may belong. The high fishing pressure might remove the large individuals (repeat spawners) and lessen the population's size returning to the main spawning grounds (Akkus and Gücü, 2018; Petitgas et al., 2006). Therefore, they may belong to young individuals who left the area where they were born for the winter and are not able to return there. Or there is no specific spawning site for the anchovy groups at all, at least for the BSa; they spawn throughout the entire basin (Gücü et al., 2016).

The Rationale Behind *E.encrasicolus ponticus* and *E.encrasicolus maeoticus* distinction

The first known taxonomic identification between anchovies in the Black Sea was performed by Aleksandrov (1927) and Puzanov (1936) using their morphological and wintering behaviors. Later on, the differences among these anchovies were tried to be revealed by other methods. The first of these was blood group research, which would be the reference of many studies later on. Blood group is an immunogenetic analysis that was conducted first time in 1963 by Altukhov et al. (1969, cf. Chashchin, 1996) with a hemagglutination test by pig and horse blood sera to reveal the intraspecific heterogeneities among the anchovy in the Black Sea. According to the results of the analysis with samples collected from the Azov and the Black Sea, three blood groups of A0=21%, A1=63%, and A2=16% were found in the Sea of Azov. From Black Sea samples, only A1=96% and A2=4% were detected (Chashchin, 1996). These results attributed to significant genetic differences between the Azov and Black Sea races as well as the genetic heterogeneity of the Azov race. To Altukhov and Samenkova (1981), the result of the experiment can be evaluated as "*Biological and immunological studies of fish sampled from each trawl disclose a further split of the subpopulations into still smaller, indivisible groups, -elementary populations-. Despite the absence of any geographical isolation, these groupings actually differ in respect of the frequency of blood groups. Such isolation has a biological character due to the fact that anchovy spawning is protracted.*" They concluded that Aa is represented by three subpopulations of an Azov, a Black Sea, which can penetrate the Sea of Azov, and a hybrid group. Similarly, Zuyev (2019) evaluated these results as the fact that the presence of the Aa in the northwestern shelf and set its reproduction in there. Moreover, this is also a sign of the migration of the BSa to the Sea of Azov and the hybrid forms of the two races. He emphasized that the rate of BSa in the Sea of Azov could reach 40-45% in the summer months.

Later, in 1965, otolith, a crystalline structure predominantly composed of calcium carbonate, which is actively used in the identification of stocks was used by Skazkina to distinguish BSa and Aa. Since then, otolith is still actively used in the diagnosis of Aa and BSa in the Black Sea. The manuscript of Skazkina's study is not available today. As it was understood from the studies that used this method, Skazkina (1965) proposed a race-specific otolith index which is the ratio between otolith length over width. In these studies, the otolith index for Aa was quoted as 1.96 by Chashchin (1996) and between the range of 1.5-2.2 by Vodyasova and Soldatov (2017). The cited ratio for the BSa was 2.15 and between the range of 1.9-2.6, respectively. Along with this information, it should be kept in mind that these race-specific otolith index differences were first noticed in 1965, predating two separate salinity-sourced regime changes in the Sea of Azov. The first was experienced in the 1970s (Berdnikov et al., 2022), and the second was at the end of the 1980s (Chashchin, 1996). For the record, the last one, the third, is happening now (Kosenko et al., 2017; Yuneva et al., 2019; Yuneva et al., 2020; Chernichko et al., 2022). Although it has been forecasted that the average salinity in the Sea of Azov would rise to 15‰ by 2020 (Kosenko et al., 2017), the measured average salinity of the Sea of Azov has already risen from 12.7‰ to 14.0‰ during 2014-2018 period (Yuneva et al., 2020). Moreover, in particular, the reason is the climate this time.

In the 1970s, due to anthropogenic reasons, a reduction of the river drainage had observed in the Sea of Azov. It triggered the extreme environmental conditions=higher salinization and it had been monitored that the salinity of the Sea of Azov in this period increased up to 14 ‰ (Chernichko et al., 2022). It means the shrinkage of isolated spawning areas of the Aa. Moreover, during these times, the mass BSa movements to the Sea of Azov were recorded (Altukhov and Salmenkova, 1981). This may not only negatively affect the Aa's gene pool but also triggered the food competition for the fingerlings which were already suffering from the salinity change in their larval period. On the other hand, in the same years, the Northwestern shelf, the main feeding area of the BSa, was also faced with another environmental crisis of eutrophication.

While all this was going on, the same blood group experiment was repeated (the 1970s), and considerable changes in the gene pool of the Aa were observed. The new result from the Sea of Azov was A0=4%, A1=88%, and A2=8%. It was explained by the dominance of the BSa in the Sea of Azov during the sampling time (Altunkov, 1974, cf. Chashchin, 1996). Anthropogenic pollution on the shelf and salinization of the Sea of Azov had been adverted to explain the divergent results of blood group tests and the reduction in number and loss of heterogeneity of Aa (Yuneva et al., 2020, Chashchin, 1998).

This rapid salinization process of the Sea of Azov led to changes in fish food resources which will eventually influence the fat content of the fish (Yuneva et al., 2020). The fat content is a substantial energy source for the anchovy, especially during its wintering period (Shulman et al., 2007), and also tells much about its story. According to Yuneva et al. (2019), the fat content is used as an energy source under scarce and ceases food sources but also helps fish survive under low temperatures. Moreover, they claimed that the fat content of anchovy also determines the adaptation duration of the Aa to the rapid salinity change between Azov and the Black Sea. The primary factor that triggers the migration is water temperature (Shulman, 2002). Together with the condition factor and food supply, fat content is another determinative factor for the wintering time (Shulman, 2002). For instance, the Aa, which has fat content lower than 14% of its total weight, fails to migrate (Shulman et al., 2007). According to observations made in the 1950s-1960s, fish with sufficient fat content (20-25% of their total weight) start their migration earlier than those with lower fat (Shulman, 2002; Chashchin,1996). On the other hand, the adult BSa starts migration with 17-18% of fat content (Shulman et al., 2007).

It is also known that the fat content of the BSa is lower than the Aa (Chashchin, 1996). Since the Aa stores more fat in their visceral cavity and muscles, they start their winter migration earlier than the BSa. Due to the temperature advantages of the Black Sea, BSa can continue feeding while the Aa prepares for migration. Therefore, at the same age, the body length of the BSa is higher than Aa (Chashchin, 1995; 1996; 2015). Even though it is used as a discrimination parameter, there is a very limited amount of study on the fat content of the BSa. In a recent study conducted by Yuneva et al. (2020), the lipid content of anchovy in the Sea of Azov varied between 5.1 and 20.4% of wet weight from 2014 to 2018. Unfortunately, the small individuals with low fat were dominant in the population. A decrease in catch weight-at-age was also observed. The demographic change in the population explains the unexpectedly low-fat situation. Moreover, the food competition with Comb jellies which is more likely to appear in the Sea of Azov as the salinity increases may be another threatening factor (Yuneva et al., 2019; Chashchin et al., 2015; Chernichko et al., 2022).

Nevertheless, extreme changes in salinity are an important factor in the survival and adaptation of organisms. In any case, it is generally accepted that these processes may have adversely affected the Aa population and accelerated the interbreeding between Aa and BSa. For instance, for the 1970s case, since this mixing occurred in their spawning period, Chashchin (1996) claimed that the hybrid level reached a point where the statistical differences between the Aa and BSa otolith index dropped from 0.19 to 0.064. He also emphasized in the same study that this increase in hybridization level makes it difficult to make the distinction between the Aa and BSa using the otolith index method. Nowadays, hybridization is likely to increase, due to the salinity increase in the Sea of Azov, and it is highly likely that this difference will fall far below 0.064.

However, despite these discrepancies, the studies addressing the differences between Aa and BSa are primarily based on the separation of the otolith index. The first distinction criteria are where the sample was collected, and the second is the otolith index (Vodyasova and Soldatov, 2017; Zuyev, 2019). These studies determine differences in otolith indices depending on the region in which the fish were caught. There are very few studies where several methods have been tested simultaneously (Chashchin, 1996; Nebesikhina and Lebedev, 2019; Nebesikhina et al., 2019). However, this is understandable because the applied methods are expensive and require expertise. Therefore, in most of the studies, since it is cheap and feasible, the samples were first separated according to the otolith index method (Zuyev, 2019; Vodyasova and Soldatov, 2017), and then the tested differences between them were revealed. After Chashchin (1996), Vodyasova and Soldatov (2017) first argued that using this index alone to separate the forms might create a significant bias. However, the source of bias they mention is due to the measurement and the significance digits applied. Thereupon, they claimed in the same study that β, which is the angle between the rostrum and anti-rostrum of anchovy's sagittal otolith (showed in Figure 1.4.), should be used together with l/d (otolith index) as another parameter to reduce the mentioned bias. Indeed, they found that using the otolith groove provided a better performance on the discrimination of the different forms than the otolith index. Moreover, the l/d and β combined methods have been applied in genetic (Vodiasova and Abramson, 2017) and biological characteristics of Aa (Chesalin et al., 2020) studies later on. However, the study of Vodyasova and Soldatov (2017) examined fish aged one year and older. The age of the fish, perhaps the most important source of error, seems to have been overlooked in this approach. The European anchovy sagittal otolith groove between the rostrum and anti-rostrum tends to fill with the accumulated otolith material as the fish get older. Therefore, the portion in which β is measured on the otolith is relatively different in young and old fish. Especially in classification studies, the age of the otolith impacts the classification accuracies by affecting the shape of the otolith (Mapp et al., 2017; Gonzalez-Salas and Lenfant, 2007). The solution for this error can be to use the same-age fish from the same years' sampling (to reduce also the year effect) during the analysis.

Apart from all these, the otolith index method continues to be used in recent studies. In Zuyev (2019), the distribution of anchovy races (Azov and the Black Sea) was demonstrated using the otolith index values from the regions: western-offshore-BS, eastern-offshore-BS, Northwestern-coastal-BS, eastern-coastal-BS, and the Sea of Azov. He used the index data in the reproductive period of anchovy collected by Danilevsky and Mayorova (1979) and the ratios proposed by Skazkina (1965). Correspondingly, the BSa race is dominated in the open Black Sea (western: 60%, eastern 55%), whereas in coastal regions of the Black Sea (northwestern shelf: 80%, eastern: 95%) and the Sea of Azov (60%) the Aa race is overpopulated. It is obvious that the coastal regions, especially the eastern Black Sea, are mainly occupied by the Aa. On the other hand, the ratios of the Aa and BSa are relatively close in the open sea and in the Sea of Azov. Indeed, the author evaluated these results as proof of Alexandrov (1927) 's suggestion of the presence and the reproductive activity of the Aa in the eastern part of the Black Sea.

On the other hand, using otolith shape analysis is the recent trend for defining different geographical groups. The environment where the fish live impacts the shape of the otolith (Mahé et al., 2019). In the study in which the European anchovy samples were collected from the eastern Atlantic coasts, the Mediterranean, and the Black Sea, it was revealed that the anchovy in the Black Sea was different from the Atlantic and the Mediterranean, according to the otolith shape analysis (Akkuş et al., 2018). The almost same method was applied by Khan et al. (2022) to lower scale sampling area of the Turkish coasts (Aegean Sea, Marmara Sea, eastern, middle, and western Black Sea). Accordingly, while the Black Sea has diverged from the other Seas, within the Black Sea, two main anchovy stocks were revealed: the western stock and the eastern-middle stock. When considering the shape's dependency on the environmental conditions, this result may show the different ecotypes exposed to different environmental conditions. Indeed, these differences were attributed to the western and eastern gyre formation that creates diverse environmental conditions (Khan et al., 2022). However, this study's drawback is collecting the samples during the wintering period, when the stocks were most probably already mixed.

The other less studied method to reveal the intraspecific heterogeneity between anchovy in the Black Sea and the Sea of Azov was parasitological analysis. The first one was conducted by Danilevsky and Kamburov in 1969 (Zuyev, 2019). They used *Contracaecum aduncum* nematode larvae and metacercariae of the trematode *Nematobothrium* sp. as biomarkers. They found no differences in infestation rate between Aa and BSa. But this study did not only focus on the Aa and BSa heterogeneity. They also collected samples from different sites of the basin (Azov-Black Sea) to be able to understand the geographical differences. Based on the results of this study, the authors identified four different herds of anchovy. They were named Eastern, western, northwestern, and Azov-Coastal Caucasian herds. The first two have higher infestation rates and live in open brackish water while the last two have lower infestation rates and prefer to live in coastal desalinated water.

On the other hand, Chashchin (1981, cf. Chashchin, 1996) claimed that the *Contracaecum aduncum* infestation rate is higher in the BSa. The infestation rate is proportional to the age of the fish. But he underlined that one should keep the possibility of fish being infected during wintering. That contradicts Danilevsky and Kamburov's (1969) results. Yet, in another study, Akkus and Gücü (2018) found that the infestation rate is higher in the western side of the southern Black Sea area. In addition, they showed that the infestation rate increased with age. They did not attribute the parasite infestation to the Aa or BSa, yet they claimed that the higher infestation rate on the western side of the Black Sea could be used as a biomarker for the hypothetical western anchovy stock.

Based on the parasite infestation, all outcomes move us to a point. The anchovy, which lives in the open sea and has a higher infection rate, has two stocks: western (probably has higher infestation) and eastern. The anchovy in the Sea of Azov, which mainly inhabits fewer saline waters has lower infestation. However, being definite in evaluating the Northwestern shelf stock separately may be erroneous because it is known that this region is the main feeding, growing, and spawning area not only for BSa but also, for some years, for Aa (Mayorova, 1954).

The Era of Genetics

The first known genetic studies to examine the intraspecific heterogeneity among anchovy in the Black Sea started with an immune-genetical blood group test in 1963 (Altukhov et al., 1969, cf. Chashchin, 1996). Later on, Limansky published another independent experiment in 1970 with the same approach. He concluded that the anchovy populations in the Sea of Azov had not reached a state of genetic equilibrium. He attributed this to the fact that the occasional entering of BSa to the Sea of Azov contributed to the genetic pool. Since there is no absolute geographical barrier between Aa and BSa, these populations may mix and influence each other's gene flow.

Kalnina and Kalnin (1984) and Kalnin and Kalnina (1985) conducted further investigations using the electrophoresis method. They found valid differences in genetic frequencies among anchovy populations and defined them as two heterogenous races (using the Soviet terminology): the BSa and the Aa. This result is significant because, after the Sea of Azov's high salinity period (1972-1978), the 1980s is defined as the medium salinity period (Berdnikov et al., 2022). The reproductive isolation during this salinity period of the Sea of Azov might be disturbed. After fewer saline characteristics are regained, reproductive isolation of the Aa in the lower salinity region may be reestablished. Before the salinity crisis, no genetic study was found other than the blood group, which examines the differences between Aa and BSa. Therefore, these genetic differences revealed by Kalnina and Kalnin (1984) and Kalnin and Kalnina (1985) also make sense when we accept that the Aa population gained a reproductive advantage in the 1980s compared to the 1970s. Moreover, the bottleneck may be established among anchovy populations due to the dramatic decline in population size in the 1980s (Vodiasova and Abramson, 2017). It may be another reason for this difference.

The popularity of genetic studies, especially after the 2000s, has increased and has become a frequently used method with their accurate genetic proximity results. But the first of its kind covering the anchovy in all Mediterranean and the Black Sea belongs to Magoulas et al. (1996). It is a significant genetic study that used Mitochondrial DNA phylogeny to evaluate the history of the European anchovy by reconstructing the populations to their mitotypes. It is known that evolution is faster in mitochondrial genes than the nuclear genes. Therefore, it is found to be more informative and frequently used in phylogenetic evolution studies, especially for classifying the inter- and intra-specific population differentiation and the level of gene flow (Hurst and Jiggins, 2005; Zhan et al., 2021). So, according to these mitochondrial DNA results, the Black Sea is thought to be the origin of Phylad A (with a frequency of 0.99; Figure 2.1a). Due to the recent geographical isolation and the re-connection events of the Black Sea and the Mediterranean Sea, the population's effective size, which had colonized through the Mediterranean at the end of the Pliocene, led to evolving Phylad A in the Black Sea. The same phylad was also observed in the Aegean, Mediterranean, and the Bay of Biscay, with frequencies of 0.85 and 0.40. The reason for this was postulated to the outflow of the anchovy from the Black Sea to the Mediterranean with the last opening of the Bosporus Strait (10 thousand years ago).

In 2005, Grant conducted a genetic study for European anchovy with a Magoulas et al. (1996) dataset from eight Mediterranean locations, including the western Black Sea. Accordingly, the anchovy in the Black Sea (from the west part) belongs to phylogroup "A" confirming the results of Magoulas et al. (1996). Due to the long-term isolation in the Black Sea, it is part of a different phylogroup than the Mediterranean relatives. The study's outcome for the BSa is that the haplotype and nucleotide diversities are lowest in the Black Sea. In other words, the BSa has the lowest degree of connection with the Mediterranean. Different oceanographic and climatic characteristics of the Black Sea and the long-term isolation from the Mediterranean Sea could be a reason for this result.

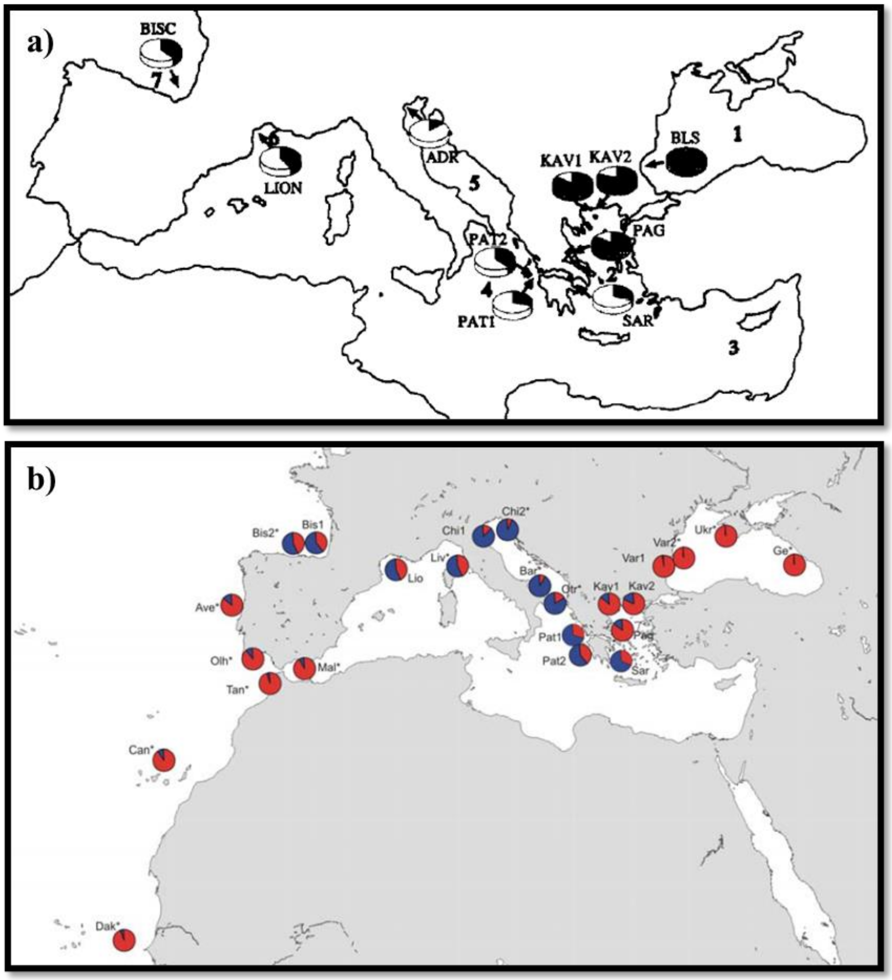

Figure 2.1: mtDNA clade frequencies a) Black: frequency of phylad A; white: frequency of phylad B (Magoulas et al., 1996) b) in red clade A, in blue clade B (Magoulas et al., 2006).

Magoulas et al. (2006) performed the same mitochondrial DNA examination as conducted by Magoulas et al. (1996) by extending the geographical coverage. This time samples of the Black Sea were from Varna, Crimea, and Batumi regions. Almost all samples (227 over 228) from the Black Sea were again found as belonging to Clade A (in this study, the term Phylad was changed to the Clade). Moreover, within all regions, the lower nucleotide diversity (genetic variability) was again observed in the Black Sea.

The same results were obtained using nuclear DNA rather than mitochondrial (Bounchenak-Khelladi et al., 2008). The Black Sea's isolated nature and the straits' hydrography prevent new groups from entering the Black Sea. It was the author's explanation for the low genetic diversity in the Black Sea. However, the main result that distinguishes Magoulas et al. (2006)'s study from the previous one was that the higher Clade-A haplotype diversity is also found extensively in the Atlantic than in the Black Sea (Figure 2.1b). It turned out that the place where Clade-A appeared was not the Black Sea but the Atlantic. So, anchovy in the Black Sea is the only Mediterranean group that carries the Atlantic-origin gene pool due to its discrete isolation from the Mediterranean.

In 2006, another study was conducted on the polymorphism of general muscle proteins to test taxonomic differences in the European anchovy by Ivanova and Dobrovolov (2006). Although the sample size was smaller than Magoulas et al. (2006), they still had 11 sampling points from the Atlantic to the Black Sea. According to the muscle protein loci analysis, Ivanova and Dobrovolov (2006) revealed that the BSa and the Aa are not subspecies as indicated by Pusanov (1936), at least not anymore. The higher hybridization among them led to the disappearance of previously detected subspecies features. This explanation may also be why the significant genetic differences found by Kalnina and Kalnin (1984) and Kalnin and Kalnina (1985) could not be found in the relatively recent research. But, considering the genetic divergence, the found low genetic distance between Aa and the BSa by Ivanova and Dobrovolov (2006) made them conclude that they are different populations, not subspecies. Moreover, in the studies conducted by Magoulas et al. (1996; 2006) and Grant (2005), they focused on the whole Black Sea's genetic variations from other seas. No separate groups are underlined within the Black Sea basin. Yet, Ivanova and Dobrovolov (2006) found differences within anchovy groups in the basins and associated these variations with the different intrusion times of the groups to the Black Sea. They concluded that the Aa entered the Black Sea in the Karangad period. In contrast, the BSa entered during the last connection of the Black Sea to the Mediterranean. This outcome and the phylogenetic tree in Figure 2.2 may raise the question of whether the process of intrusion from the Mediterranean to the Black Sea is still occurring. Or, it raises the question of what effect the rate of environmentally-induced introgressive intraspecific hybridization has on this.

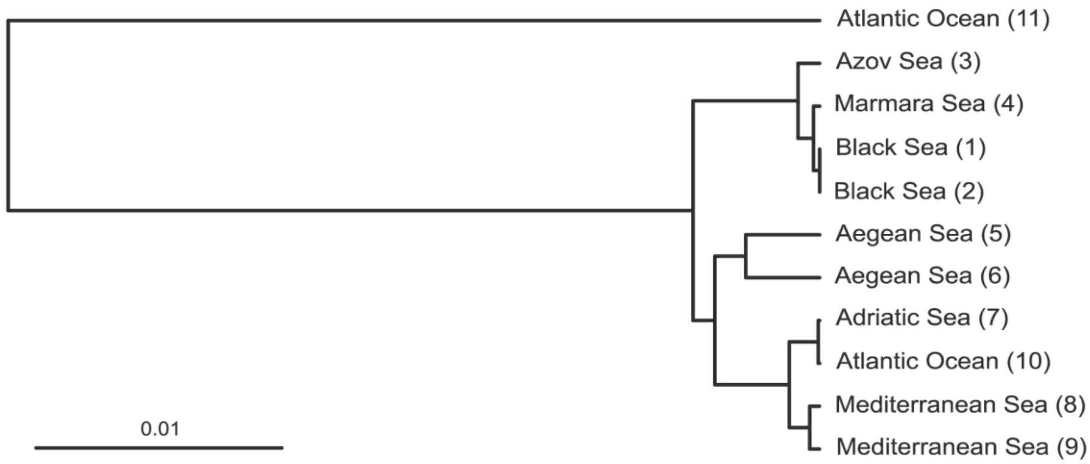

Figure 2.2: UPGMA tree, derived from allozyme data for 22 loci using NEI'S genetic distance (DNei), illustrating the relationships among 11 anchovy populations (Ivanova and Dobrovolov, 2006)

Another genetic study was conducted by Erdoğan et al. (2009) using allozyme analysis. The samples were collected by commercial fishing vessels from the Turkish Aegean, Marmara, and Black Sea (west, mid, and east) regions. As the results of the study, although the genetic similarity between Marmara and the western Black Sea was revealed, the authors emphasized the eastern Black Sea population is genetically different than the others. This situation was explained by the effect of the hybridization with Aa or another self-recruiting stock in this specific region. However, although the differences in the eastern population confirmed Alexandrov (1927) 's opinion about the occupation of the eastern Black Sea mainly by the Azov population, it does not coincide with the inference of Mayorova (1954) that both Azov and Black Sea races share the eastern Black Sea.

Keskin and Atar (2012) also used the mitochondrial DNA sequence method to test the European anchovy's intraspecific genetic heterogeneity around Anatolia. Samples represented 16 populations collected using fishing vessels from the Anatolian coasts of the Black Sea, the Marmara Sea, the Aegean Sea, and the Mediterranean Sea. They found ten genetically diverged populations. The lowest nucleotide divergence was found between the Eastern Black Sea and the Marmara Sea. Higher similarities between eastern and western BSa were also found (Figure 2.3). The study did not include samples from the Azov region, yet the differences between the east and west sides confirmed the previous studies. However, the higher rate of similarities between eastern samples with Marmara is inconsistent with the similarity between the Marmara and the western Black Sea presented in previous studies. Moreover, it contradicted especially with Pusanov (1936) who accepted the Aa as another species and connected the Black Sea and Mediterranean anchovy and defined them as one race. Overall, they underlined that eastern and western populations in the Black Sea have the lowest pairwise distance values, which means they have the most conserved intragenic features compared to the other regions.

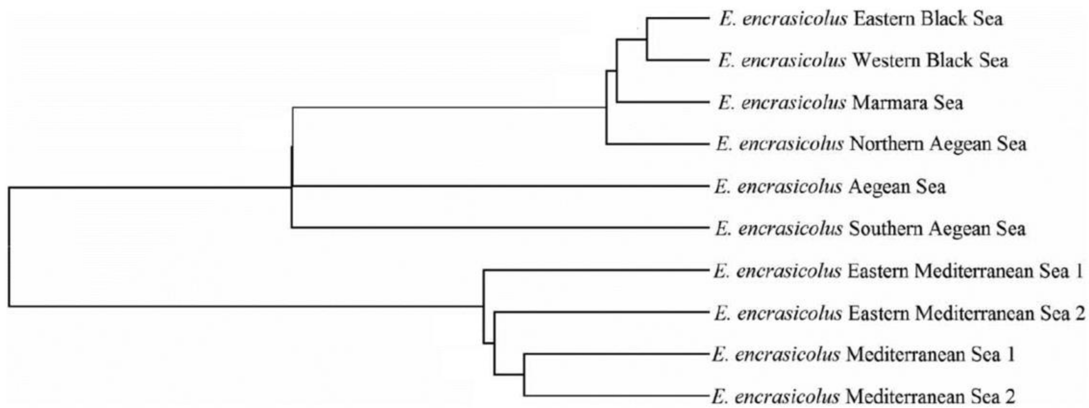

Figure 2.3: Evolutionary relationship of *E. encrasicolus* taxa for sampling sites (Keskin and Atar, 2012).

In the following year, another genetic study was conducted by Ivanova et al. (2013) with the approach of using unspecified muscle esterase polymorphism as genetic-biochemical markers. The study aims to identify the population structure of the anchovy groups in the Sea of Azov and the Black Sea. The samples were collected from Bulgarian, Turkish, Ukrainian, and Georgian coasts and the Sea of Azov. This is the last study in which the genetic differences between Aa and BSa were found. These differences were attributed to the presence of two anchovy subspecies (races) by underlining the hybridization process. The outcome of the study is the alternative migration and summer spawning pattern of the Aa. By the allelic frequencies, Aa was observed on Bulgarian and Turkish coasts in the winter and even in the summer period mixed with the BSa. Although Ivanova et al. (2013) evaluate this mixing as an alternative migration route for Aa, it confirms the co-occurrence conclusion of Zuyev (2019). Moreover, the found genetic variation and the presence of Aa in the western and the southern Black Sea agreed with the results of Kalnina and Kalnin (1984), who used the same method and found the differences.

Tuncay (2014) used mitochondrial and microsatellite DNA analyses to examine the population genetic structure and genetic diversity of European anchovy in the Turkish Seas. Similarly, the highest gene flows were found among Marmara, east and west Black Sea coast of the Türkiye. Moreover, the haplotype diversity is lowest in the Black Sea region. Indeed, microsatellite, mtDNA allelic, and haplotype diversity showed that anchovy in the Eastern Black Sea have less genetic diversity than other sampled regions. She concluded that the genetic diversity of BSa has decreased due to overfishing or other ecological and historical reasons. Her conclusion can be read as an alarming issue in the future of this species in this particular region, especially under the threat of climate change. Nonetheless, the loss of reproductive isolation characteristic of the Azov population due to the salinity increases in the Sea of Azov may diminish the population abundance. Therefore, it raises the question of whether the hybridization effect in the eastern Black Sea region as proposed by Erdoğan et al. (2009) may have diminished in the mid-2010s.

After several years, in 2017, a new genetic study was conducted by Vodiasova and Abramson using the mtDNA cytb gene as a tool to explain the intraspecific genetic variability of European anchovy in the Azov-Black Sea basin. Samples were derived from seven different locations of Crimea, Türkiye, Georgia coasts, and the Kerch and Bosporus straits. Collected samples were pre-separated into subspecies concerning the otolith index (l/w) and the angle between the rostrum and anti-rostrum (β), as proposed by Vodyasova and Soldatov (2017). After the subspecies assignment was completed, subspecies ratios by location were demonstrated. The highest proportion of Aa was encountered in the Kerch Strait (100%). In contrast, for BSa, Trabzon, Sinop, and the Bosporus Strait were the locations with the highest ratio (90%, 89%, and 80%, respectively). The Aa, which was said to be found only in the Sea of Azov, but later on the northwestern shelf and all eastern black sea regions, has been proven to be found even on the Bulgarian coast in later studies. In this study, surprisingly, the existence of Aa was detected even in the Bosphorus Strait. This is a result that will make the separate subspecies concept questionable once again.

We can mention that hybridization is high in regions where the Aa, whose main breeding areas are considered less salty estuaries, and BSa, which can reproduce in the whole sea, mix. They may be found together in coastal regions, but the Aa ratio decreases in the open sea due to the low salinity preferences. Even though there are separate breeding areas (the Sea of Azov and the northwestern shelf) where the ancient features are transferred separately to new generations, we can talk about high gene flow hybridization in the whole sea, depending on environmental conditions. Indeed, according to the results of the study of Vodyasova and Soldatov (2017), two dominant haplotypes were found in all samples. However, their frequencies were the same in both subspecies, so the factor that caused this haplotype differentiation could have been evolutionarily influential in both groups at the same rate. That outcome can turn the question of interspecific differentiation of anchovy groups into regional differentiation. This could of course reintroduce the marine and coastal groups hypothesis. For the Black Sea, this hypothesis was first started to be discussed in 1926 by Pusanov and Tzeeb. Indeed, Pusanov classified coastal groups with different taxonomic names of *E. maeticus* and its features attributed to the Aa. On the other hand, the marine group (*E. encrasicolus*) started to be known as the BSa. However, this hypothesis did not become prominently studied in the Black Sea until Bonhomme et al. (2021) brought it up again with their new genetic study covering the whole Mediterranean. The study aimed to re-determine the taxonomic status of the European anchovy. Accordingly, the revealed genetic difference was found to be significant, and the anchovy stocks in the Mediterranean were defined as marine (*E. encrasicolus*) and coastal (*E. maeticus*). Moreover, it was also noted that anchovy is divided into two as marine and coastal in the Black Sea.

If we go back to Vodyasova and Soldatov (2017), they also found the genetic homogeneity of the Azov and Black Sea subspecies regarding the Cytb. The highest diversity was detected in the southeastern part of the Black Sea. This is quite the opposite of Tuncay's genetic diversity results (2014). The reason for these differences may be the time of the sampling. While the samples of the Vodyasova and Soldatov (2017)'s study were collected during the anchovy's spawning period-anchovies disperse in the basin-, Tuncay (2014) collected them during fishing season -different groups overwinter in the same area-. Differently, Vodyasova and Soldatov (2017) explained this higher genetic diversity in the southeastern Black Sea by a higher population growth rate in the eastern part of the basin compared to the western one by referring to the high catch rate in this particular region. Finally, the study's primary conclusion was that there were no genetically distinct subspecies of Aa and BSa. Based on the mitochondrial cytochrome b gene variability, they can only be defined as local populations. The possibility that both subspecies evolved from a single ancestor was emphasized. Although they had colonized different regions, it has been said that since there is no definite geological barrier between them, the gene flow between them is such high that it prevents genetic differentiation. Still, it has been deduced that this semi-isolation may create some morphological differences between Aa and BSa populations under the influence of environmental factors at their feeding grounds, such as temperature, salinity, food type they feed on, etc.

In 2018, Düzgüneş et al. conducted a genetic study using mitochondrial DNA polymerase chain reaction-restriction fragment length polymorphism to be able to reveal the phylogenetic relationships in anchovy populations in the Black Sea (South-east part), Sea of Azov, and the Marmara Sea. The samples were collected during the winter period from the Marmara Sea, Sea of Azov, Abkhazia, Georgia, Trabzon-Türkiye, and in summer from Trabzon-Türkiye. The results of the study showed that the populations of the Marmara Sea compared to the Black Sea and the Sea of Azov populations showed distinct genetic characteristics, which is consistent with previous studies. Moreover, the Aa populations have similar attributes to the Georgia and Abkhazia populations. Similar to the finding of Vodyasova and Soldatov (2017) about the higher diversity in the southeastern Black Sea, Düzgüneş et al. (2018) underlined the haplotype and genotypic richness of the Trabzon (southeastern Black Sea) samples. However, as the authors indicated, no evidence was found for the recent population bottleneck as an answer to the genetic differences among populations.

One of the most recent studies on the genetic diversity of the anchovy in the Black Sea was completed by Nebesikhina and Lebedev in 2019. By referring to the study of

Vodyasova and Abramson (2017), the authors claimed that mitochondrial DNA (Cytb) is not useful enough for revealing the genetic differentiation between Azov and Black Sea anchovy. Therefore, they used the polymorphic microsatellite markers to demonstrate the population structures of anchovy in the Black Sea. Moreover, they believed that this method makes the measure of gene flow level among the populations more possible. The samples were collected from the Black Sea and the Sea of Azov during summer and winter. The interpopulation differences were found as 4%, whereas intrapopulation differences were 96%. They affirmed the active genetic exchange between the Aa and the BSa by the migrants per generation percentage value (22.5%).

Furthermore, Nebesikhina and Lebedev (2019) asserted that the BSa and Aa might be a single stock by testing the microsatellite loci. According to the results of this study, the absence of two separate subspecies was also proven by polymorphic microsatellite marker tools. They concluded that the phenotypic differences between the BSa and Aa are epigenetic.

The latest genetic research was conducted by Nebesikhina et al. in 2019. The same microsatellite marker method was used, but the sampling area was extended to the Adriatic Sea and the Bay of Biscay. In this study, the result did not change from the one found in the previous one, and no genetic difference was found between the Aa and the BSa. The genetic variations among them are just at the intrapopulation level. For this reason, their phenotypic features were identified as paratypical this time. Nebesikhina et al. (2019) defined the BSa -Aa as different stocks with similar genetic features but exposed to particular environmental conditions.

Anchovy in the Changing Black Sea

As emphasized more loudly nowadays, the Black Sea and the Sea of Azov are on the verge of climate change. The disappearance of the salinity barrier between the two seas, which was observed before, especially in the 1970s, is on the agenda again. This appears to be a factor that will increase hybridization among paratypical anchovy groups. Their different proportioned temporally and spatially overlapping presence may affect the rate of this hybridization process. The

differences found in the first genetic studies were probably due to the reproductive isolation of the Azov population to less saline water. But, under the condition of the rapid salinization process of the Sea of Azov and the reduced river input due to anthropogenic and climatic reasons, the distinct spatial regions in both seas may diminish. This, in turn, can remove reproductive barriers between groups. As Mayorova and Chugunova stated in 1954, this may raise the possibility that the already dominant in number Black Sea anchovy group may have achieved absolute dominance and that this group may have formed different environmental ecotypes.

On the other hand, according to Zuyev (2019), the subsequent hybridization process between the BSa and the Aa has occurred since their first meeting after the last connection of the Mediterranean and the Black Sea. The evolution of anchovy in the Black Sea may oscillate between those that came earlier and adapted to the less saline environment and those that come through the Bosporus Strait from the saltier Mediterranean region. These dynamics are shaped by environmental conditions, primarily temperature, salinity, and food availability. Of course, this is natural selection based on environmental conditions. Due to their highly adaptive short-life-span features and the overfishing they expose, anchovy may be susceptible to these rapid ecological condition changes.

As the genetic studies showed, there are no genetically different groups in the Black Sea. Depending on the environmental conditions, especially where they were born and fed, they may have a shared gene pool that constitutes their ecotype. They may vary in specific characteristics due to genetic responses to certain environmental factors. Probable intraspecies groups may have remained separate as long as these phenotypic differences could develop (Ihssen et al., 1981).

In any case, the deficiency of the studies in the southern part of the sea, especially underlined by Chashchin (1996) and Zuyev (2019), leaves the inference with a holistic approach incomplete and the final classification controversial. In addition, as it is understood from the literature, the environment plays an important role in the dynamics of anchovy and, thus, in its classification. Therefore, in intraspecies heterogeneity studies, it is essential to understand the characteristics of the water in which the samples were collected, for clues it may contain, and to test the environmental ecotype hypothesis.

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CHAPTER 3

An Otolith-Based Approach to Estimate the Growth Parameters: A Case Study for the Anchovy in the Black Sea

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Abstract

The Black Sea anchovy is a small pelagic fish that is aimed for sustainable exploitation and managed using scientific stock assessment models. The growth is one of the important parameters estimated outside of the models and used as a component within the model. Therefore, using uncertain growth parameters can cause unrealistic estimations of the stock condition, the stock's reproductive potential, recruitment, fishing mortality, and consequently the mismanagement of the targeted stock. For the Black Sea anchovy, the von Bertalanffy growth curve is commonly used for parametrization of the length-at-age information. Using fish length information may bring some observational error sources together, such as aging, measurements, and sampling. This study aims to eliminate these biases using the otolith-based chronological increment width in growth parametrization and compare the obtained results with published growth parameters. This method was found to be more convenient to use in estimating the growth parameters of L_∞ = 12.9 cm, K=0.765, and t_0 = -0.134 and representing the stock more precisely. Moreover, decreasing Linf throughout the sampling years was another remarkable outcome of this study. Consequently, the employed otolith increment information is

believed robust and demonstrative of enhanced reliability in portraying the anchovy stock dynamics. Therefore, it is suggested to use growth parameters obtained by this approach which promises more accurate stock assessment results and consequently the more effective sustainable management of the Black Sea anchovy stock.

Keywords: Black Sea anchovy, growth, otolith increment, von Bertalanffy, stock assessment

Introduction

In this new decade, the resilience and the sustainable usage of marine food sources, especially fish, are becoming more and more substantial (FAO, 2021; UNESCO-IOC-Sustainable Development Goal 14, 2021). In this context, the Black Sea demonstrates its importance by accounting for about 7.9 % of the total marine fisheries revenue in the General Fisheries Commission for the Mediterranean (GFCM) areas (FAO, 2022). In this special sea, ~90% of this marine capture is represented by eight species which are priority managed under the supervision of GFCM. Among them, anchovy *Engraulis encrasicolus* (Linnaeus, 1758) inhabited the Black Sea, a fast-growing, short-lived small pelagic fish species, which accounts for 64.7% of the total landings in 2018-2020 alone, is economically prominent. Being a top-target species for commercial fisheries, anchovy stock is managed according to the results of an age-based, advanced, scientific stock assessment model.

While these models are specifically based on fishery data, they also use some other important parameters and the parameters derived from them. One of the most important of these is the fish's growth information. The growth, a mathematical description of how much and how fast the fish increases its size in time, is a central phenomenon in fish population dynamics and fisheries assessment (Lorenzen, 2016; Maunder and Piner, 2015). It is a parameter calculated outside of these mathematical models using, generally, length-at-age data (Francis, 2016). Thereafter, this life history information is used within the model to estimate the biomass, convert catch estimates from biomass to the number, yield-per-recruit analysis, and selectivity estimation (Schueller et al., 2014; Maunder and Punt, 2013). Moreover, it is also used in the calculation of natural mortality which eventually affects the fishing mortality rate estimations (Williams and Shertzer, 2003; Pauly, 1980). Therefore, using uncertain growth parameters can cause unrealistic estimations of the stock condition, the stock's reproductive potential, recruitment, fishing mortality, and consequently the mismanagement of the targeted stock (Maunder and Piner, 2015).

Otoliths are the calcified structures found in the teleost fish. They are the structural units of fish biology for hearing and balance (Popper and Lu, 2000; Campana and Thorrold, 2001; Rodríguez Mendoza, 2006; Payan et al., 2004). They are biologically inert and therefore mainly used in age and growth studies since 1899 (Bilton, 1974 cited by Campana and Thorrold, 2001; Ricker, 1975; Basilone et al., 2020). Due to the slow and fast growth periods of fish in temperate regions, the accumulation of the materials on the otolith decreases and increases depending on these cycles. These cyclical changes form opaque and translucent zones known as annual rings. These incremental rings allow the age of fish to be determined on both daily and annual time scales (Campana and Thorrold, 2001). As underlined by Francis (1990), the age increments on the otolith represent also the growth of the fish and can be used to reconstruct the fish length-at-age as well as the growth characteristics in individuals (Morrison et al., 2019; Morrongiello et al., 2012). The major assumption for using growth increment widths in growth parameter estimation is to prove the linear relation between fish length and the otolith increment width (Vigliola and Meekan, 2009; Campana and Neilson, 1985). The other assumption is to define the age increment with accuracy and precision (Campana,1990; 1992). Although in a few fish species correlation between fish and otolith size cannot be shown, for the anchovy it has been proved (Basilone et al., 2004; Zengin et al., 2015; Carbonara et al., 2023).

The first available information about anchovy growth in the Black Sea is from 1949 by Berg et al. Thereafter other studies were conducted which mainly focused on the growth of anchovy (compiled in Table 3.2). It is known that the growth of anchovy living in temperate regions is slower in winter and higher in summer (Huret et al., 2019). Therefore, it can be readily anticipated that fish of the same age can be of different sizes at different times of the year. However, in the studies conducted for the Black Sea anchovy, the issue that is believed to have a direct effect on the growth parameter estimations is that the calculated growth parameters are based on fish size, and the sampling was done at different times of the year. When compiling the length intervals at age data, the anchovies of the same age but sampled at the beginning and end of the year may remain in different size ranges. Due to the probability of moving from one size class to the other size classes at age, this can lead to an incorrect growth result and may not represent the stock correctly. Additionally, the samples used in the studies are generally from commercial fisheries (Solak and Bilgin, 2020; Bilgin et al., 2013; Şahin et al., 2006; Samsun et al., 2004; 2006). Due to the minimum landing size rule since 1989 (Üstündağ, 2010), the smaller and younger samples in the specimen can be neglected, leading to questionable growth estimates. Therefore, in this study, age increment widths were measured from otoliths obtained from anchovy samples collected by scientific surveys were measured. It is aimed to recalculate the growth parameters for Black Sea anchovy with the fish size information obtained from the otolith corresponding to the completed age.

Material and Methods

Study materials

The data used in this study were obtained within the framework of the scientific surveys conducted with the financial and technical support of the Turkish Scientific and Technical Council (TUBITAK KAMAG-110G124), the Ministry of Food, Agriculture, and Livestock; the Office of Naval Research Global (Grant No: N62909- 16-1-2092); and the BlackSea4Fish Project of the General Fisheries Commission for the Mediterranean and the Black Sea.

All surveys were conducted with RV Bilim-2 of the Middle East Technical University (METU). Sampling stations, demonstrated in Figure 3.1, covering the Turkish Exclusive Economic Zone represent coastal and offshore regions of the Southern Black Sea (40.9^o – 43.4^o N and 28.0^o - 41.4^o E). However, the 2013 winter covered only the South-Eastern part of the Turkish Black Sea coast. For the winter of 2016, data from three stations representing the east, west, and central black sea were used in the study.

Figure 3.1: Stations in the southern Black Sea where the samples used in this study were collected.

A total of 3150 fish samples collected in 2012 - 2016, 2018, and 2020 were included in the analysis. These samples are from two different seasons; summer (July and August; 2013, 2015, 2018) and winter (October 2014; November 2012, 2013, 2016, and 2020; and December 2012, 2020) (Table 3.1). For fitting the growth curve, otolith increment lengths representing the length of fish at a certain age were back-calculated instead of the measured total fish length. Therefore, sampling time within the years does not affect the results of the study. This is one of the advantages of this approach.

Table 3.1: Total sample size used in this study to the seasons

Sampling Years	**Summer sample size in #**	**Winter sample size in #**	**Total sample size in #**
2012	-	323	323
2013	472	160	632
2014	-	610	610
2015	654	-	654
2016	-	56	56
2018	496	-	496
2020	-	379	379
Total	1622	1528	3150

Collected frozen samples were analyzed at the METU Marine Sciences Institute Marine Biology and Fisheries Department Laboratory. Total length in cm (±1 mm) and total weight in grams (±0.001g) were measured. Subsequently, the dissection of individual samples was performed. Following these measurements, otolith extraction was performed. The extracted sagittal otolith pairs were cleaned, dried, and stored in small plastic tubes for later age readings.

Fish ages from the otoliths were determined under the reflected light against a black background using a binocular microscope (Olympus SZX16) with a magnification of 25X while immersed in 70% ethyl alcohol and oriented as the distal surface (convex side) up. During age reading the Common Age Reading Protocol for the Black Sea anchovy prepared by Black Sea experts under the supervision of the BlackSea4Fish Project (GFCM, 2019) was followed. This protocol was presented in Chapter 1.

As soon as the age was determined, the photographs of the otolith pairs in *tiff* format (to retain the image detail and colors) were taken with the Olympus UC30 Microscope Camera. The aimed measurements on the otoliths (Figure 3.2) were done on these photographs by using the right-side otolith. It was preferred to make the measurements on the photograph rather than the live view from the microscope. In this way, inaccurate measurements due to possible light instabilities are believed to be avoided. For all measurements, Stream Basic Version 1.8 Software was used.

The increment measurements (±0.001 mm) were done for each corresponding age ring from the nucleus (approximate center of primordia) to the posterior edge along the long axis (Figure 3.2). The radius of the first true hyaline ring is used as a representative of the length of the fish at its age 1. The further ring radii are for the following ages. Each increment radius is assigned to a formation year by back-counting from the catch date. Nevertheless, in order to solve the fitting of the growth curve to the zero-age, the translucent core radius (R0) was also used. Whereas the R0 is not so clearly visible in all otolith samples, only the samples in which this ring is 100% visible were selected and measured (n=1436).

Based on the linear regression relationship between otolith radius and fish length, the mean back-calculated length-at-age was used to estimate the growth.

a) b)

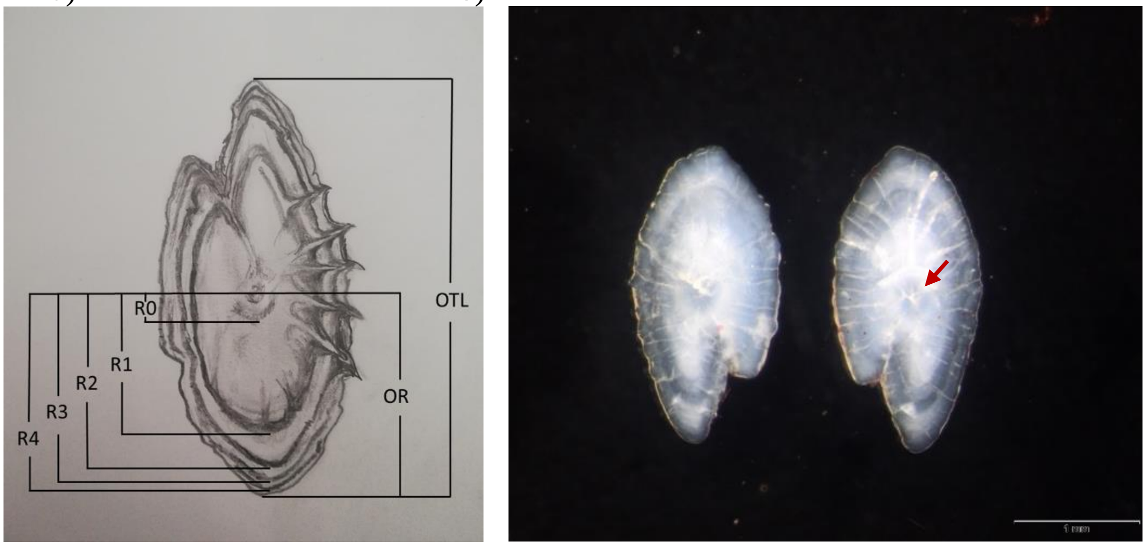

Figure 3.2: a) Increment measurements on the otolith: OTL: otolith's total length; OR: otolith's total radius; R0 (b): translucent core zone radius represented the zero-age increment; R1: first annulus' radius; R2: second annulus' radius; R3: third annulus stands for age 3; R4: fourth year's annulus for age 4.

Growth Information Collected from the Literature

The growth parameters of anchovy in the Black Sea for the region covering the entire Black Sea and Sea of Azov between 1949 and 2018 were compiled from the literature and summarized in Table 3.2. To reach these sources, all available publications were searched. Moreover, the FishBase and the database of the GFCM BlackSea4Fish Project were used.

The ranges for Linf: 10.3-23.5 cm in total length, K: 0.138-2.064 per year, and t0= (-6.165)- (-0.070). Considering the 5% probability that the true value is not within the confidence interval, the Linf and K parameters within a 95% confidence interval to the mean (μ±2σ) were selected. Accordingly, parameters 11.53<Linf<21.57 and 0.126<K<1.520 were selected. On the other hand, unrealistically low t0 values, lower than -1, were removed from the analysis. These selected growth parameters were used in comparing the results of the current approach.

Table 3.2: von Bertalanffy growth parameters for anchovy published in the Black Sea and the Sea of Azov

Selected	Year	Location	Linf (cm)	K (1/ year)	t0 (year)	a	b	ϕ	Sex	References
L1	1949	Azov	11.7	1.16	-0.17	NA	NA	2.20	C	Berg et al., 1949
L2	1954	Russia//Eastern BS	14.2	0.81	-0.54	NA	NA	2.21	C	Majarova and Chungunova, 1954
L3	1954	Romania// Western BS	15	1.26	-0.23	NA	NA	2.45	C	Majarova and Chungunova, 1954
L4	1964	Russia//Eastern BS	14.1	1.01	-0.28	NA	NA	2.30	C	Svetovidov, 1964
L5	1961	Bulgaria// Western BS	16.6	0.581	-0.92	NA	NA	2.20	C	Stoyanov, 1961
	1983	Azov basin	13.30	0.610	-1.800	NA	NA	2.03	C	Volovik and Kozlitina (1983)
	1985	Turkish Coast	16.7	0.33	-2.27	NA	NA	1.96	C	Özdamar et al., 1991
	1990	central-eastern Black Sea	16.80	0.324	-2.070	NA	NA	1.96	C	fishBase
	1990	Turkish Coast	23.5	0.14	-3.08	0.0065	2.978	1.89	C	Okur (1991)
	1996	Turkish Coast	11.04	0.634	-0.746	NA	NA	1.89	C	Bingel et al., 1996
	1996	Turkish Coast	19.70	0.224	-1.101	NA	NA	1.94	C	Bingel et al., 1996
	1996	Turkish Coast	23.38	0.174	-1.330	NA	NA	1.98	C	Bingel et al., 1996
	1999	Turkish Coast	15.50	0.420	-1.830	NA	NA	2.00	C	Mutlu, 2000
	2002	Turkish Coast	15.3	0.39	-2.11	NA	NA	1.96	C	Şahin et al., 2003
	2003	South East BS	18.91	0.163	-3.700	0.0080	2.8695	1.77	C	Samsun et al., 2006
	2006	South East BS	15.272	0.284	NA	0.0055	3.0425	1.82	C	GFCM
	1979-1993	Black Sea	17.09	0.308	NA	NA	NA	1.95	C	Prodanov and Stoyanova, 2001
	1985-1986	Central and South East BS	16.7679	0.3235	-2.0695	0.0023	3.4157	1.96	C	Erkoyuncu and Özdamar, 1989
	1986-1987	South East BS	16.85	0.3241	-1.9882	0.0025	3.3832	1.96	C	Karacam and Düzgüneş, 1990
L6	1987-1988	South East BS	14.14	0.918	-0.320	0.0025	3.3868	2.26	C	Düzgüneş and Karaçam, 1989
	1987-1988	Turkish Coast	17.99	0.294	NA	NA	NA	1.98	C	Özdamar et al., 1994
	1987-1989	Turkish Coast	17.51	0.2773	-2.397	0.0047	3.1002	1.93	C	Özdamar et al., 1994

	1988-1989	Turkish Coast	15.65	0.28	-3.0224	NA	NA	1.84	C	Özdamar et al., 1994
	1988-1989	Turkish Coast	15.73	0.3166	-2.1966	0.0064	2.974	1.89	C	Ünsal, 1989
	1988-1992	Turkish Coast	16.16	NA	NA	NA	3.1275	NA	C	Bingel et al., 1996
L7	1990-1991	South East BS	15.01	0.610	-0.070	0.0049	3.1230	2.14	C	Genc and Basar, 1991
	1991-1992	South East BS	18.30	0.250	-2.140	0.0055	3.0360	1.92	C	Genc and Basar, 1992a
L8	1992-1993	South East BS	16.72	0.500	-0.350	0.0053	2.9990	2.15	C	Genc and Basar, 1992b
	1993-1994	South East BS	15.82	0.340	-2.144	NA	NA	1.93	C	Mutlu et al., 1993
	1994-1995	South East BS	16.83	0.3102	-2.2093	0.0047	3.0975	1.94	C	Özdamar et al., 1995
	1995-1996	South East BS	16.65	0.300	-2.490	0.0052	3.0300	1.92	C	Mutlu, 1996
	1996-1997	South East BS	17.00	0.310	-2.160	0.0073	2.9030	1.95	C	Mutlu, 2000
	1996-1997	Turkish Coast	17.42	0.284	-2.108	0.0057	3.1170	1.94	C	Kayalı, 1998
	1997-1998	Turkish Coast	15.57	0.417	-1.826	0.0055	3.027	2.00	C	Mutlu, 2000
	1997-1998	South East BS	16.97	0.260	-6.165	0.0057	3.0150	1.87	C	Gözler and Çiloğlu, 1998
	1998-1999	Turkish Coast (Sinop)	15.66	0.3368	-2.526	0.0083	2.872	1.92	C	Samsun et al., 2004
	1999-2000	Turkish Coast (Sinop)	17.07	0.2836	-2.1047	0.0076	2.919	1.92	C	Samsun et al., 2004
	2000-2001	Turkish Coast (Sinop)	16.84	0.233	-3.080	0.0118	2.7101	1.82	C	Samsun et al., 2006
	2000-2003	Turkish Coast (Sinop)	18.91	0.163	-3.7	0.008	2.8695	1.77	C	Samsun et al., 2006
	2001-2002	Turkish Coast (Sinop)	18.46	0.217	-2.860	0.0051	3.0568	1.87	C	Samsun et al., 2006
L9	2002-2003	South East BS	14.98	0.840	-0.200	0.0049	3.0922	2.28	C	Çiloğlu and Şahin, 2022
	2002-2003	Turkish Coast (Sinop)	18.73	0.156	-3.969	0.0075	2.8946	1.74	C	Samsun et al., 2006
L10	2003-2004	South East BS	15.70	0.710	-0.250	0.0073	2.9476	2.24	C	Çiloğlu and Şahin, 2022
	2004-2005	South East BS	15.123	0.3701	-2.0944	NA	NA	1.93	M	Şahin et al., 2006
L11	2004-2005	South East BS	15.79	0.690	-0.260	0.0068	2.9710	2.24	C	Çiloğlu and Şahin, 2022
	2004-2005	South East BS	16.036	0.3121	-2.3341	NA	NA	1.90	F	Şahin et al., 2006

	2004-2005	South East BS	16.114	0.2919	-2.5626	0.0101	2.7948	1.88	C	Şahin et al., 2006
	2004-2005	Turkish Coast	21.17	0.196	-2.314	0.0101	2.7900	1.94	C	Bilgin et al., 2006
L12	2006-2007	South East BS	15.38	0.760	-0.230	0.0084	2.8291	2.25	C	Çiloğlu and Şahin, 2022
	2006-2010	Bulgarian Coasts	14.60	0.479	-1.760	NA	NA	2.01	C	Yankowa, 2014
L13	2007-2008	South East BS	13.34	0.700	-0.270	0.0051	2.0974	2.10	C	Çiloğlu and Şahin, 2022
L14	2008-2009	South East BS	14.28	0.900	-0.180	0.0103	2.7826	2.26	C	Çiloğlu and Şahin, 2022
	2008-2009	Turkish Coast (Samsun)	17.01	0.230	NA	0.0096	2.8166	1.82	C	Özdemir et al., 2018
	2008-2011	South East BS	16.52	0.360	-2.020	0.0124	2.7110	1.99	C	Kasapoğlu, 2018
	2010-2011	Mid-BS and Samsun	13.063	0.978	-0.8	NA	NA	2.22	M	Bilgin et al., 2013
L15	2010-2011	Mid-BS and Samsun	13.68	1.188	-0.86	NA	NA	2.35	F	Bilgin et al., 2013
	2010-2011	SE-BS, Rize	13.85	0.855	-0.25	NA	NA	2.21	M	Bilgin et al., 2013
L16	2010-2011	SE-BS, Rize	13.955	0.993	-0.06	NA	NA	2.29	F	Bilgin et al., 2013
	2010-2011	South East BS	16.368	0.425	-1.35	0.011	2.742	2.06	C	Sağlam and Sağlam, 2013
L17	2012-2013	South East BS	14.09	0.910	-0.180	0.0051	3.1487	2.26	C	Çiloğlu and Şahin, 2022
	2013-2014	South East BS	10.80	1.027	-1.197	NA	NA	2.08	M	Solak and Bilgin, 2020
	2013-2014	South East BS	11.10	2.064	-0.611	NA	NA	2.41	F	Solak and Bilgin, 2020
L18	2014-2015	South East BS	13.98	0.990	-0.150	0.0088	2.8950	2.29	C	Çiloğlu and Şahin, 2022
L19	2015-2016	South East BS	14.47	0.880	-0.180	0.0067	3.0136	2.27	C	Çiloğlu and Şahin, 2022
L20	2016-2017	Sea of Azov	12.20	1.220	-0.380	NA	NA	2.26	C	Chesalin et al., 2020
L21	2017-2018	South East BS	12.86	0.880	-0.190	0.0178	2.7738	2.16	C	Çiloğlu and Şahin, 2022
	2017-2018	Kerch Strait	14.00	0.440	-1.670	NA	NA	1.94	C	Chesalin et al., 2020

*C: combined sex; F: female, M: male

Statistical analysis

The correlation (Pearson's Product Moment Correlation; Eq (1.1)) and linear regression (Eq (1.2) between the total fish length in cm and the total otolith radius in mm were calculated. The asymptotic length was estimated with the information derived from the otolith. Then, by the linear regression equation, it was converted into total fish length.

Pearson's correlation coefficient (r) is calculated as (x and y are variables),

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}} \tag{3.1}$$

where r is Pearson's Product Moment Correlation coefficient, x_i is the -ith value of the independent variable, y_i is the -ith value of the dependent variable, x and y mean are the mean values of the x and y variables.

$$TL = a + bOR \tag{3.2}$$

where TL is the measured fish length, OR is the otolith radius, a is the intercept-fish length at otolith formation- and b is the slope of the TL-OR relationship.

The total length-weight relationship (LWR) was estimated following the equation

$$W = a * TL^b \tag{3.3}$$

by transformed into

$$\log \mathrm{W} = \log \mathrm{a} + \mathrm{b} \log \mathrm{TL} \tag{3.4}$$

where W is an independent variable-the total weight in gr of the individual anchovy, TL is a dependent variable- the total length in cm, and as regression coefficients: a is an intercept and b is the allometry coefficient.

The most commonly used method in growth studies in fisheries studies is the von Bertalanffy growth model (Lorenzen, 2016; Flinn and Midway, 2021). It was developed essentially as part of the general application of open systems theory to biological systems by von Bertalanffy with different mathematical versions. But the version proposed by Beverton and Holt (1957) is the one mostly used in fisheries science (Allen, 1966) is used in this study. The von Bertalanffy growth (vBG) parametrization of a nonlinear regression equation (von Bertalanffy, 1938, 1957; Beverton and Holt, 1957) is simply,

$$Lt = L\infty(1 - e^{-K*(t-t0)}) \qquad (3.5)$$

where; L_t is the length at time t, L_∞ is the theoretical asymptotic length, K is the growth rate: the rate at which the asymptotic size is approached and t_0 is a hypothetical age at which fish has zero length. It is the equation that analyzes the functional relationship between the mathematical variables of fish length and age, by including the parameters (estimated by the best least-squares) of L_∞, K, and t_0 (Kirkwood, 1983).

The growth performance index (ϕ: phi-prime) (Pauly and Munro, 1984),

$$\phi = \log10(K)+2\log10(L\infty) \qquad (3.6)$$

where ϕ is the growth performance index used as a tool to compare the growth of the same species, K is the growth rate parameter obtained from the vBG model together with L_∞ which is the asymptotic length of the fish. This index is used to overcome the correlation between K and L_∞ (Pauly and Munro, 1984).

A total of 4887 back-measured annual radii at the age were used to fit the vBG curve. However, the increment width numbers in each age group were unequal. The specimen numbers of smaller and younger were higher. This eventually may affect the growth estimation for the older and bigger anchovy. To address this imbalance, random subsampling with a sample size of 7 was applied for each age. The sample size of 7 was chosen because it was the entire sample size that was 5 years old. Every subsampled set had an equal number of length information at age. The parameters of L_∞, K, and t_0 were estimated for each set by the *optim* function in R, utilizing nonlinear least squares optimization with 2e+10 iterations to minimize the sum of squares of deviation (Nash, 2014). This calculation and the fitting process were repeated 1000 times and the average of the calculated parameters is used as the final estimate.

Considering the effects of different years on growth, the overall composition of fish length for each age group was subjected to an analysis of variance over the sample years. An alpha level of .05 was used for all statistical tests.

All analysis was performed in R version 4.2.2 (within R Studio), using the packages of ggstatsplot_0.10.0 (Patil, 2021), tidyverse_1.3.2 (Wickham, 2019), dplyr_1.0.9 (Wickham, 2022), and ggplot2_3.4.0 (Wickham, 2016).

Results

The primary assumption of the model was tested and a very high positive Pearson's correlation coefficient of 0.96 (p-value < .001) between total fish length and total otolith radius was found. Moreover, the statistically significant linear relationship between total fish length and the otolith radius is 0.92 (p-value < .001) (Figure 3.3) also quite high enough to indicate how these two variables are associated. It was revealed that overall regression between OR and TL was statistically significant (R^2 = [0.92], F (1, 3147) = [3.649e+04], p-value < .001) was linear and highly significant (Table 3.3). The fitted regression model: [OR= 0.0399188+0.1162938*TL].

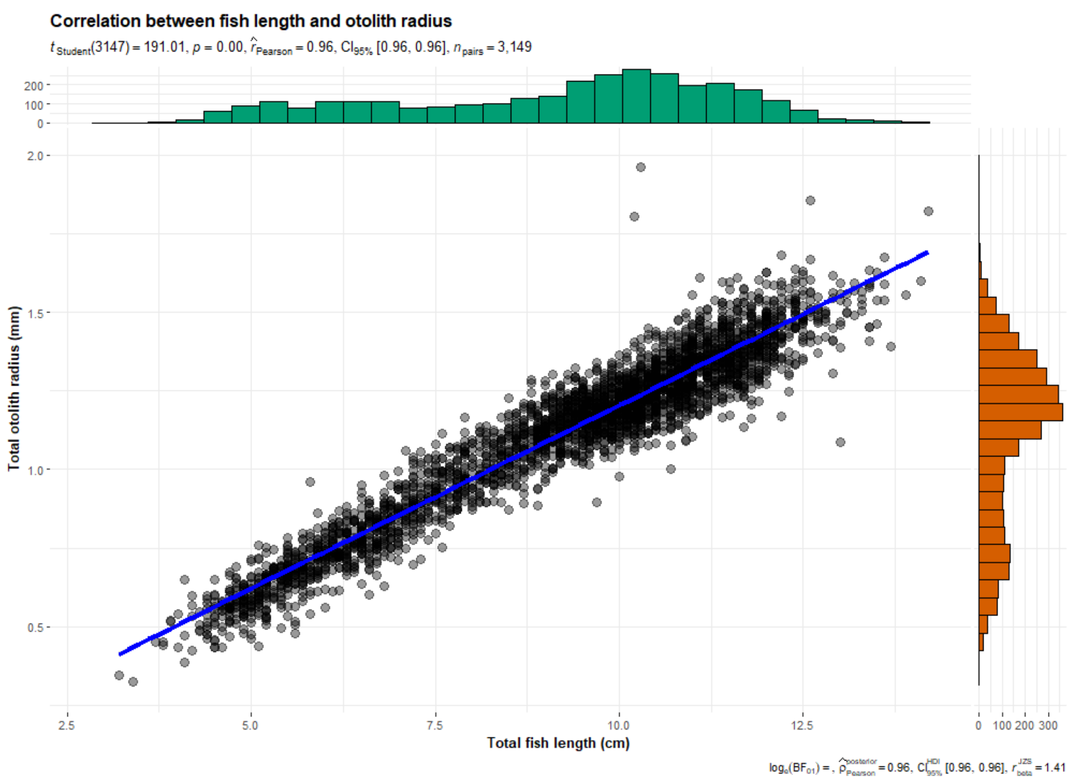

Figure 3.3: Correlation tests between total fish length in cm and otolith radius in mm

Table 3.3: The results of the linear regression analysis between total fish length and total otolith radius

Coefficient	Estimate	Std. Error	t value	Pr (>|t|)
(Intercept)	0.0399188	0.0057574	6.933	4.96e-12 ***
Total Length (cm)	0.1162938	0.0006088	191.014	< 2e-16 ***

The usual approach for fitting the growth curve is to use fish length-at-age data with length class intervals for small species, usually 0.5 cm (Erzini, 1990). In Figure 3.4 below, same-length class intervals were used while fitting the growth curves with 21 different growth parameters selected from the literature along with length-at-age data from 3150 individuals collected as part of this study.

As a result of this study, the initial estimates of vBG parameters based on total fish length were found to be L_∞ = 12.2 cm, K = 0.789, and t_0 = -1.016, independent of time and place of sampling and sample size representing each age. The calculated ϕ was 2.07. From the length-weight relationship, the estimated a was 0.0027 and b was 3.3783. Average Linf in the 2010s has decreased by 18% since the 2000s. Looking at the 90s, this figure rises to 22%.

What is also recognized in Figure 3.4 is the high variety and inconsistency in the length-at-age data in the literature, especially in the length-at-age 0. While the minimum length at age 0 was 0.6 cm, the maximum estimated length for age 0 was 8.8 cm.

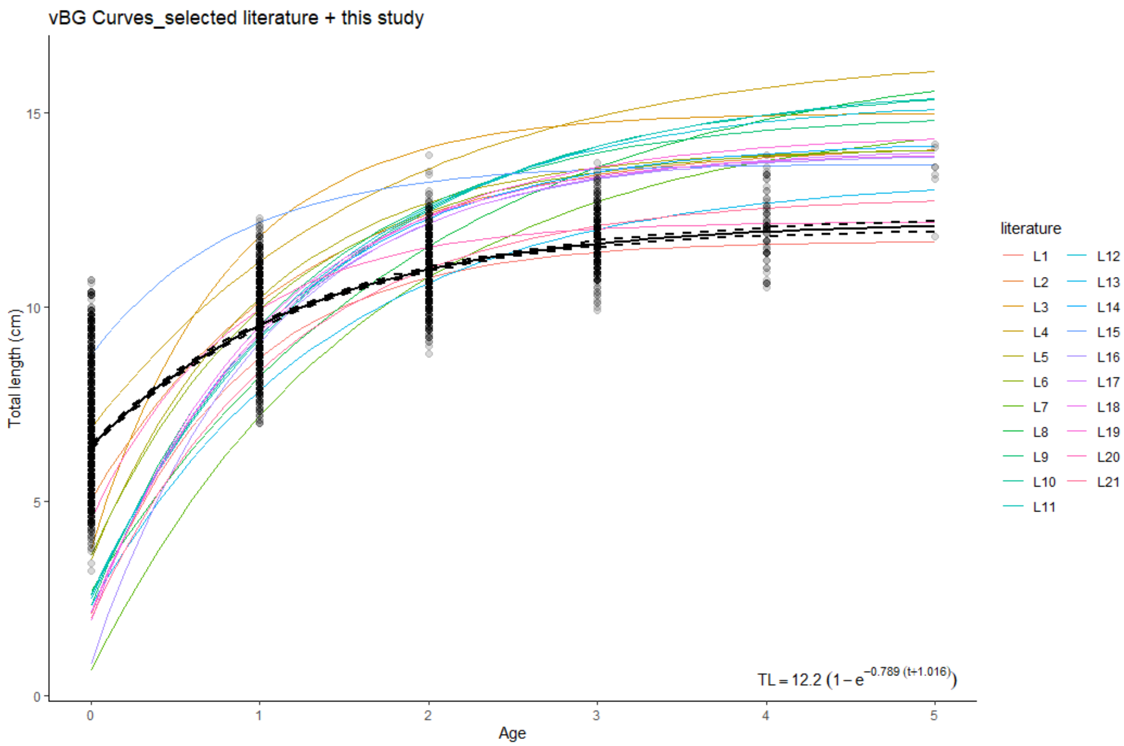

Figure 3.4: vBG curves from the selected literature and the growth curve fitted by data of the current study (black line) with its 95% CI (black-dashed lines). The black dots are the length-at-age data of current studies' samples (the darker the color means increasing the number of samples in that particular age and length)

The 'black line' in Figure 3.4 shows the estimated growth parameters by following the traditional total fish length at age approach using the data of this thesis study. Accordingly, even though the no-size selectivity was applied during sampling in the fishery-independent survey, the anchovies smaller than the recruitment size could not be caught. Moreover, although length stratification was taken into account when sampling, the number of older anchovies is remarkably lower than that of young and small anchovies. There are 1041 specimens at zero years of age, 1192 at one year of age, 566 at two years of age, and 282 at three years of age, while there are 61 fish specimens at four years of age and only 7 at five years of age (dots in Figure 3.4). Therefore, the consequent low L_∞ and high t_0 were most probably the results of unevenly distributed sampling. Furthermore, the combined length-at-age data were fitted in Figure 3.4 by the samples collected at different times of the year.

Hence, the growth curves were fitted with lower and balanced sample sizes were performed by using otolith increment widths at age as a representativeness of the exact length at age. All fitted curves were demonstrated in Figure 3.5. With this approach, both the sampling time problem in length-at-age and the high variation in age-0 were minimized.

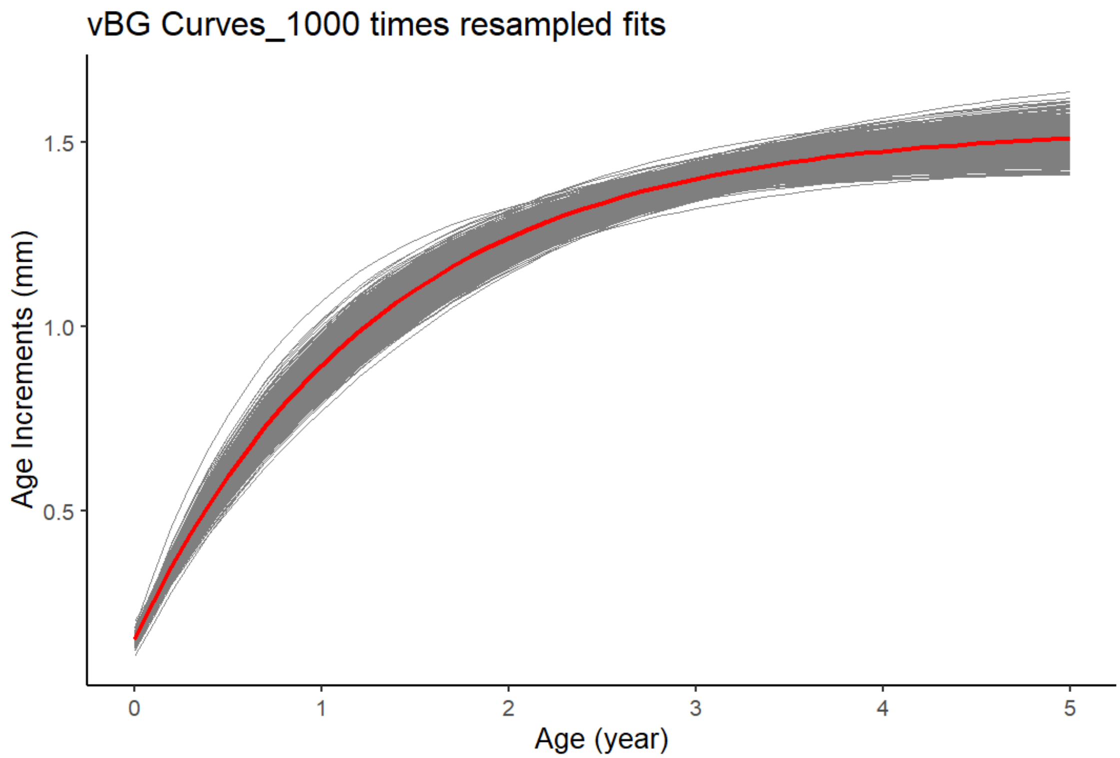

Figure 3.5: The fitted 1000 independent growth curves. The red is the average of all.

The data set was selected from a total of 4887 increments of radii: 1436 for age-0, 2108 for age-1, 917 for age-3, 68 for age-4, and 7 for age-5. 1000 independent subsamples were randomly selected with respect to the smallest number of samples (age-5, n=7). The growth curve was fitted for per dataset that has an equal number of increment lengths at each age. The otolith-based von Bertalanffy growth equation for the average estimation (red line in Figure 3.5) was shown in Equation 3.7 below. When the conversion was based on the linear relation of otolith radius and the fish length, Linf was found as 12.9 cm in total length (3.8). In this case, the calculated ϕ became 2.10.

$$ORt = 1.5\left[1 - e^{-0.765(t+0.134)}\right] \quad (3.7)$$

$$TLt = 12.9[1 - e^{-0.765(t+0.134)}] \quad (3.8)$$

Significant year effects were particularly observed at age 0 (ANOVA; p-value: 0.001492**) and age 1 (ANOVA; p-value: 0.011493*). Therefore, the growth parameters of the anchovy in the Black Sea were estimated for every independent sampling year using the same otolith-based approach (Table 3.4). Sample size in each age was taken into account and growth parameters were obtained from random re-sampling methods. The increase in the growth rate was observed in the latest years which would prove the lowest L_{inf} in 2018 and 2020. Moreover, the growth performance index per sampling year is significantly different than the mean ϕ from the literature (t-test; p-value: 0.0273; rejected H0). Even though a small fluctuation observed over the years, L_{inf} was decreased by 14% from 2012 to 2020. As it was expected the K increased by 16% in the same time intervals.

Table 3.4: Estimated growth parameters for corresponding sampling years

Catch Year	Sample size in #	# of age	Linf TL in cm	K (1/Year)	t_0 (Year)	ϕ	a	b
2012	323	5	13.6	0.943	-0.133	2.24	0.0027	3.3620
2013	632	5	12.4	0.875	-0.118	2.13	0.0026	3.3764
2014	610	4	11.4	0.990	-0.113	2.11	0.0027	3.3938
2015	654	4	12.9	0.765	-0.135	2.10	0.0028	3.3568
2016	56	4	12.8	0.849	-0.113	2.14	0.0036	3.2412
2018	496	5	12.2	1.025	-0.113	2.18	0.0024	3.4236
2020	379	5	11.7	1.098	-0.099	2.17	0.0028	3.3688

Discussion

An otolith-based back-calculation approach was used to estimate the growth parameters of the Black Sea anchovy for the first time. It was found that this method could be an alternative to the traditional mean fish size at age method. Using otolith growth (increment width) as a proxy for fish somatic growth requires a strong linear relationship (Vigliola and Meekan, 2009; Campana, 1990). In this study, statistically significant linear relationship was revealed (Table 3.3). The strength of the relationship (p-value < .001) confirms that fish growth is represented by otolith growth radii.

Growth curve based on mean fish size at age was also fitted for 2012-2020 in this study (Figure 3.4). This method, which was used in previous studies referenced in Table 3.2., was used to compare the results of these studies with the current thesis study. The possible sources of errors, which time of year it was caught, and whether it had a length-at-age composition representing the stock were ignored at this stage. The L_{inf} found was 15% lower than the average L_{inf} from the selected literature. However, the 2016-2017 (Chesalin et al., 2020) and 2017-2018 (Çiloğlu and Şahin, 2022) research findings, which are also below the average, show a reduction in anchovy asymptotic length in recent years. This is a matter of concern and above all, should be underlined. Anchovy is a thermophilic species. Yet, there could be a sign of the Temperature–Size Rule (Atkinson, 1994) started to work for the Black Sea anchovy with the increasing sea-water temperature (Ozkan and Tutak, 2022; Mohamed et al., 2022). So, Global warming (Wright et al., 2020; Hattab et al., 2021; Lefevre et al., 2021; Lindmark et al., 2022; Lavin et al., 2022; Berdnikov et al., 2022) and size-selective fishing (Hamilton et al., 2007; Swain et al., 2007) could be quick explanations for increasing growth rate and decreasing Linf. More research is needed on this topic for sustainable stock management.

In addition to the results of the decreasing fish length, it was also focused on several known bias sources in estimating the growth parameters. Observational error sources of aging, measurements, and sampling errors were considered (Piner et al., 2016). To solve these errors, otolith-based growth increment widths were used by reconstructing the past somatic growth.

Using otolith is reliable. Because, it can be stored as an inert biological material, possibly for a long time, allowing retrospective analysis (Campana and Thorrold, 2001). Also, since it is already used for age reading, it makes it easier to reach more life history information about the fish with the measurements to be made on it. Still, it requires expertise in defining the age rings and measuring the radii of these increments. Erroneous age reading can lead to misestimates of the growth rate, mortality rate, and productivity and eventually affect the management of the stock (Basilone et al., 2020; Beamish and McFarlane, 1983; Goldman, 2005). For many species, age reading is still a controversial issue, but for the anchovy in the Black Sea, the common age reading protocol has been prepared (GFCM, 2019) and improved (GFCM, 2023, in press) by the experts from the all-riparian countries. In this study, the ages were determined with respect to this protocol. Therefore, the other important assumption of reading increments with accuracy and precision (Campana, 1992) was also significantly fulfilled.

t_0 is another problematic issue in growth parametrization. Although the t_0 parameter is not of biological interest (Beverton and Holt, 1957), it may affect the model results and consequently the growth parameters. Using fish samples in growth studies from the commercial fishery increases this uncertainty indeed. Uncertainty in the length-based growth model often emanates from a lack of data on the growth of juveniles (Helidoniotis and Haddon, 2013) and the overrepresentation of the slow-growing old fish and/or fast-growing young fish (Taylor et al., 2005). For the Black Sea anchovy case, it is hard to know to what extent the number-of-length-at-age data is evenly distributed in the samples used in the growth model estimations in the literature. But since it is known that they are from commercial catches, it is possible to say that the inclusion of smaller fish (<9cm) is open to discussion. So, commercial catch sampling is most probably the reason for the unrealistically high t_0 values estimated in the literature (Gözler and Çiloğlu, 1998; Samsun et al., 2006; Şahin et al., 2006; Mutlu, 1996; Özdamar et al., 1994; Erkoyuncu and Özdamar, 1989). To solve this, the option of fixing t_0 to zero is widely used (Taylor et al., 2005; Gwinn et al., 2010). Still, even though it is complex, requiring skilled researchers and time, the increment-based studies give more accurate growth estimates (Neves et al., 2022).

Age increments on the otolith start at age 1, but it was also needed the 0-year-old increment information to fit the growth curve correctly. For this, we used the radius of the translucent core zone (Figure 3.2, b), representing the R0. The spawning of the Black Sea anchovy starts in the middle of May (Lisovenko and Andrianov, 1996) and the defined birth date is the 1st of June (GFCM, 2019). After the anchovy starts to feed (Aldanondo et al., 2008) and a rapid growth process takes place (Aldanondo et al., 2011). It is known that as soon as the fish starts feeding, the opaque formation will start together (Panfili et al., 2002). Therefore, the formation of the opaque region represents the life interval of fish where intense nutrition and growth (Beckman and Wilson, 1995).

Moreover, this translucent increment around the center is also called as the “larval ring” in the Black Sea region (Anonymous). Since this translucent core layer represents the region between the first feeding and the rapid growth zone, it can represent the beginning of age zero, that is, at the age of zero with small decimal-only days. This zone is most probably the only macroscopic zone that can be practically used in the assessment purposed annual increment-based growth studies. It is also believed that this is the optimum approach to both fit the growth curve accurately to the age zero and eliminate the uncertainties due to the lack of juvenile information.

This otolith back measurement method also solves the sampling problem by itself. The size-selected fishery sampling is not the only sampling problem. Sampling in the different time periods and fitting the growth curve with the assumptions of each age contained randomly chosen length information (Francis, 2016; Piner et al., 2016) are other sources of bias. Moreover, while slicing, the separation of the length data with 0.5 cm class intervals may also cause shifts between the length groups within the ages depending on the sample collection time. Furthermore, considering the birth date, if the fish was caught on November 21 and aged as 1-year-old, it does not mean the exact age. It means this fish's life span is 538 days. So, the fish’s biological age is 1.5 and the age should be written decimally. However, while estimating the vBG parameters, this knowledge is generally disregarded. Therefore, it creates deviations in the correct estimation of growth parameters. On the other hand, by measuring the incremental width of the rings, estimating the growth parameter with the length corresponding to the exact age allows for minimizing the errors in the sampling time and the data processing part.

At this point, the t0 value in the growth parameters obtained by this method was higher than that estimated with length, as expected. However, the estimated Linf with increment method still shows a decreasing trend in asymptotic length. The reason for this is thought to be due to two things. Either, the data collected in fishery-independent scientific research could not completely sample the larger and older fish. Or, this is the worst-case scenario, the signs of a dramatic decrease in anchovy size have already appeared, as reported by Dagtekin et al. (2022).

It is also known that growth is affected by many environmental and biological factors. Therefore, it is inevitable that the growth will not remain the same from year to year. However, unfortunately, there are not enough studies that represent each year and cover large regions. In stock assessment studies, the growth information in the publications is sometimes combined and sometimes used for the years it represents. This causes the growth information of one year to be used simultaneously in many years in management models. Naturally, this leads to model results that do not represent the stock. Therefore, as part of this study, the growth parameterization was carried out separately for each sample year. These growth parameters obtained by the otolith-based back-calculated length are the results that have eliminated the observation error concerns. Hence, it is believed to better reflect the stock. Thus, it can be directly used in the estimation of M and other parameters for use in management models. When the parameters in the literature were compared with these research years, it was seen that only 2012 was compatible with the study of Çiloğlu and Şahin (2022) for the same year (Table 3.2, Table 3.4). These differences in the literature may be due to observation errors in the data calculated with the length-based approach or may be due to growth variation. In other words, both in the sample of this study and in the samples of previous studies, there may be groups with different growth characteristics. The presence of these groups at different proportions in the sampling area may make it questionable whether the growth parameters found reflect the stock, even if the observation errors are removed entirely (Helidoniotis and Haddon, 2013). Therefore, testing the possible differential growth of fish is also crucial for population dynamics and stock assessment studies.

Finally, growth modeling is an essential part of modern fisheries stock management aimed at the sustainable use of fish stocks with growth-sensitive models. Therefore, to achieve the sustainability goals, among other proper data collection attentions, the parameters as growth estimation should be robust and represent the stock itself. For this reason, using the otolith-based growth increment approach is suggested as an accurate and practical approach for parametrization of the fish growth by removing the observation errors' effect on growth estimation.

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CHAPTER 4

Detection of Anchovy (*Engraulis encrasicolus*) Groups with Different Growth Characteristics in the Southern Black Sea Using Otolith

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Abstract

Anchovy *Engraulis encrasicolus* (Linnaeus, 1758) is a fast-growing, short-lived small pelagic fish species that is economically important in the Black Sea. Accepting anchovies in the basin as a unit stock is the major presumption of the current assessment models used for sustainable stock management. Whereas, the existence of at least two distinct intraspecific stocks of anchovy has been an ongoing debate since the early 20th century and is still being discussed. Many methods, from blood groups to otoliths to advanced genetic tests, have been attempted to separate these groups. Although it is claimed that these two groups grow differently, no detailed study has been conducted on the subject so far. Anchovies are estimated to reach ~72% of their asymptotic total length in their first year of life. Therefore, this study aims to define possible distinct groups according to their first-year growth inferred from the otolith. For this purpose, 3150 anchovies' right sagittal otolith samples collected from the entire southern Black Sea in 2012-2016, 2018, and 2020 were used. Two groups with growth performance indices of 2.05 and 2.29, called "Group 1" and "Group 2", with different growth rates of K=0.726 and K=1.347, respectively were identified (likelihood-ratio-test;chi-square-statistics: p-value< .001, df=3). Within the born years, the proportion of Group 2 in the population has decreased significantly, while Group 1 has tended to increase. Based on this, it can be claimed that the sustainable management of this valuable fish can be ensured by knowing different growing groups and their proportional changes over the years and managing the stocks accordingly.

Keywords: growth, otolith increment, first-year growth**,** Black Sea anchovy

Introduction

Anchovy *Engraulis encrasicolus* (Linnaeus, 1758) living in the Black Sea is a fast-growing, short-lived small pelagic fish species. This species, which is the target species for commercial fishing with 64.7% of the total catch only in 2018-2020 (FAO, 2022), is ecologically very important for its trophic position in the basin as well as its economic value. In the literature, the intraspecies structure of the known anchovies has been assumed to have two hierarchically upper forms, defined according to their morphological and behavioral characteristics. One of them is the Black Sea anchovy *Engraulis encrasicolus ponticus* (Alexandrov, 1927), which feeds and reproduces mainly in the entire Black Sea (Chashchin, 1995), and the other is the Azov anchovy *Engraulis encrasicolus maeticus* (Pusanov, 1923;1936) which prefers to forage and spawn in the Sea of Azov (Chashchin, 1995). Different groups are known to exist, but their nomenclature and how to be distinguished are still a matter of debate (see Chapter 2).

To date, several methods have been used to separate these anchovy groups (the terminology "group" is used in this study) such as parasite infestation (Danilevsky and Kamburov, 1969; Chashchin,1981) fat content (Shulman et al., 2008; Shulman, 2002; Chashchin, 1996, blood type (Limansky, 1970; Altukhov and Salmenkova, 1981) otolith index (Skazkina, 1965; Chaschin, 1996; Vodyasova and Soldatov, 2017; Zuyev, 2019), body morphometry (Şahin, 2014) genetic studies (Nebesikhina et al., 2019; Düzgüneş et al., 2018; Vodiasova and Abramson, 2017) (for more detailed information see Chapter 2). In line with the applied methods, two different groups of anchovy inhabiting the Black Sea were classified as “races” (Aleksandrov, 1927; Altukhov and Salmenkova, 1981; Kalnin and Kalnina, 1985; Chashchin, 1995; Melnikova, 2013; Chashchin et al., 2015), “populations” (Ivanova and Dobrovolov, 2006), “subpopulations/elementary populations" (Altukhov et al., 1969), "subspecies" (Zuyev, 2019; Ivanova et al., 2013; Ivanov and Beverton, 1985; 1996; 1998; 2015; Shulman, 2002), "herds-as authors called" (Danilevsky and Kamburov, 1969 cf. Zuyev, 2019), "stocks" (Nebesikhina et al., 2019) and "ecological morphs" (Vodiasova, 2020). Despite the fact that the issue is still being debated (Zuyev, 2019), according to the WoRMS Editorial Board (2023), these two groups were taxonomically classified as subspecies. Nevertheless, the hybridization hypothesis put forward by Chashchin (1995; 1996) has been disregarded.

In addition, when separating the Black Sea and Azov anchovies morphologically, their growth rates were also taken into account. Literature has repeatedly emphasized that the Black Sea anchovy of the same age is longer than the Azov anchovy (Chashchin, 1995; 1996; 2015). So far, however, there is no available study on the growth differences. At the point reached it was an outcome that there is no genetic difference between the aforementioned Azov and Black Sea anchovies and that differentiation can occur due to their exposure to certain environmental conditions (Nebesikhina et al., 2019). So, this situation brought to mind that anchovies exposed to different environmental factors can have different growth characteristics depending on the conditions. However, in order to understand how and what conditions affect growth, it is first necessary to determine whether Black Sea anchovies have a different growth tendency.

The separation of these possible groups is also important for stock management (Begg et al., 1999; Secor, 2014). It is crucial to define the stock used as a unit (Gulland, 1983). For instance, applying the same minimum catch size rule to different growing groups can be a disadvantage for fast-growing groups. In other words, if there are groups with different growth and mortality parameters in a region, the assessment should be performed on a stock basis (Gayanilo, 1997). In addition, knowledge of growth parameters is also required to estimate natural mortality (Pauly, 1980; Gislason et al., 2010). The combination of all these reasons has increased the need to identify possible distinct growth groups in the Black Sea.

Otolith is a calcified and biologically inert structure that functions as hearing and balance in teleost fish (Campana and Thorrold, 2001). The oscillations in the intensity of increments form the hyaline and opaque formations. These annual formations are used in fish age estimation and growth studies in fisheries science (Campana and Thorrold, 2001; Mendoza, 2006). Age increments on the otolith, which represents the growth of the fish, can be used to reconstruct fish length-at-age as well as the growth history of individuals (Francis, 1990; Morrison et al., 2019; Morrongiello et al., 2012). In the Black Sea, Azov and Black Sea anchovies have been separated using the otolith index (length/width of otolith) and the angle between rostrum and anti-rostrum so far (Chashchin, 1996; Zuyev, 2019; Vodyasova and Soldatov, 2017). However, this method could not achieve the expected separation in the southern Black Sea (see Figure 9).

Otolith was also used in this study but from a different perspective. It was focused on growth in the first year of life when anchovies grow fastest. By using the increment width, which represents the growth of the first year on the otolith, an attempt is made to determine possible distinct groups that grow at different rates at this time interval of their life. Namely, the hypothesis tested in the study is that in the Black Sea, there are different anchovy groups with different growth tendencies and these can be determined by their growth in the first years of life. This hypothesis is based on the four basic assumptions: (i) there is a strong linear relationship between fish size and otolith size, (ii) anchovy reached most of its maximum size in the first year of its life, which makes it more possible to measure and compare, (iii) all specimens were born on June 1, which is accepted as a thumb rule in the age reading, and (iv) the size of individuals in each age group shows the Gaussian distribution.

Material and Method

Study materials and Measurements

A total of 3150 fish samples collected in 2012 (323), 2013 (632), 2014 (610), 2015 (654), 2016 (56), 2018 (496), and 2020 (379) were included in the analysis. These samples are from two different seasons; summer (July and August; 2013, 2015, 2018) and winter (October 2014; November 2012, 2013, 2016, and 2020; and December 2012, 2020). All surveys were conducted with RV Bilim-2 of the Middle East Technical University (METU). Sampling stations, demonstrated in Figure 4.1, covering the Turkish Exclusive

Economic Zone represent coastal and offshore regions of the Southern Black Sea (40.9° – 43.4° N and 28.0° - 41.4° E).

Figure 4.1: Stations in the southern Black Sea where the samples used in this study were collected.

Collected frozen samples were analyzed at the METU Marine Sciences Institute Marine Biology and Fisheries Department Laboratory. Total length in cm (±0.1 cm) was measured and sex was determined (ICES WKSPMAT Report, 2008). Subsequently, the dissection of individual samples was performed and otoliths were extracted. The extracted sagittal otolith pairs were cleaned and stored in small plastic tubes for later age readings.

The ages of the samples were determined under the reflected light against a black background using a binocular microscope (Olympus SZX16) with a magnification of 25X while immersed in 70% ethyl alcohol and oriented as the distal surface (convex side) up. During age reading the Common Age Reading Protocol for the Black Sea anchovy prepared by Black Sea experts under the supervision of the BlackSea4Fish Project (GFCM, 2019) was followed. This protocol was prepared under the scope of this thesis study and presented in Chapter 1. The exact ages (decimal years) were determined by using information that the catch date and the accepted birth date of the 1st of June.

As soon as the age was determined, the photographs of the otolith pairs in *tiff* format were taken with the Olympus UC30 Microscope Camera. The aimed measurements on the otoliths were done on these photographs by using the right-side otolith. It was preferred to make the measurements on the photograph rather than the live view from the microscope. In this way, inaccurate measurements due to possible light instabilities are believed to be avoided. For all measurements, Stream Basic Version 1.8 Software was used.

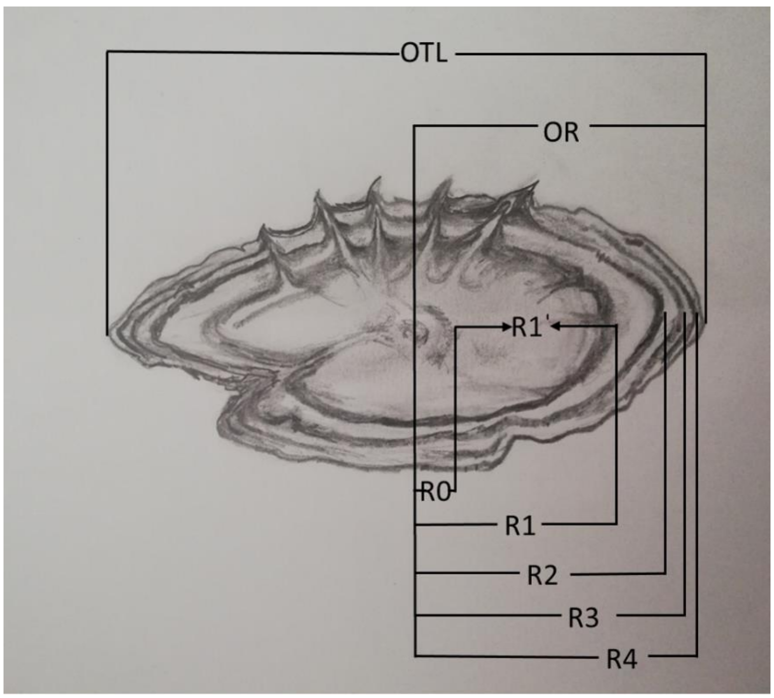

Figure 4.2: Increment measurements on the otolith: OTL: otolith's total length; OR: otolith's total radius; R0 translucent core zone radius-theoric the zero-age increment; R1ˈ: the region between R0 and R1 represents the fastest growing zone; R1: first annulus' radius; R2: second annulus' radius; R3: third annulus stands for age 3; R4: fourth year's annulus for age 4.

The increment measurements (±0.1 μm) were done for each corresponding age ring from the nucleus (approximate center of primordia) to the posterior edge along the long axis (Figure 4.2). The radius of the first true hyaline ring started from the beginning of the first opaque after larval hyaline ring (R0) is used as a proxy of the length of the fish at its age 1. The further ring radii are for the following ages. Each increment radius is assigned to a formation year by back-counting from the catch date. Nevertheless, to solve the fitting of the growth curve to the zero-age, the translucent core radius (R0) was also used. Whereas the R0 is not so clearly visible in all otolith samples, only the samples in which this ring is 100% visible were selected and measured (n=1436). All increment measurements were used in growth estimations. However, the R1 prime[3] (R1': hereinafter referred to as R1) was used to separate the groups according to their fastest-growing period in the first year (n=923).

Statistical Analysis

The correlation (Pearson's Product Moment Correlation) and linear regression were used to reveal the relationship between the total fish length in cm and the total otolith radius in mm. The length frequency at age data was subjected to Hartigans' Dip Test for Unimodality to identify possible patterns. Density frequency distribution for R1 was also tested for its modality character considering the skewness and kurtosis (Pfister et al., 2013; Ameijeiras-Alonso et al., 2018). Normality was tested by the Shapiro-Wilk test. To compare the variances across the means of different groups the Analysis of Variance (ANOVA) was used. Multimodality in the R1 density distribution was tested by the excess mass test (Ameijeiras-Alonso et al. (2018). An alpha level of .05 was used for all statistical tests.

Two consecutive steps were applied to detect possible differently growing groups within the R1 distribution. To do this, the unsupervised classification technique of the Expectation-Maximization (EM) algorithm was implemented to estimate the parameters of the Gaussian mixture models (GMM) (Dempster et al., 1977; Redner and Walker, 1984; Hamerly, 2003). This algorithm was implemented as a practical tool to assign each data point to each possible Gaussian group, with its iterative optimization of the final probabilities, called expectation. After the initialization, by the final step, called

[3] To fit the growth curve, the measurement designated as R1 (measurement from the middle to the outer part of the first-year hyaline) was used. However, since the aim was to make group distinctions by taking into account the portion of growth gains in the first year after starting feeding, the measurement from R0 to the outer part of the first year of hyaline is defined as the R1 prime. Groups with different growth rates were separated according to this measurement.

Maximization, the means, variances, and mixing proportions were iteratively estimated using the maximum likelihood approach until no further improvement could be achieved and the model converged. This approach ensured that the algorithm stably determined which group each fish belonged to. Then, for further analysis, 1-year-old fish were selected from both groups that experienced a winter migration and dispersed for the first time in the basin.

A total of 4887 age-corresponding increment widths were determined. However, the increment width numbers in each age group were not equal due to the smaller sample size of older ages. Therefore, the estimation of growth parameters was performed using the von Bertalanffy growth equation (4.1) (von Bertalanffy, 1938, Beverton and Holt, 1957) with the randomly selected same sample-sized otolith increment-at-age data.

$$Lt = L\infty(1 - e^{-k*(t-\mathrm{t0})}) \qquad (4.1)$$

where; L_t is the length at time t, L_∞ is the theoretical asymptotic length, K is the growth rate: the rate at which the asymptotic size is approached and t_0 is a hypothetical age at which fish has zero length. It is the equation that analyzes the functional relationship between the mathematical variables of fish length and age, by including the parameters (estimated by the nonlinear least squares optimization with 2e+10 iterations to minimize the sum of squares of deviation) of L_∞, K, and t_0 (Kirkwood, 1983). This calculation and the fitting process were repeated 1000 times and the average of the calculated parameters was used as the final estimate.

The growth performance index (ϕ: phi-prime) (Pauly and Munro, 1984),

$$\phi = \log10(K)+2\log10(L\infty) \qquad (4.2)$$

where ϕ is the phi-prime growth performance index used as a tool to compare the growth of the same species, K is the growth rate parameter obtained from the vBG model together with L_∞ which is the asymptotic length of the fish. This index is used to overcome the correlation between K and L∞ (Pauly and Munro, 1984)

Finally, the likelihood ratio test was also used to test differences between fitted growth curves (Kimura, 1980, Kirkwood, 1983; Cerrato, 1990). In this approach, using the nonlinear least squares function a general model for each group (with Linf, k, and t0) and hypothesized four sub-models for testing were fitted. For each general model-sub-model comparison, likelihood ratios are calculated by using the residual sum of squares. The outcomes were tested with chi-square statistics with suitable degrees of freedom (Nelson and Nelson, 2023).

All calculations and statistical tests were performed under the R version 4.2.3 (R Core Team, 2023) within the R Studio (Posit team, 2022), using the packages of *tidyverse_1.3.2* (Wickham, 2019), *dplyr_1.0.9* (Wickham, 2022), *ggplot2_3.4.0* (Wickham, 2016), *Mclust_ 6.0.0* (Scrucca et al., 2016), *mixtools_ 1.2.0* (Benaglia et al., 2009), *LambertW_ 0.6.7.1* (Goerg, 2022), and *fishmethods_ 1.11.3* (Nelson, 2022).

Results

The accepted arbitrary birth date of the anchovy is the 1st of June (GFCM, 2019). Two other major assumptions were tested. Accordingly, a highly significant relationship was found between total fish length and otolith radius (linear regression analysis, OR=0.1162938*TL+0.0399188, R^2=0.92, n=3150; see Chapter 3). Furthermore, from the growth parameters estimated in Chapter 3 and the literature (see Chapter 3; Table 3.2), it has been calculated that the anchovy attains an average of ~72% of its total asymptotic length in the first year of its life (Table 4.1).

Table 4.1: Sources and results used to calculate what percentage of first-year asymptotic growth is complete

Data Sources	Growth equation	% Of the growth in the first year by Linf
From current study	$L_t=12.2[1-e^{-0.779(t+0.956)}]$	78.2%
Average Linf from the literature (for Southern Black Sea)	$L_t=16.1[1-e^{-0.508(t+1.670)}]$	74.2%
Min Linf from the literature*	$L_t=10.8[1-e^{-1.027(t+1.197)}]$	89.5%
Max Linf from the literature**	$L_t=23.5[1-e^{-0.140(t+3.08)}]$	43.5%

*The minimum Linf value is 10.8 cm and the samples were collected from commercial purse seines in September 2013 and April 2014 (Solak and Bilgin, 2020). ** The maximum estimated Linf is 23.5 cm which was obtained from samples from the commercial fishery in November 1990 (Okur, 1989).

The length frequency distribution was normally distributed for the older ages of 2-, 3-, and 4- (Shapiro-Wilk normality test; p-value>0.05). Whereas, ages 0 and 1 significantly deviated from a normal distribution (Shapiro-Wilk normality test; p-value < .001). The unimodality hypothesis was rejected and alternative hypothesis (non-unimodal, i.e., at least bimodal) was accepted for both Age-0 (Hartigans' dip test for unimodality/ multimodality: D = 0.016795, p-value = 0.0461) and Age-1 (Hartigans' dip test for unimodality/ multimodality: D = 0.025566, p-value = 1.491e-05) (Figure 4.3). The growth-deviated modes were more observable and more feasible to distinguish. Therefore, focusing on the first year of growth (R1) will provide more insight into differentiated growth groups.

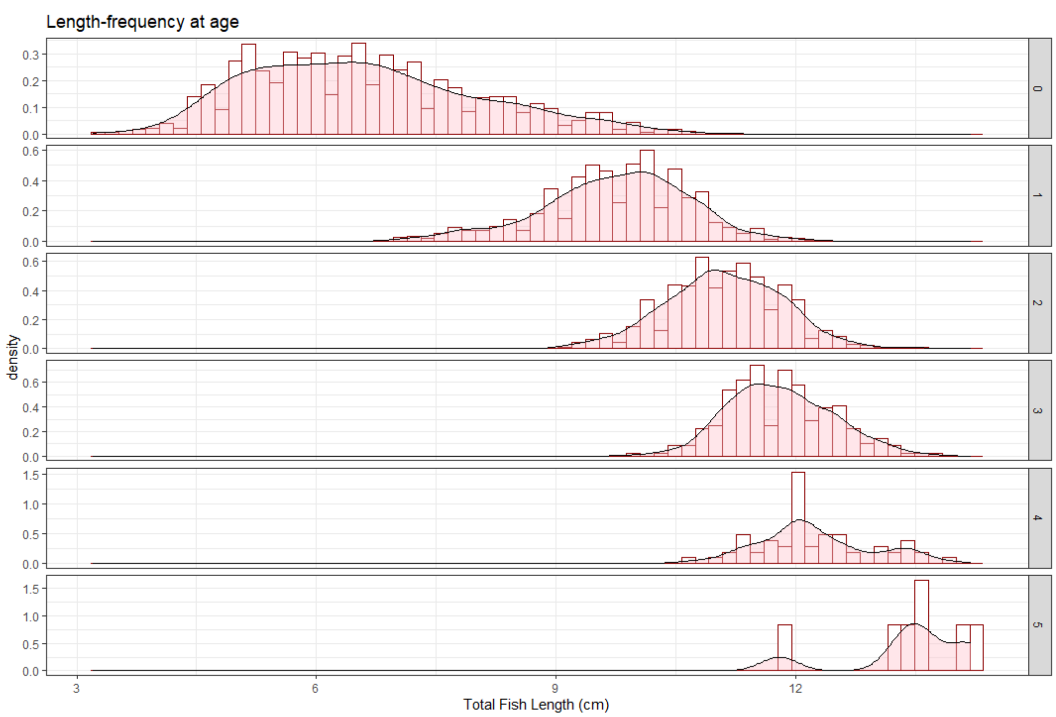

Figure 4.3: Total length (TL) density frequency distribution.

What was observed from the sampling data was that the length variation in the first year of life, when anchovies grow fastest, was very high compared to other age groups (Figure 4.4). The expected increase in average length with advancing age was not observed particularly in the case of 0- and 1-year-olds (Figure 4.4). Although discrete modes are expected to become more difficult to observe in later life due to the decreasing growth rates as the fish age (Laslett et al., 2004), the variation in the first year of life may indicate different growing groups in the population.

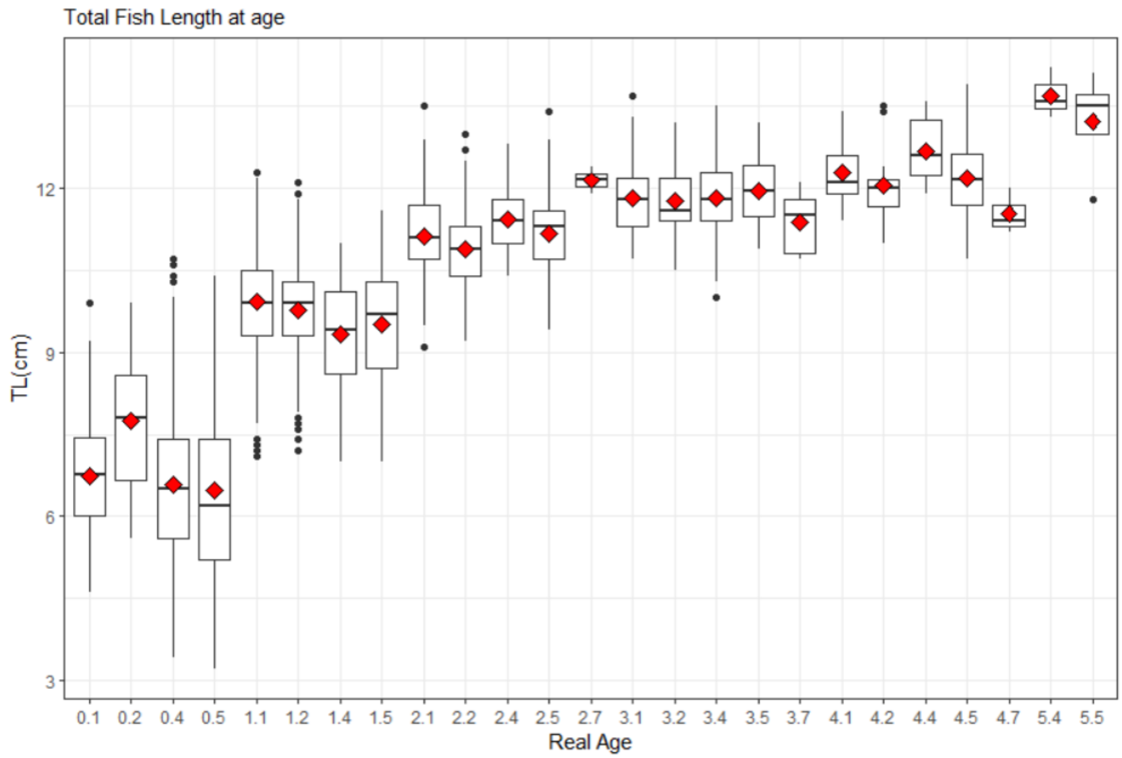

Figure 4.4: Total length (TL) versus decimal age (with respect to birth and catch dates) boxplot (The mean fish length was demonstrated by red diamonds).

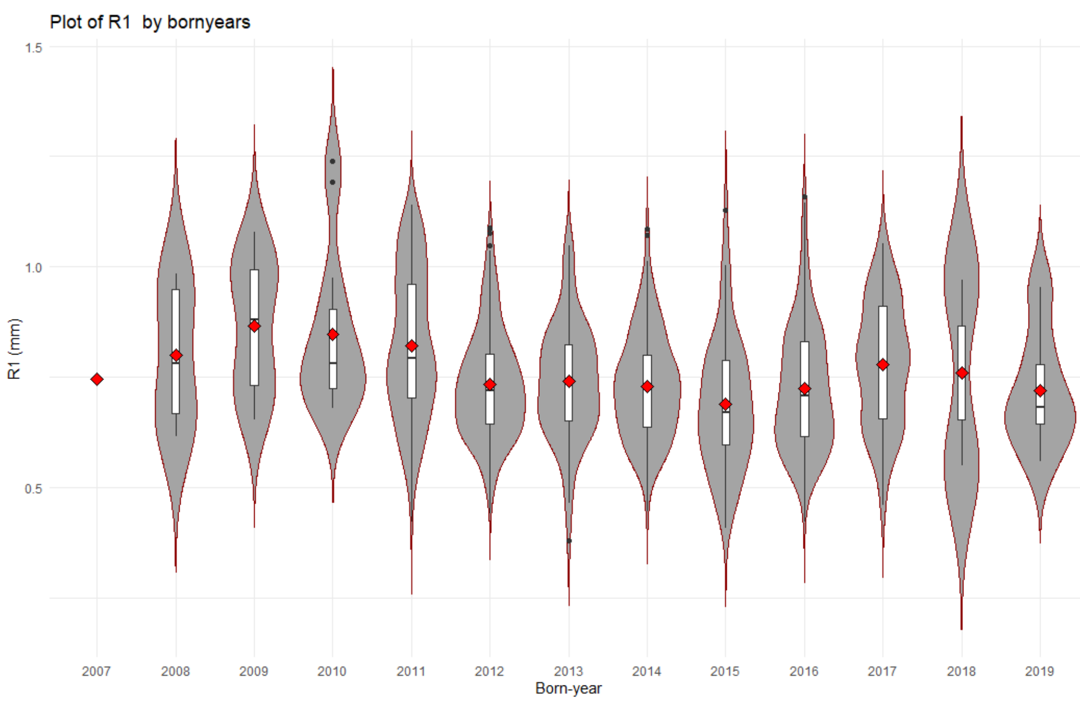

Figure 4.5: R1 distribution over the born years of the fish (red dots are the mean R1)

Since the R1 values were obtained by the measurements from the otolith, the time and the location of the sampling were ignored. Yet, the existence of different growing groups within the years of born was especially striking in some years (Figure 4.5). Indeed, the R1 distributions over the years were not normally distributed (Shapiro-Wilk normality test; p-value < .001). There is strong year effect was observed in R1 (ANOVA; p-value < .001). Moreover, the sex of the individuals had no statistically significant effect on the R1 (ANOVA; p-value >0.05).

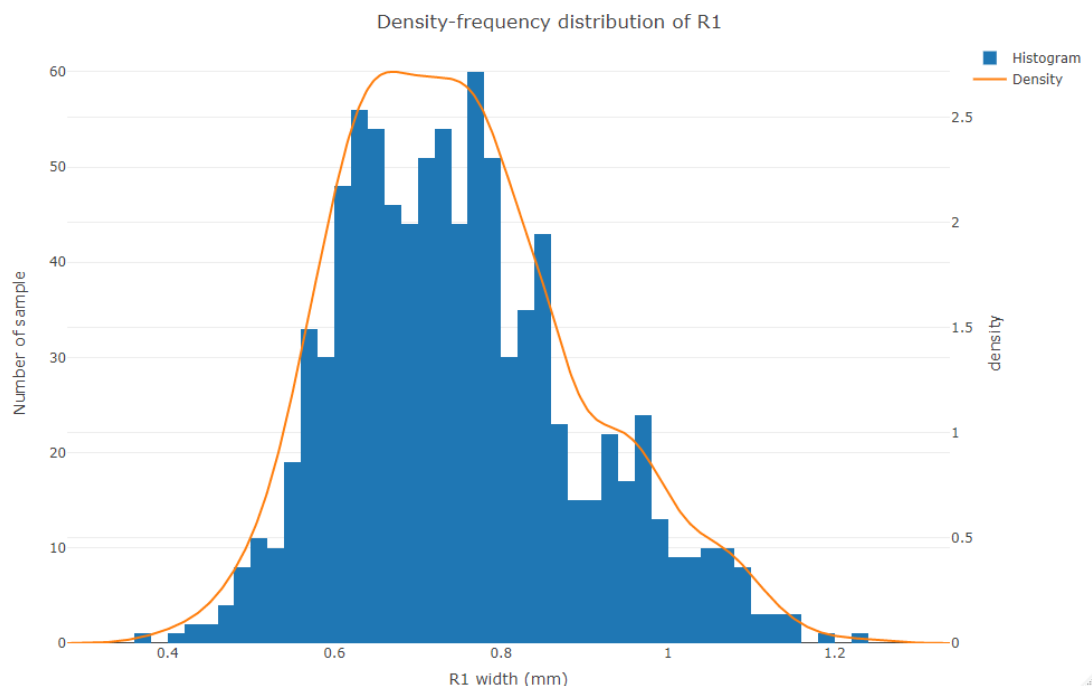

Figure 4.6: R1 Histogram with probability density distribution for R1_Bandwidth= 0.03311

The density frequency distribution of R1 was shown in Figure 4.6. Applied Shapiro-Wilk normality test showed that R1 values were not normally distributed (n=923, W = 0.98092, p-value = 1.258e-09). Yet, even though there are several ridges, neither it can be concluded a clear bimodality (bimodality coefficient = 0.421 by skewness and kurtosis, less than 0.555; Pfister et al., 2013). However, the hypothesis of a multimodal distribution cannot be rejected (excess mass test, p-value = 0.344).

Assuming the fish growths within age are normally distributed, different Gaussian distributions within the R1 distribution were searched to identify possible groups of growth variables. The Gaussian Finite Mixture Model fit results showed that there were two components (log-likelihood: 507.6511; BIC: 987.9917). The Gaussian distribution was determined based on the group probability estimation that each fish could belong to (Figure 4.7; Table 4.2). The overlapping area was expected because of the high rate of hybrid forms of the anchovy in the Black Sea is known.

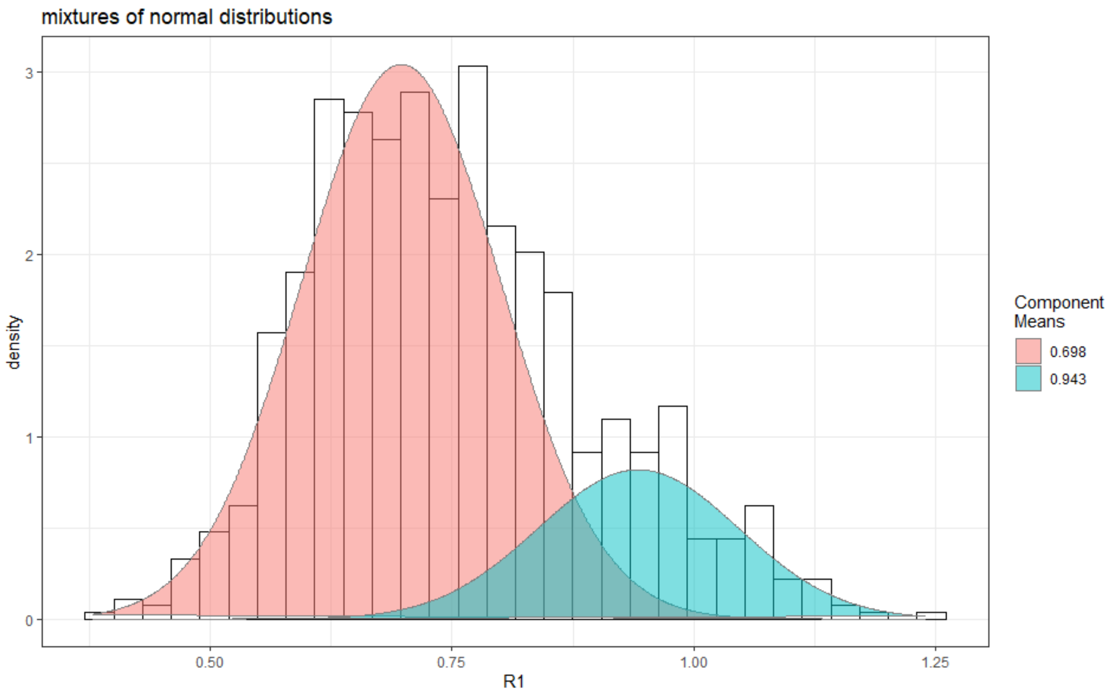

Figure 4.7: Probability distribution of each observation to which Gaussian distribution they fall.

Table 4.2: Results of the EM algorithm for mixtures of univariate normal calculation

	Component 1	**Component 2**
Final mixing proportions	0.786777	0.213223
Component means	0.697536	0.942577
Standard deviations	0.103179	0.103955

***loglik at estimate: 507.6648

The components were designated Group 1 and Group 2. Group 2, which represented a smaller proportion of the southern Black Sea population studied, grew more than Group 1 in the first year of life (Table 4.2). The majority of the population (~79%) consists of Group 1, which were relatively small fish in their first year.

After the groups were determined the von Bertalanffy growth parameters were estimated for each group (Figure 4.8; Table 4.3). Accordingly, the growth rate of Group 2 was 85% higher than that of Group 1. The estimated length at age 1 was 7.0 cm for Group 1 while, for Group 2 it was 9.2 cm. In other words, the individuals in Group 1 can achieve 56% of their asymptotic length in their first year of life. Whereas for Group 2 this rate increases to 76%. Indeed, the growth performance index (ϕ) was 10% higher in Group 2. Expectedly, the asymptotic length was lower in Group 2 than in Group 1. Even though the growth rates of Group 2 were relatively high, its proportion in the population was considerably low when compared with Group 1.

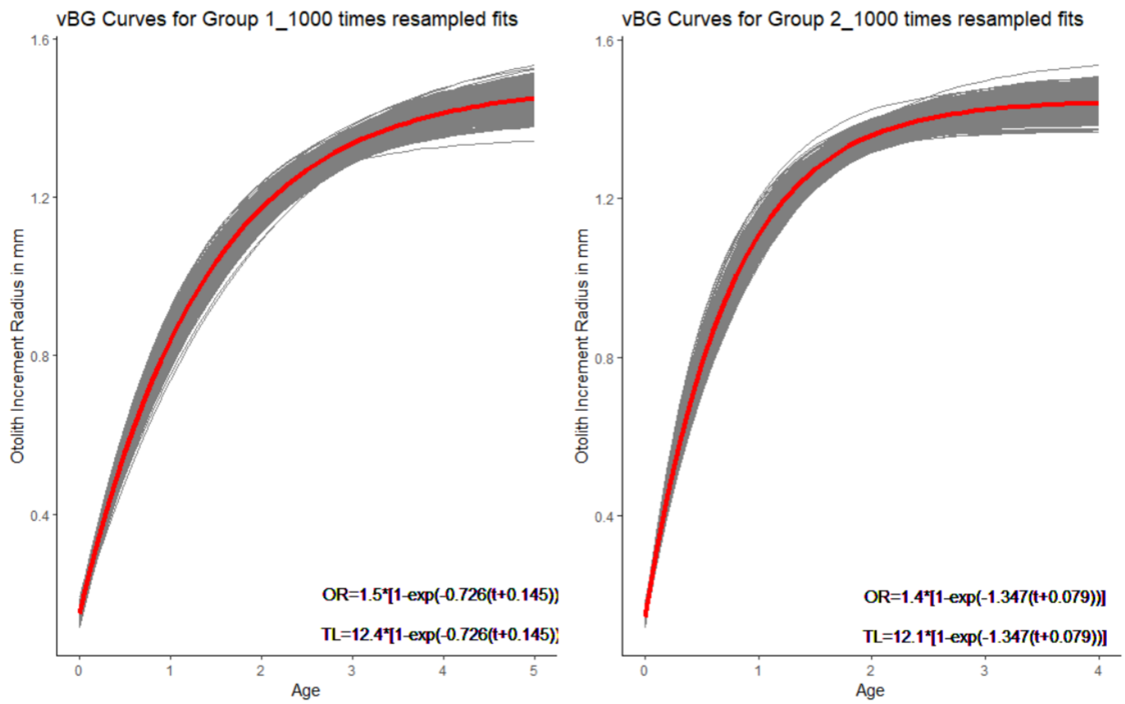

Figure 4.8: von Bertalanffy growth fits for Group 1 (left-hand side) and Group 2 (right-hand side.

Table 4.3: Estimated von Bertalanffy growth parameters for each defined group.

Groups	Mean R1 (mm)	L_{inf} (cm)	K(1/year)	t0	ϕ
Group 1	0.698	12.4	0.726	-0.145	2.05
Group 2	0.943	12.1	1.347	-0.079	2.29

The statistical differences between these growth curves were tested by testing four hypotheses ($L_{inf1}=L_{inf2}$, $K_1=K_2$, $t_{01}=t_{02}$ and $L_{inf1}=L_{inf2}$, $K_1=K_2$, $t_{01}=t_{02}$; Appendix) with the likelihood ratio test. Accordingly, the differences between these models were found to be statistically significant and the H0 was rejected for model 2 (p-value < .001), model 3 (p-value = 0.042), and model 4 (p-value < .001). It was concluded that these growth curves were significantly different (for more detailed results, see Appendix).

The proportion in the percentage of the groups according to the date of birth was found by counting the age backward from the otolith was shown in Figure 4.9. It is noteworthy that the proportion of individuals belonging to Group 2 has decreased significantly in recent years.

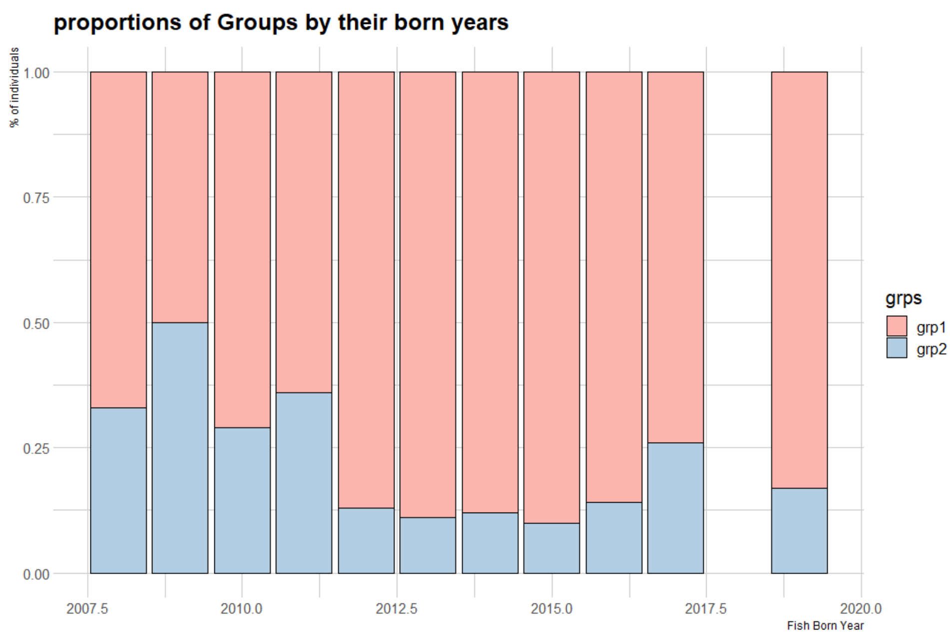

Figure 4.9: Proportion distribution of the groups within their born years (2008:2019)

Discussion

Growth, one of the characteristics of biological life history that is continuously recorded for management purposes, is also one of the descriptive parameters used to compare fish stocks (Begg et al., 1999). In this study, we attempted to detect different anchovy groups that showed different growth characteristics in the Black Sea by focusing their first-year growth increment widths on the otolith. Our results reflect the presence of two main anchovy putative groups with different growth characteristics in the Southern Black Sea region.

The two most commonly used data in estimating the growth parameters are length-age and just length composition, which is generally sliced into length-age data. Age is a key component in fisheries science. In this study, our approach includes length increment data obtained from measurements of increment widths on otoliths corresponding to certain ages. In this method, which eliminates all sources of error from using length-at-age data in growth estimation, there may still be uncertainty due to aging. Because defining the true age rings requires expertise and experience. All the age reading of the anchovy in the Black Sea, is based on a protocol developed by experienced experts from riparian countries since 2019 (GFCM, 2019; GFCM, 2023, in press). In this study, the ages that were read within the framework of the rules outlined in this protocol are considered to have largely eliminated inconsistencies in terms of repeatability and consistency.

The otolith-based methods of separating stocks from first-year growth increment (R1) (Ventero et al., 2017) and estimating growth parameters from increment width at age (Carbonara et al., 2023) have previously been used for anchovy in different parts of the Mediterranean Sea. However, this study is the first for anchovies in the Black Sea region.

In the Black Sea, although no strong bimodality was observed in the growth frequency distribution in the first year (Figure 4.5), clear divergence was expected to be inevitable in the case of the anchovy in the Black Sea. It could be explained by the higher rate of hybridization among anchovies in the basin (Chashchin, 1996; Kalnin and Kalnina, 1985). Although there are no geological barriers between Azov and the Black Sea, some water mass characteristics such as the lower salinity of the Sea of Azov act as a reproductive barrier. Occasional changes in the water's physical conditions and the concurrent reproduction seasons can accelerate the hybridization (Mayr, 1970; Chashchin et al., 2015; Zuyev and Skuratovskaya, 2023). Considering what has changed so far (Balykin et al., 2019; Yuneva et al., 2019; Yuneva et al., 2020) in the environmental conditions in the Sea of Azov and what will change from now on (Berdnikov et al., 2022;

Chernichko et al., 2022; Kosenko et al., 2017), we may no longer just talk about hybridization, but about the ecological morphs.

As a result of the existence of different groups revealed in the study, the presence of more than one group in the basin was consistent with predictions from other studies (Chashchin, 1981; 1995; 1996; Altukhov et al., 1969). However, in this study, the growth traits were not directly attributed to the Azov and Black Sea anchovy as the source of the divergence. Instead, this situation was evaluated as the groups, were most probably, exposed to different environmental conditions during their first year of life. Because, in recent genetic studies, it has been proven that they do not show genetic divergence (Vodyasova and Soldadov, 2017), but their differences have their origin in epigenetics (Nebesikhina and Lebedev, 2019).

Morphometric and meristic features may show differences among the groups to the place where the juveniles grow up. This situation is explained by ecophenotypic variation (Kinsey et al., 1994). As emphasized above, the most important factor in the formation and disappearance of morphometric and meristic characters in Azov and Black Sea anchovy is the reproductive barrier (Vodiasova and Abramson, 2017). The accumulation of generations in the breeding areas can be seen as the main reason for the development of specific characteristic dynamics. In addition, physical conditions can affect generations in a variety of ways, from feeding to reproduction, from winter migration to growth (Petitgas et al., 2013; Brosset et al., 2016; Albo-Puigserver et al., 2021).

This could explain how the Azov and the Black Sea anchovy groups formed. But with climate warming, it is also known that the Sea of Azov is beginning to resemble the Black Sea (Berdnikov et al., 2022; Yuneva et al., 2020). In this scenario, it is expected that a faster-growing group to form due to Azov anchovy depletion or hybridization shifting toward Black Sea anchovy traits. In fact, in the study conducted in the 2016-2017 season in the Sea of Azov, the growth rate (K) was estimated at 1.220, higher than average (Chesalin et al., 2020). Salinization will lead to an expected decline in Azov anchovies. However, the presence of the relatively slow-growing southern Black Sea stock and its proportional increase over the years may indicate the presence of separate stock from these two known groups (Niermann et al., 1994; Gücü et al., 2016).

On the other hand, the decrease in the proportion of Group 2, which is a relatively fast-growing group and shows Black Sea anchovy characteristics according to the literature (Chaschin, 1996), leads us to suspect that hybridization in favor of Black Sea anchovy could not be achieved. Therefore, the groups in this study were not directly assigned to Black Sea anchovy or Azov anchovy and were considered as two separate groups with growing differences in the southern Black Sea. Based on this, it is highly recommended to re-make the stock separation focused on first-year growth with samples covering the entire Black Sea. Thus, the environmental-based growth effect will be understood more comprehensively.

If we go back to the southern Black Sea region, it is worth assessing the decrease in the share of the fast-growing Group 2 and the increase in Group 1 over the years and looking for the reason. For example, the increase in the proportion of the smaller group in the first year of life is reminiscent of the selective fishing effect (Shephard et al., 2012; Hsieh et al., 2010). Most of the anchovy fishing in the Black Sea is carried out in the southeastern Black Sea during the winter months. While the 9 cm minimum catch length rule is applied in fishing on the Turkish coasts, no quota is applied (Üstündağ, 2010). However, while the quota is applied every year in high-catch territorial waters of Georgia, the minimum catch length limit is implemented as 7 cm (GFCM, 2022). Juveniles caught off the coast of Georgia can cause stocks to be withdrawn from the system before they have a chance to breed. On the Turkish coast, the fast-growing group, which is exposed to high fishing pressure, may be more disadvantaged in the first year of life compared to the slow-growing group. This may have accelerated an increase in the slow-growing Group 1 population, while it may have triggered the collapse of the stock for Group 2. This decline can be seen as a warning. It also highlights the importance of stock-based fisheries management. Likewise, considering that Group 2 is 9.2 cm at age 1 and 7 cm at age 1 of Group 1, it is evident that this situation does not appear to be an unrealistic scenario. In addition, the loss of the maternal effect is another issue (Hsieh et al., 2010; Brosset et al., 2016). So, for the sustainable management of stocks, it is important to keep large fish in the sea to ensure stock resilience (Birkeland and Dayton, 2005; Tu et al., 2018). The stock collapse of the Arctic cod (Heino et al., 2002), Norwegian spring spawning herring

(Engelhard and Heino, 2004), and North Sea plaice (Grift et al., 2003) were some examples of the serious impact of size-selective fishing on stocks.

Another situation that should be brought to the fore according to the results of the study is that the growth rate of Group 1, which we defined as a low growth rate, was actually relatively low compared to Group 2. Because if we look at the literature, the estimated average K(1/year) value for anchovy in the Black Sea is 0.548 (Chapter 3; Table 3.2). In other words, the group we called the slow-growing is growing 70% faster than average compared to historical data. The trend in the K value in the literature increased between 2000-2017 and during this period the average was 0.645 (Chapter 3; Table 3.2). Whereas, in this study, this value for Group 1 was estimated to be 0.726 for the years 2012-2020 (Figure 4.7; Table 4.3). Consequently, this increase in growth rate and decrease in asymptotic length might be associated with rises in seawater temperature in the Black Sea, particularly in recent years due to climate change (Stanev et al., 2019; Mohamed et al., 2022). Because, increasing temperature can drive somatic growth rate (Jonsson et al., 2013) and fish body size can decrease with the increasing temperature (Bergmann's rule: Bergmann, 1848; Hattab et al., 2021).

While relatively faster fish growth due to temperature sounds good for stock conditions, it does not always mean more yield. This situation can reduce the larger individuals in the stock (by decreasing the asymptotic length) and eventually lead to lower spawning stock biomass (Lindmark et al., 2022). Therefore, defining stocks and their growth characteristics under specific environmental conditions is crucial for the sustainable management of anchovy stocks in the Black Sea. Through this study, two groups with different growth characteristics were identified in the Southern Black Sea Region. Based on this situation, it is recommended to consider each of them separately in terms of stock management, taking into account the proportional changes over the years.

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Appendix

Likelihood ratio test results

`results`	list elements with the likelihood ratio tests comparing von Bertalanffy models.
`model Ho`	list elements with the `nls` fit for the general model.
`model H1`	list element with the `nls` for model H1 (Linf1=Linf2=..=Linfn) where n is the number of groups.
`model H2`	list element with the `nls` fit for model H2 (K1=K2=..=Kn).
`model H3`	list element with the `nls` fit for model H3 (t01=t02=...=t0n).
`model H4`	list element with the `nls` fit for model H4 (Linf1=Linf2=..=Linfn, K1=K2=..=Kn, t01=t02=...=t0n).
`rss`	list element with the residual sum-of-squares from each model.
`residuals`	list element with the residuals from each model.

```
$results
     tests                hypothesis chisq df     p    model
1 Ho vs H1              Linf1=Linf2  1.91  1 0.167 von Bert
2 Ho vs H2                    K1=K2 18.72  1 0.000 von Bert
3 Ho vs H3                  t01=t02  4.14  1 0.042 von Bert
4 Ho vs H4 Linf1=Linf2,K1=K2,t01=t02 26.13  3 0.000 von Bert

$`model Ho`

Formula: len ~ (Linf1 * grp1 + Linf2 * grp2) * (1 - exp(-(K1 * grp1 +
    K2 * grp2) * (age - (t01 * grp1 + t02 * grp2))))

Parameters:
      Estimate Std. Error t value Pr(>|t|)
Linf1  1.49534    0.03497  42.764 1.32e-07 ***
Linf2 -0.04316    0.04405  -0.980  0.37220
K1     0.70943    0.06237  11.375 9.19e-05 ***
K2     0.60591    0.13838   4.379  0.00716 **
t01   -0.14848    0.04069  -3.649  0.01476 *
t02    0.06822    0.04641   1.470  0.20156
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.03413 on 5 degrees of freedom

Number of iterations to convergence: 7
Achieved convergence tolerance: 1.188e-06


$`model H1`
```

```
Formula: len ~ (Linf1 * grp1) * (1 - exp(-(K1 * grp1 + K2 * grp2) * (age
-
    (t01 * grp1 + t02 * grp2))))

Parameters:
      Estimate Std. Error t value Pr(>|t|)
Linf1  1.46975    0.02105  69.814 5.81e-10 ***
K1     0.74936    0.05071  14.778 6.04e-06 ***
K2     0.50727    0.09279   5.467  0.00156 **
t01   -0.13888    0.03769  -3.684  0.01028 *
t02    0.05498    0.04334   1.268  0.25163
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.03398 on 6 degrees of freedom

Number of iterations to convergence: 7
Achieved convergence tolerance: 6.242e-06


$`model H2`

Formula: len ~ (Linf1 * grp1 + Linf2 * grp2) * (1 - exp(-(K1 * grp1) *
    (age - (t01 * grp1 + t02 * grp2))))

Parameters:
      Estimate Std. Error t value Pr(>|t|)
Linf1  1.41662    0.04936  28.700 1.19e-07 ***
Linf2  0.12124    0.05896   2.056 0.085490 .
K1     0.93132    0.12403   7.509 0.000289 ***
t01   -0.09140    0.06221  -1.469 0.192156
t02   -0.04597    0.08011  -0.574 0.586958
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.07295 on 6 degrees of freedom

Number of iterations to convergence: 8
Achieved convergence tolerance: 6.282e-06


$`model H3`

Formula: len ~ (Linf1 * grp1 + Linf2 * grp2) * (1 - exp(-(K1 * grp1 +
    K2 * grp2) * (age - (t01 * grp1))))

Parameters:
      Estimate Std. Error t value Pr(>|t|)
Linf1  1.48263    0.03513  42.201 1.18e-08 ***
Linf2 -0.02402    0.04554  -0.527  0.61683
K1     0.75246    0.06281  11.981 2.05e-05 ***
K2     0.50398    0.13166   3.828  0.00868 **
t01   -0.10054    0.02229  -4.509  0.00406 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.03761 on 6 degrees of freedom

Number of iterations to convergence: 8
Achieved convergence tolerance: 2.221e-06


$`model H4`
```

```
Formula: len ~ (Linf1 * grp1) * (1 - exp(-(K1 * grp1) * (age - (t01 *
    grp1))))

Parameters:
      Estimate Std. Error t value Pr(>|t|)
Linf1  1.45784    0.05353  27.233 3.56e-09 ***
K1     0.96834    0.15525   6.237 0.000249 ***
t01   -0.11109    0.05584  -1.990 0.081835 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.08847 on 8 degrees of freedom

Number of iterations to convergence: 7
Achieved convergence tolerance: 1.263e-06


$rss
  model                  rss     AIC
1    Ho 0.00582445178342532  -37.76
2    H1 0.00692891661442394  -37.85
3    H2  0.0319292152847308  -21.05
4    H3 0.00848611728961102  -35.62
5    H4  0.0626190207250464 -17.638

$residuals
         resid0        resid1      resid2       resid3       resid4
1  -0.004042516  0.0001921591  0.02986530  0.037439333 -0.003227829
2  -0.002982813 -0.0045524335 -0.04215801 -0.030565579 -0.006169123
3   0.010521073  0.0001105978 -0.06015340  0.008907997 -0.116924253
4   0.022773309  0.0309724950  0.11961099  0.031602122  0.163529070
5   0.010379698  0.0062113412 -0.03456875  0.002627721 -0.089038150
6  -0.043036843 -0.0475730687 -0.01272731 -0.039407627  0.045946449
7  -0.029876918 -0.0246189086 -0.03176151 -0.033552202 -0.080907519
8  -0.010594770 -0.0229198250 -0.03873834 -0.012622990  0.030166199
9  -0.015571710 -0.0026724884  0.01570986 -0.013897649 -0.029661540
10  0.033841118  0.0181816672 -0.02598734  0.029081311  0.048625497
11  0.028590371  0.0466684634  0.08090849  0.034471570  0.037661196
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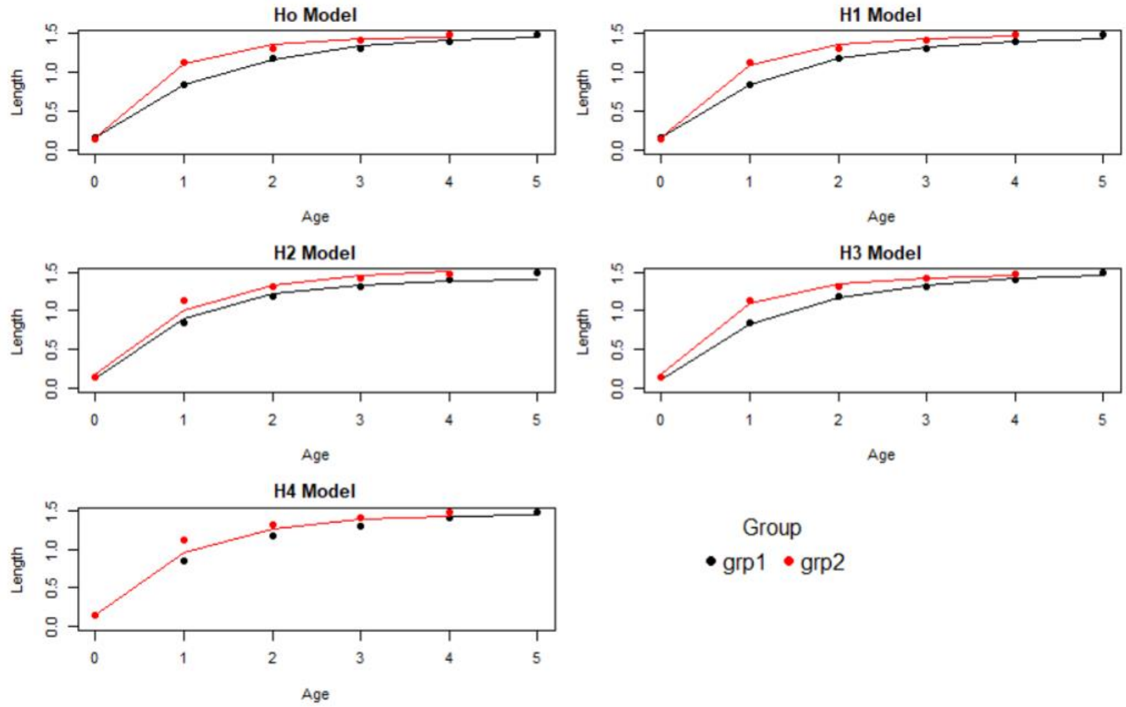

Figure 1: observed versus predicted model results for each hypothesis

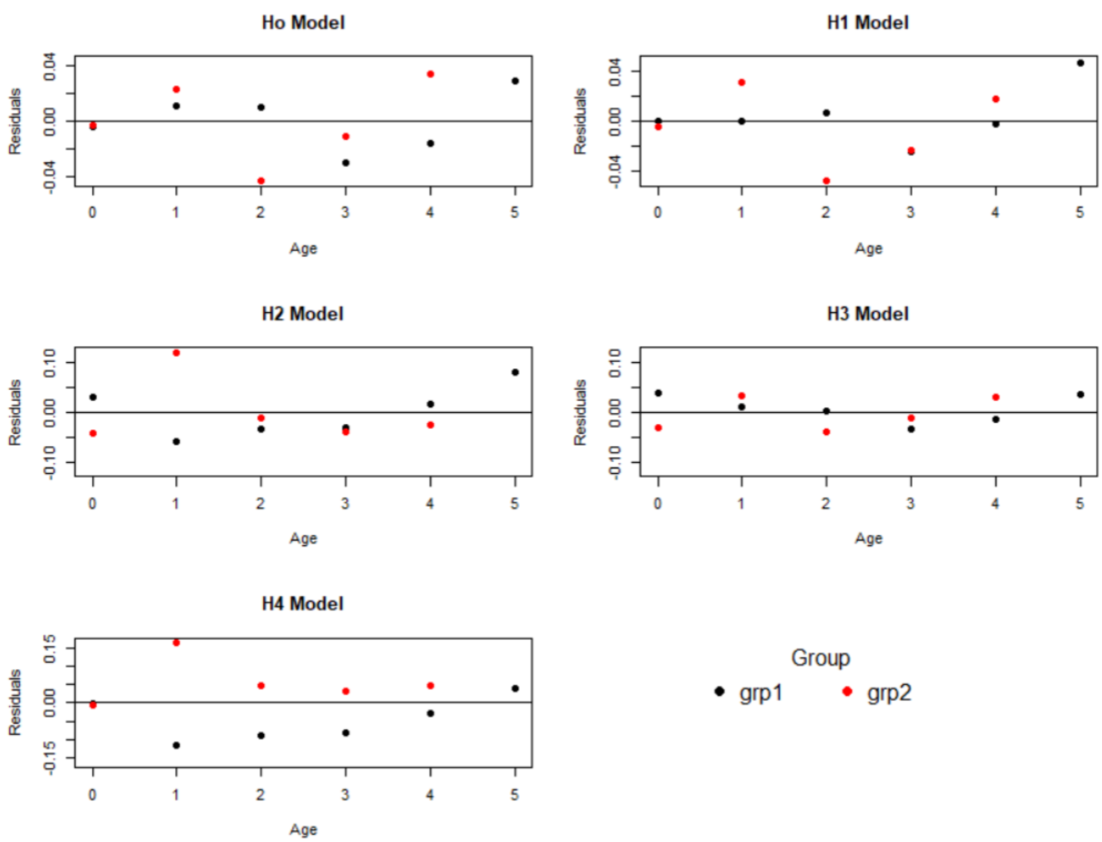

Figure 2: Residual distributions for all models established for each hypothesis

CHAPTER 5

Impact of Environment on the Anchovy Growth: A Hydrographic Perspective

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Abstract

The European anchovy, *Engraulis encrasicolus* (Linnaeus, 1758) in the Black Sea is an ecologically and economically very important fish species. Furthermore, like many other small-pelagic fish, its biomass significantly is driven by the climatic changes in the basin. So, to better manage this precious stock, it is very important to understand how these fish respond to their environment in terms of growth and seawater preferences. Theoretically, there are two different anchovy stocks, that have different growth rates, in the region and therefore they are scientifically assessed by assuming them as separate unit stocks. Growth is one of the traits used in fisheries science to parametrize the rate of change in fish length/weight over time. On the other hand, the otolith is the calcified hard structure that records the growth oscillations of the individual fish.

This is the first study in the southern Black Sea using the otolith increment signals to elucidate the seawater preferences of different growing anchovies during their reproductive period and the young-of-year anchovy growth according to the water mass where they caught. For this purpose, the hydrographical characteristics of the southern spawning ground of the Black Sea anchovy were identified using temperature, salinity, and fluorescence measured at 363 stations. These environmental descriptors were used to compare the anchovies sampled at 75 stations during the scientific expeditions conducted in 2013, 2015, and 2018 (July-August) in the southern Black Sea (Turkish

EZZ). According to the growth differentiation, two different anchovy groups were found. Different water masses, characterized by temperature, salinity, and fluorescence, were defined for each sampling year. Our results show no statistically significant association between different adult anchovy groups' water preferences. Indeed, they mixed in their reproductive period. However, a significant relationship was found between young-of-year growth and the water masses in which they were caught.

In order to understand the impact of characteristic changes in environmental water masses on anchovy growth, the results of this study serve as a proxy for future studies. Furthermore, the identified mixed anchovy groups in the same region indicate the need to reconsider the currently accepted unit-stock approach.

Keywords: Black Sea anchovy, hydrography, growth, unit stock, young-of-year

Introduction

The European anchovy, *Engraulis encrasicolus* (Linnaeus, 1758) in the Black Sea, is an economically and ecologically important fish species. Therefore, sustainable management of this precious species is crucial. Indeed, its stock has been considered an important asset for the Black Sea nations, and the efforts to understand these fish go back to the 1950s (Mayorova, 1954; Pektas, 1954). The attempts to reveal the stock status and provide scientific management advice to better manage anchovy fishery started after the 1990s (Prodanov et al., 1997; Prodanov and Stoyanova, 2001). In the last two decades, more comprehensive stock assessment studies have been carried out (Scientific, Technical, and Economic Committee for Fisheries (STECF), 2010:2017; General Fisheries Commission for the Mediterranean (GFCM), 2012 - continues).

Although anchovy live in a closed basin in the Black Sea, some important issues make assessment difficult and sometimes controversial. The 'unit stock' is at the forefront of these problems. Due to the geographical isolation of the foraging, spawning, and nursery grounds displaying significant hydrographic conditions (mainly salinity), the Regional Fisheries Organizations (STECF and GFCM) in the Black Sea assess the status of the anchovy assuming that, i) there are two anchovy stocks in the Black Sea, ii) they do not mix and, ii each are exploited by separate fishery (STECF 17-14).

Besides Kerch Strait, there is no physical barrier, rather than distinct salinity differences between the Sea of Azov and the Black Sea (Zavialov et al., 2020). This salinity barrier is claimed to result in reproductive isolation for the anchovies living in the Black Sea and the Sea of Azov during the anchovy spawning season (Chashchin, 1998). However, the salinity barrier was reported to weaken a few times in the 1970s and 1980s (Chernichko et al., 2022; Berdnikov et al., 2022; Chashchin, 1996). It has also been known that at such times the hybridization between these two anchovy groups increases, and the distinction becomes difficult (Chashchin, 1998). Moreover, during this period, the food sources of the anchovy also changed, eventually affecting the anchovy's survival in the Sea of Azov (Yuneva et al., 2020). Furthermore, the simultaneous co-occurrence of these anchovies in summer was observed previously not only in the Sea of Azov (Altukhov and Salmenkova, 1981) but also in the Black Sea on the Bulgarian and Turkish coasts (Ivanova et al., 2013; Zuyev, 2019). Likewise, recent studies have shown that the salt barrier between the Black Sea and the Sea of Azov is being weakened again, mainly due to climate change (Kosenko et al., 2017; Yuneva et al., 2019; Yuneva et al., 2020; Ginzburg et al., 2021; Chernichko et al., 2022).

Discrimination of the two anchovy forms in the Black Sea has been addressed in several ways. With that regard, phenotypic features (Maksimov, 1927; cf. Zuyev, 2019; Şahin, 2014), blood groups (Limansky, 1970; Altukhov and Salmenkova, 1981), fat content (Shulman et al., 2008; Shulman, 2002), parasite infestation rate (Danilevsky and Kamburov, 1969) otolith morphology (Skazkina, 1965; Chaschin, 1996; Vodyasova and Soldatov, 2017; Zuyev, 2019) and genetic structure (Nebesikhina et al., 2019; Düzgüneş et al., 2018; Vodiasova and Abramson, 2017) studies have been conducted so far. However, all the attempts to distinguish these anchovy forms from each other have been disputed since the beginning of the 20th century (Zuyev, 2019). But it is known that the Azov anchovy’s growth rate is slower than the Black Sea anchovy which has a longer body length-at-age (Gubanov and Limansky, 1968; Shevchenko, 1980, cf. Chashchin, 1996; 1998). Applying the same management rules to different stocks that have various biological, spatial, and temporal characteristics could be detrimental to the sustainability of certain groups. Therefore, managing these two distinct growth groups simultaneously as a single group creates very serious problems within an assessment, the fundamental step in fisheries management. Indeed, in the latest assessments, it was noted the importance of resolving this issue for properly managing the anchovy in the Black Sea (GFCM, 2015; 2017; 2018).

The otolith, which functions for hearing and balance in teleost fish, is a biologically inert body compound composed largely of calcium carbonate (Popper and Lu, 2000; Campana and Thorrold, 2001; Rodríguez Mendoza, 2006; Payan et al., 2004). It has been actively used in fisheries science mainly for the age and growth history of individual fish (Bilton, 1974 cited by Campana and Thorrold, 2001). The otolith's hyaline and opaque ring patterns are formed from the temporal oscillations in the intensity of increments that increase proportionally with fish growth (Campana and Neilson, 1985; Thresher and Wright, 1995; Campana, 1999; Campana and Thorrold, 2001; Rodríguez Mendoza, 2006; Morrongiello et al., 2012). One hyaline and one opaque increment together represent the fish’s annual growth in the temperate regions. The annual growth of the otolith can be measured with the width of the annual growth bands [4](Stevenson and Campana, 1992).

The Black Sea, a nearly enclosed basin, lies between Southeast Europe and Western Asia and is a unique body of water with complex dynamic hydrography which mainly affects the physical, chemical, and biological properties (Özsoy and Ünlüata, 1997). The oxygenated upper layer hydrography (100-150m), separated from anoxic bottom water by a permanent halocline, exhibits seasonal temperature variations (Oğuz et al., 1998; Murray et al., 1991). Although the oxygenated water column is well-mixed during winter, a pronounced thermocline forms in summer. It creates a strong temperature gradient that separates the warm surface water from the colder deep water. This stratification, together with the water carried, affects the movement of water, nutrient distribution, and distribution of dissolved oxygen. Anchovy tend to inhabit this upper warmer water, where temperatures are suitable for their growth and feeding activities (Lisovenko and

[4] The distance from the center of the sagittal otolith to the last edge of this annual growth ring is called as increment width (Panfili et al., 2002).

Andrianov, 1996; Sirotenko and Danilevskiy, 1977). Indeed, the thermal window for the spawning of the anchovy in the Black Sea is reported to range between 16 to 28 °C, and the optimum spawning was recorded at 19-24 °C by Lisovenko and Andrianov (1996). Temperature is the key environmental parameter for anchovy. With the decreasing water temperature in winter, the anchovies, which are dispersed in the Black Sea basin and the Sea of Azov, form large schools and migrate to the southeastern part of the Black Sea for overwintering (Chaschin, 1996).

The Black Sea has a large catchment area with a positive water budget (Stanev, 2005). The Black Sea water is classified as brackish with 17-18 psu. Salinity is varied by river run-off, evaporation, and precipitation. So, the variation in salinity can affect anchovy growth and survival (Fernández-Corredor et al., 2021; Ospina-Álvarez et al., 2012; Chashchin, 1996). For instance, the Azov anchovy reproduces at 10-17 ‰ (Dementeva, 1958, cf. Yuneva et al., 2020; Mayorova, 1950; Danilevsky and Mayorova, 1979, cf. Zuyev, 2019), but for the egg and larvae development, the needed salinity is 10-12‰ (Bokova, 1955, cf. Yuneva et al., 2020). However, the Black Sea anchovy can spawn and the larvae can survive at a salinity of 17-20 ‰ (Danilevsky and Mayorova, 1979, cf. Zuyev, 2019). Hence, the salinity gradient with the impacts of the freshwater input in the Black Sea can affect the spatial distribution, growth, and abundance of anchovy populations.

Their main spawning and feeding grounds for anchovies are the most productive regions of the Black Sea, where the regions of freshwater influence, ROFIs (salinity less than 16psu), like the Northwestern Shelf. (Ivanov and Beverton, 1985; Shulman, 2002; Zaitsev, 2006; Oğuz and Velikova, 2010). These regions are very important nursery areas for the fish with their diverse habitat and abundant food source, such as plankton (phyto- and mainly zoo-), the primary food source for anchovy (Bulgakova, 1993). This highly productive, riverine shelf water is transported to the southern Black Sea through the Black Sea currents (Miladinova et al., 2020) (Figure 5.1.).

The water masses at the surface are very dynamic with the presence of turbulent eddies in its two-gyre system surrounded by a seasonally modified, with year-to-year variability, cyclonic Rim Current. (Oğuz et al., 1993; Korotaev et al., 2003). Intense Rim Current forms, after the permanent thermocline breaks up at the beginning of winter and is amplified by the coastal freshwater, which flows into the sea. (Murray et al., 2007; Oğuz et al., 1993; Stanev, 2005). On the other hand, during the summer and autumn months, the two-gyre winter circulation system gradually disintegrates into interconnected eddies characterized by mesoscale activity (Korotaev et al., 2003). Thus, due to the weakened thermohaline circulation and stratification, the strength of the surface current decreases and the surface water becomes trapped, which has more stable mesoscale activity properties compared to winter (Oğuz and Malanotte-Rizzoli, 1996). Thus, when all this knowledge is incorporated, in the southern Black Sea region, the major riverine water mass carried from the Northwestern shelf in the west (Oğuz et al., 2002; Miladinova et al., 2020), the warmer sea water characteristics of the eastern Black Sea (Mohamed et al., 2022; Sakalli and Başusta, 2018), and the coastal regions with meandering sea waters with the weakened rim current in summer, upwelling, the influence of the river discharge (Sur et al., 1996; Oğuz et al., 2002; Korotaev et al., 2003) can create water masses with different properties.

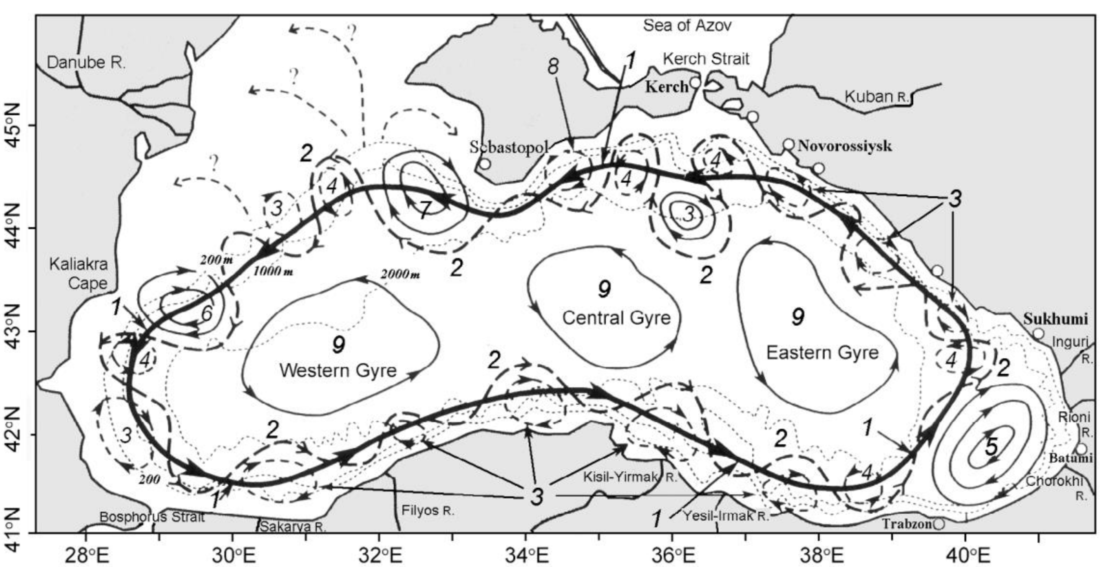


Figure 5.1: Schematic representation of the Black Sea circulation during summer observations [1: mean position of the cyclonic Rim Current jet current; 2: Rim Current meanders; 3: anticyclonic coastal eddies; 4: cyclonic eddies; 5: Batumi anticyclonic eddy; 6:Kaliakra anticyclonic eddy; 7: Sebastopol anticyclonic eddy; 8: Crimea anticyclonic eddy; and 9: quasi-stationary cyclonic gyres] (Korotenko et al., 2010 and the references therein).

Considering the importance of environmental factors on anchovy and the complex interplay between hydrographical variables in the Black Sea, this study seeks to accomplish two main objectives by referencing the different growth rates (Chashchin, 1995;1996) and seawater preferences of the Black Sea anchovy and the Azov anchovy in terms of salinity and temperature (Mayorova, 1950; Chashchin, 1995;1996; Ilyin, 2009; Ivanov and Belokopytov, 2013; Zuyev, 2019; Yuneva et al., 2020), it was firstly aimed to reveal distinct anchovy groups exhibiting divergent growth patterns through the analysis of otolith growth increments. The second is to characterize the water masses in which the samples were collected to understand the impact of the water preferences of these different adult anchovy groups and the growth of the young of the year. In this study, firstly, water masses are more stable in summer than in winter; secondly, young-of-years were most likely born in the water masses in which they were captured; thirdly, the 1-year-old adult anchovy, after the first winter migration, may naturally prefer water masses with similar characteristics to the one in which it was born, were assumed. Accordingly, with these three main assumptions, the two hypotheses that are at the center of the study objectives and tested are: (i) the optimum temperature conditions above the thermocline in the relatively still water masses formed during the summer season, combined with adequate salinity and food availability, can eventually influence the water preferences of 1-year old adult anchovies of different growing groups according to their first-year-growth, (ii) possible different water masses with different temperatures, salinity, and fluorescence characteristics in the Southern Black Sea during summer can affect the growth of the young-of-years caught in these waters.

Material and Methods

Sample Collections and measurements

Anchovy sampling and otolith measurements

The anchovy samples used for otolith analysis were collected during scientific research surveys conducted with RV Bilim-2 of the Middle East Technical University (METU) using midwater trawls on July 12-30, 2013, July 13-31, 2015, and July 13-August 2,

2018. Samples were collected in the Southern Black Sea region (40.9° – 43.4° N and 28.0° – 41.4° E), covering the Turkish Exclusive Economic Zone (TEEZ) during the reproductive time of anchovy. Sampling was done at the same time almost every year, preventing intra-annual variations. A total of 1000 sagittal anchovy otolith specimens were used in this study.

Frozen samples were transferred to the METU Fishery Laboratory for analysis. The samples' total length (±1 mm) was measured before otolith extraction. Ages of anchovy from the otoliths were determined under the reflected light against a black background using a binocular microscope (Olympus SZX16) with a magnification of 25X while immersed in 70% ethyl alcohol and oriented as the distal surface (convex side) up. During age reading the Common Age Reading Protocol for the Black Sea anchovy prepared by Black Sea experts under the supervision of the BlackSea4Fish Project (GFCM, 2019) was followed.

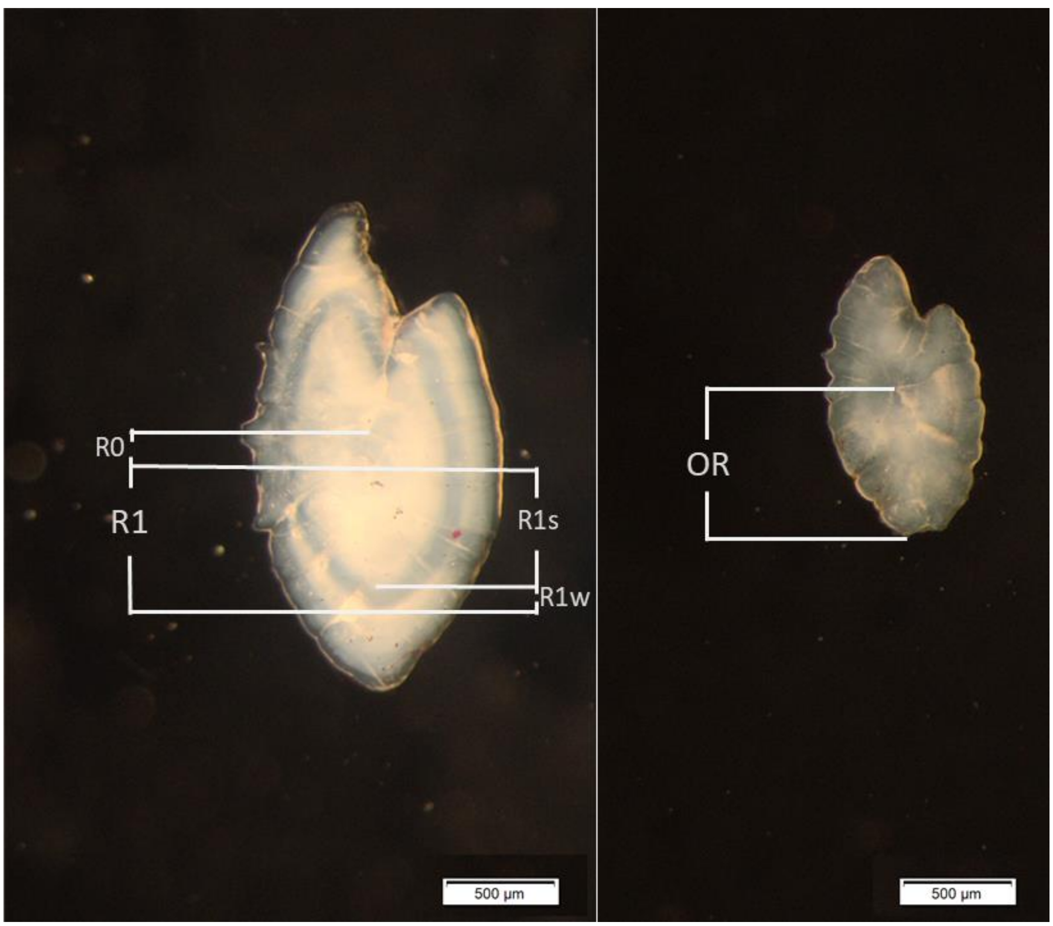


Figure 5.2: First-year growth increment width (R1) of the 1-year-old anchovy, summer increment width (R1s), Winter increment width (R1w) (Left), and the total otolith radius (OR) of the 0-year-old anchovy (Right).

As soon as the age was determined, the photographs of the otolith pairs in .tiff format were taken with the Olympus UC30 Microscope Camera. The measurements were made on the photographs rather than the live view under the microscope to avoid inaccurate measurements that might be caused by light instabilities. Stream Basic Version 1.8 Software was used for all measurements. The first-year growth increment (R1) of the 1-year-old anchovy and the otolith radius (OR) of the 0-year-old anchovy were measured in these photographs by using the right-side otolith (Figure 5.2).

CTD Measurements

During the expedition in the southern part of the Black Sea, covering the entire TEEZ, vertical temperature, salinity, and fluorescence profile measurements along the water column were acquired using a calibrated CTD Seabird SBE 911(Sea-Bird Scientific Inc.). The accuracies of temperature, salinity, and pressure sensors are 0.002°C, 0.010, and 5 m, respectively. With a CTD profiler equipped with a fluorometer, castings were made at 137 different stations in 2013, 120 in 2015, and 106 in 2018 during the sampling period. The stations were positioned 0.5 degrees apart for systematic representation.

In the analysis to reveal the characteristic structure of the water body, measurements at 7 meters depth were used as a basis to depict the mixed layer above the thermocline where the thermophilic anchovy lives. The average thermocline depth was between 10-22 m. Furthermore, the first 5m depth had variations caused by possible differences arising from near-surface measurement, and the ship effect was eliminated by using the 7m that considered representative depth for upper water mass over the thermocline.

Statistical Analysis

Defining the water masses

In order to test the hypothesis that anchovies, which are believed to have different origins and therefore different growth characteristics, live in water masses with different hydrographic characteristics, the waters in the southern Black Sea were discovered. To do so, first, the spatial patterns and variability of the direct CTD measurements of temperature, salinity, and fluorescence were visualized using the ODV (Ocean Data View) Version 5.6.3 (Schlitzer, 2022) by employing the "scope: surface" setting in conjunction with the DIVA gridding technique to show all stations' isosurface values on the map by variable-scaling resolution grid. Moreover, the differences in temperature, salinity, and fluorescence among the sampling years and which specific variables are different from each other were tested by Kruskal-Wallis and Wilcoxon Rank Sum tests, respectively.

In this study, considering the contrasting water preferences of Azov and Black Sea anchovy, water masses defined by their distinct combinations of salinity, temperature, and fluorescence attributes and possibly differentiated under the influence of riverine inputs, coastal dynamics, and offshore seawater interactions were investigated in the southern Black Sea. For this purpose, two methods - principal component analysis (PCA) and agglomerative hierarchical cluster analysis -were performed for three measurements- temperature, salinity, and fluorescence. PCA helped identify the key variables and primary axes of the variances by lowering data dimensions (Lever et al., 2017). All variables were standardized (mean=0 | var=1) before performing the PCA to ensure a comparable scale and prevent a single variable from dominating the analysis. The components that best explain the variance in the data set were selected based on the cumulative explained variance vs eigenvalues. Subsequently, agglomerative Euclidean hierarchical clustering with Ward's linkage was performed to cluster this dimension-reduced dataset to obtain clustered water mass. Different water masses were independently labeled Cluster 1, Cluster 2, and Cluster 3 for each year.

Detection of different growing anchovy groups in the first year of their life

A significant linear relationship was tested between the otolith growth (Otolith radius) and fish growth (Total length) to meet the prerequisite for using fish otolith as a representation of body growth (Stevenson and Campana, 1992).

The study is based on the assumption that there are two groups of anchovies in the Black Sea that intrinsically display different growth rates. It was also assumed that the size of the radius of the first annual ring (R1) is an indicator of the growth rate, and the R1 measurements taken from the mixed population will be represented by bimodal distribution. To distinguish slow- and fast-growing anchovies, the unsupervised classification technique of the Expectation-Maximization (EM) algorithm was implemented to estimate the parameters of the Gaussian mixture models (GMM) was used (Dempster et al., 1977; Redner and Walker, 1984; Hamerly, 2003). By this way, the means, variances of the different R1 populations, and mixing proportions were estimated. Then, for further analysis, 1-year-old fish were selected from both groups that experienced a winter migration and dispersed for the first time in the basin.

An alpha level of .05 was used for all statistical tests. All calculations and statistical tests used in this study were performed under the R version 4.2.3 (R Core Team, 2023) within the R Studio (Posit team, 2022), using the packages of ggplot2_3.4.0 (Wickham, 2016), mixtools_ 1.2.0 (Benaglia et al., 2009), LambertW_ 0.6.7.1 (Goerg, 2022), and FactoMineR_2.8 (Le et al., 2008).

Results

Water Mass Characteristics

The results of in situ temperature, salinity, and fluorescence measurements at 7 meters depth were summarized in Table 5.1 and demonstrated in Figure 5.3 for each sampling year. Accordingly, there were significant temperature, salinity, and fluorescence variations across the sampling years (Kruskal-Wallis chi-squared = 150.7, df = 2, p-value < .001). The pairwise comparisons for 2013, 2015, and 2018 showed, regarding the measured seawater temperature, the warmest conditions were recorded in 2018 (Wilcoxon rank sum test; p-value<0.05). However, no significant temperature differences were found between 2015 and 2013 (p>0.05). As for salinity and fluorescence, mean salinity and fluorescence were higher in 2015 (p<0.05). However, even though the lowest salinity measurement and lowest fluorescence measurements were recorded in 2013 and 2018, respectively, no significant differences in mean salinity and mean fluorescence were found for 2013 and 2018 (p>0.05). These results provide insights into the annual temperature, salinity, and fluorescence variability and their potential impact on anchovy growth.

Table 5.1: Data summary of in situ seawater temperature, salinity, and fluorescence measurements for each sampling year

Year	Sampling time	CTD depth	Temperature [deg C]		Salinity [PSU]		Fluorescence [ug/l]	
			mean	***95% CI***	***mean***	***95% CI***	***mean***	***95% CI***
2013	12-30 July 2013	7m	24.85	24.71, 24.99	17.74	17.63, 17.85	0.07	0.055, 0.077
2015	13-31 July 2015	7m	24.91	24.58, 25.24	17.98	17.88, 18.08	0.25	0.208, 0.287
2018	13 Jul-2 Aug 2018	7m	26.55	26.4, 26.71	17.88	17.81, 17. 96	0.06	0.056, 0.063

Table 5.2 shows the contribution of each variable to the principal components (PCs) according to the PCA result applied to measured variables of temperature, salinity, and fluorescence. The first two components explain 94%, 94.5%, and 73.3% of the total variance for 2013, 2015, and 2018, respectively (Figure 5.4). Accordingly, while the first two PCs were used for 2013 and 2015, three PCs were used for 2018 in order not to lose information. By the hierarchical clustering on these PCs, three distinct seawater clusters were identified for each year. The regions represented by these clusters are presented in Figure 5.6. The variables used in PCA (salinity, fluorescence, and temperature) are unique to each cluster. Based on their similarity in terms of these variables, the clustering analysis reveals specific groupings of observations. The mean values within category and overall (Table 5.3), were used to evaluate how each cluster is differentiated from the overall dataset. Accordingly, Cluster 1 is represented by relatively higher fluorescence and lower salinity (Table 5.3). From this, it can be concluded that this body of water represents the region under the influence of the Danube, both in terms of its location (Figure 5.6; map 2013: orange colored cluster labeled as 1, in the western part of the sampling area) and in terms of its characteristic features. For this reason, this water mass was identified as a “Danubian Arm (DA)”. Cluster 2, defined in the same year, was associated with relatively low temperature and salinity (Table 5.3). It differs from the Danubian arm by the lack of fluorescence representation. This water mass has been

referred to as the “Coastal West (CW)”. Cluster 3, named the “Eastern Warm (EW)” seawater mass, exhibits low fluorescence and high salinity and temperature properties.

Table 5.2: Contribution of the original variables captured by each principal component for each year

Years	Variables	PC1	PC2	PC3
2013	Temperature	0.4875605	0.8652593	0.1166663
	Salinity	0.6285606	-0.2551180	-0.7347288
	Fluorescence	-0.6059673	0.4315566	-0.6682534
2015	Temperature	0.642465	0.75577713	0.1266479
	Salinity	0.836609	-0.49104464	0.242818
	Fluorescence	-0.9509521	0.07860331	0.2991849
2018	Temperature	0.77233858	0.10996793	-0.6256198
	Salinity	-0.01892486	0.99218333	0.1233454
	Fluorescence	0.77423825	-0.08544597	0.6270998

The first water mass discovered in 2015 was characterized by high fluorescence and low temperature. It was entitled “Upwelling Water (UW)”, associated with the region it represented. On the other hand, Cluster 2, which has river traces with its high fluorescence and low salinity feature, is categorized as an "expanded DA" water mass compared to 2013. The EW water mass, defined in 2013 and characterized by relatively high temperature and salinity and low fluorescence, appears to have extended further west in 2015 as Cluster 3.

In 2018, the hottest year among the sampling years (Table 5.1), three different seawater clusters were detected (Figure 5.4). Accordingly, the main defining variables of Cluster 1, which is named "Offshore West (OW)", were higher salinity and relatively low temperature, and fluorescence (Table 5.3). Cluster 2, associated with lower salinity and fluorescence, covered mainly the Anatolian coasts. Thus, this water mass was called "Anatolian Coastal (AC)". The water body identified as Cluster 3 in 2018 is similar to the EW water mass identified in 2013 and 2015 in terms of its characterization with temperature (v-test and mean in category > overall mean; p-value<0.05; Table 5.3). However, this water body, which covers a narrower area (Figure 5.6), is characterized by relatively high fluorescence in contrast to the EW (Table 5.3). This body of water was, therefore, labeled "Eastern Mix (EM)".

Table 5.3: Description of each cluster by quantitative variables

			v.test	Mean in category	Overall mean	sd in category	Overall sd	p.value
2013	**Cluster 1 DA**	**Fluorescence**	9.515922	3.798197	-2.60E-17	1.632821	0.996317	1.80E-21
		Temperature	-4.26041	-1.70051	-7.17E-16	0.515188	0.996317	2.04E-05
		Salinity	-9.0473	-3.61115	-2.19E-15	0.660274	0.996317	1.47E-19
	Cluster 2 CW	**Salinity**	-3.62494	-0.63935	-2.19E-15	0.78281	0.996317	2.89E-04
		Temperature	-7.05978	-1.24518	-7.17E-16	1.061117	0.996317	1.67E-12
	Cluster 3 EW	**Temperature**	8.607149	0.4094	-7.17E-16	0.52841	0.996317	7.49E-18
		Salinity	7.740412	0.368174	-2.19E-15	0.360071	0.996317	9.91E-15
		Fluorescence	-5.09047	-0.24213	-2.60E-17	0.418631	0.996317	3.57E-07
2015	**Cluster 1 UW**	**Fluorescence**	3.570218	1.755105	-3.52E-17	0.503143	0.995825	3.57E-04
		Temperature	-8.79262	-4.32242	4.49E-16	1.598887	0.995825	1.46E-18
	Cluster 2 DA	**Fluorescence**	8.446584	1.542128	-3.52E-17	0.997038	0.995825	3.00E-17
		Salinity	-9.05479	-1.65317	2.65E-15	0.971272	0.995825	1.37E-19
	Cluster 3 EW	**Salinity**	8.359178	0.420977	2.65E-15	0.392037	0.995825	6.32E-17
		Temperature	5.57216	0.28062	4.49E-16	0.451404	0.995825	2.52E-08
		Fluorescence	-9.50345	-0.4786	-3.52E-17	0.19941	0.995825	2.03E-21
2018	**Cluster 1 OW**	**Salinity**	5.535447	0.66369	-1.45E-15	0.527855	0.995272	3.10E-08
		Fluorescence	-3.59797	-0.43139	-1.33E-16	0.828414	0.995272	3.21E-04
		Temperature	-6.0522	-0.72565	1.90E-15	0.612547	0.995272	1.43E-09
	Cluster 2 AC	**Fluorescence**	-2.17472	-0.31416	-1.33E-16	0.688117	0.995272	2.97E-02
		Salinity	-7.68166	-1.1097	-1.45E-15	0.781263	0.995272	1.57E-14
	Cluster 3 EM	**Temperature**	6.370164	0.962382	1.90E-15	0.700499	0.995272	1.89E-10
		Fluorescence	6.08232	0.918895	-1.33E-16	0.867002	0.995272	1.18E-09

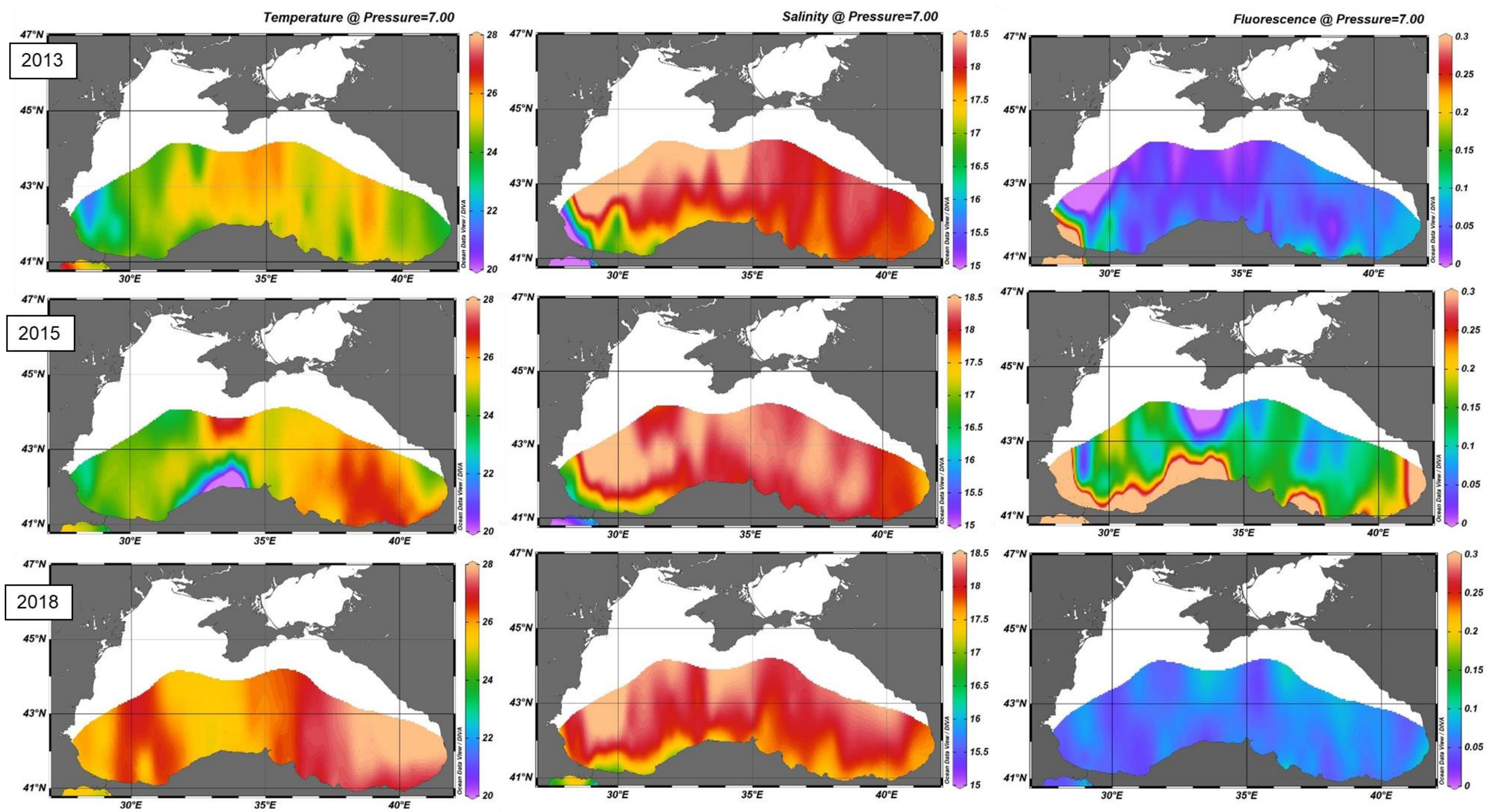
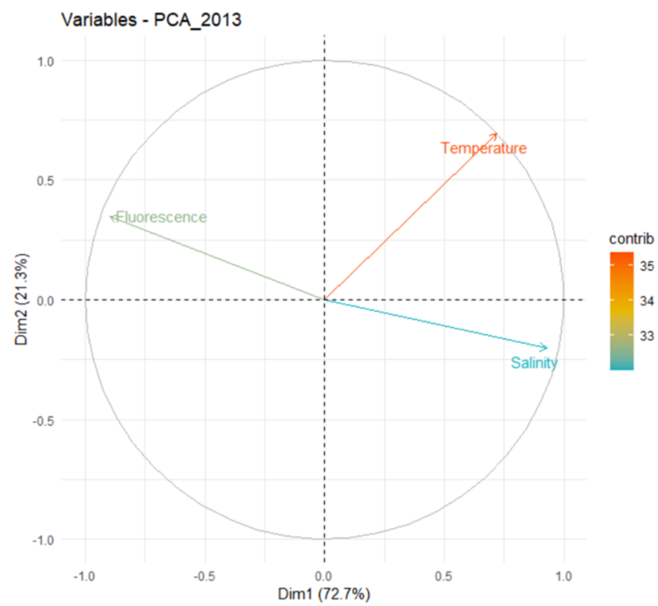


Figure 5.3: Temperature, Salinity, and Fluorescence measurements at 7m depth for the years 2013, 2015, and 2018.



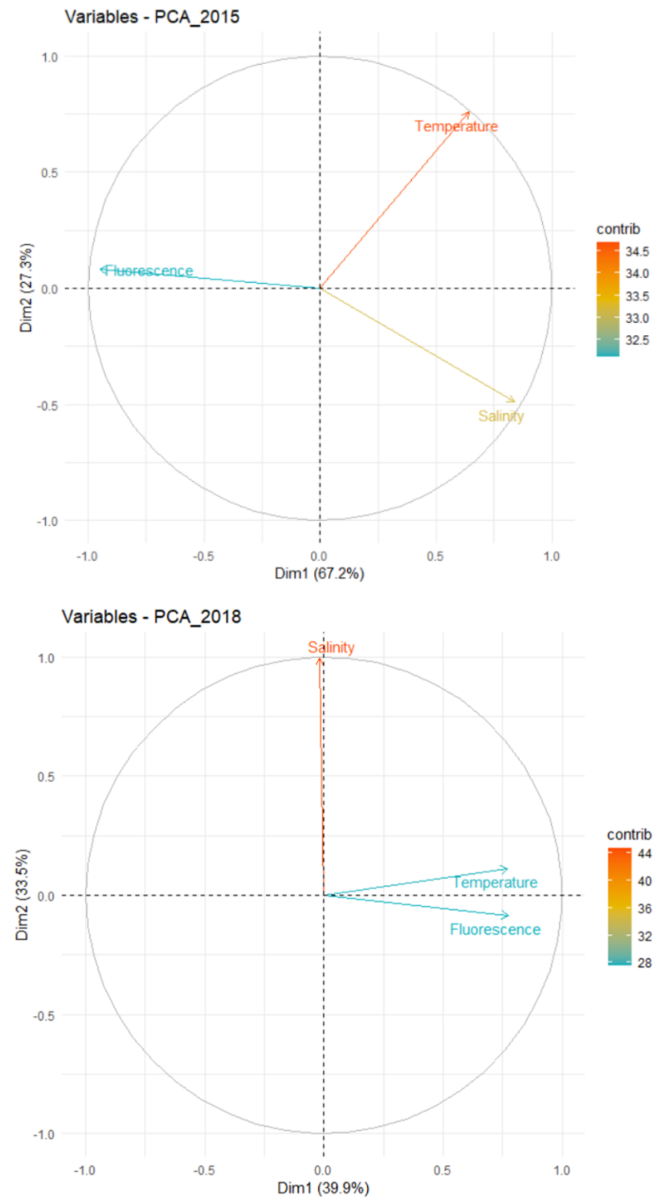


Figure 5.4: graphical representation of the variables and their contributions in the space of the first two principal components.

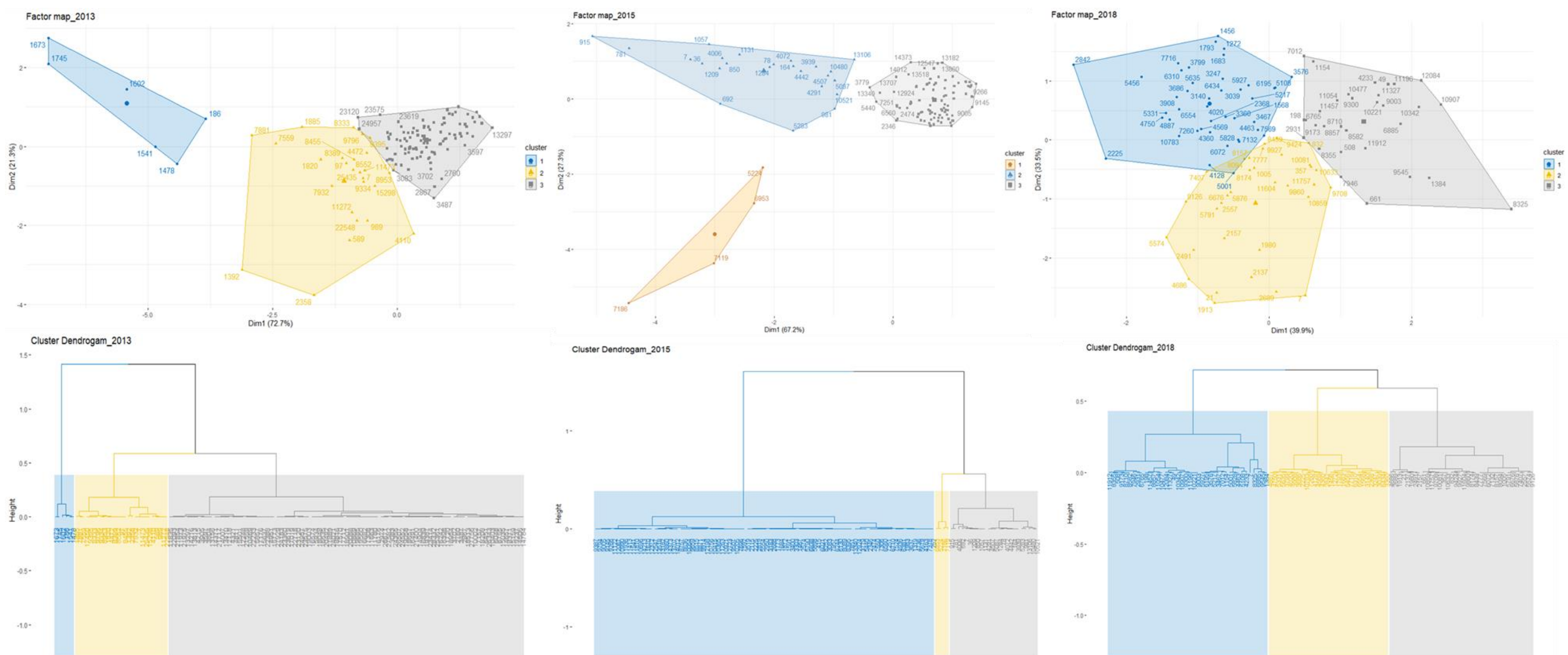


Figure 5.5: In the upper half of the figure: Representation of the seawater clusters by the first two principal components. In the lower half: The hierarchical dendrograms for each measurement year.

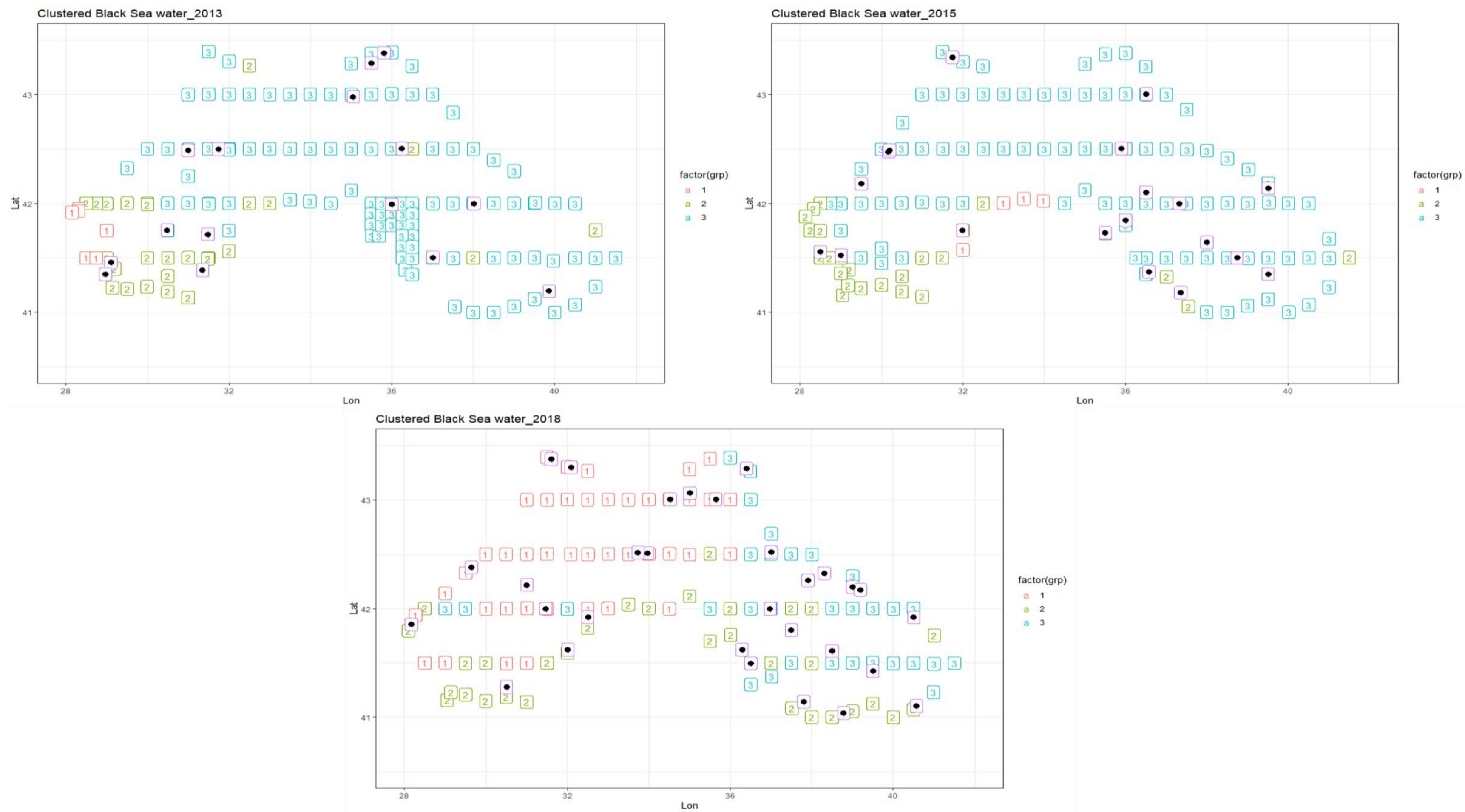


Figure 5.6: Demonstration of the clustered water masses in Turkish EZZ for 2013, 2015, and 2018. Black dots are the stations where the anchovy samples were collected.

Growth Variations of Anchovy in Different Water Masses

A linear and highly significant (OR= 0.0399188+0.1162938*TL; R^2=0.92; p-value < .0001; Chapter 3: Table 3.3) relationship was found between total fish length and otolith radius. Thus, the assumption of a relationship between otolith and fish size was satisfied, and the radius of the first ring and the radius of the otolith (for age zero) were used to represent fish length with statistical confidence.

Two differently growing anchovy groups were identified according to their first-year growth increment widths (EM algorithm for mixtures of normal distributions: log-likelihood=254.6817). 81% of these were represented by Group 1, while 19% represented the fast-growing Group 2 (Table 5.4, Figure 5.7).

According to the summer (R1s) and the winter (R1w) growth increment measurements, it was found that while the summer growth among the anchovy groups showed a statistically significant difference (ANOVA; F(1, 409) = [270.35], p-value < .001), the winter growths were not different (ANOVA; F(1, 409) = [1.6791], p-value=0.1958). These results were evaluated as that the variation in summer growth of the 1-year-old anchovy samples could be a sign of their exposure to different environmental conditions during the first summer of their life.

Table 5.4: Results for the EM algorithm for mixtures of univariate normal calculation

	Component 1	**Component 2**
Final mixing proportions	0.8142129	0.1857871
Component means	0.6960822	0.9395499
Standard deviations	0.1116945	0.1055005

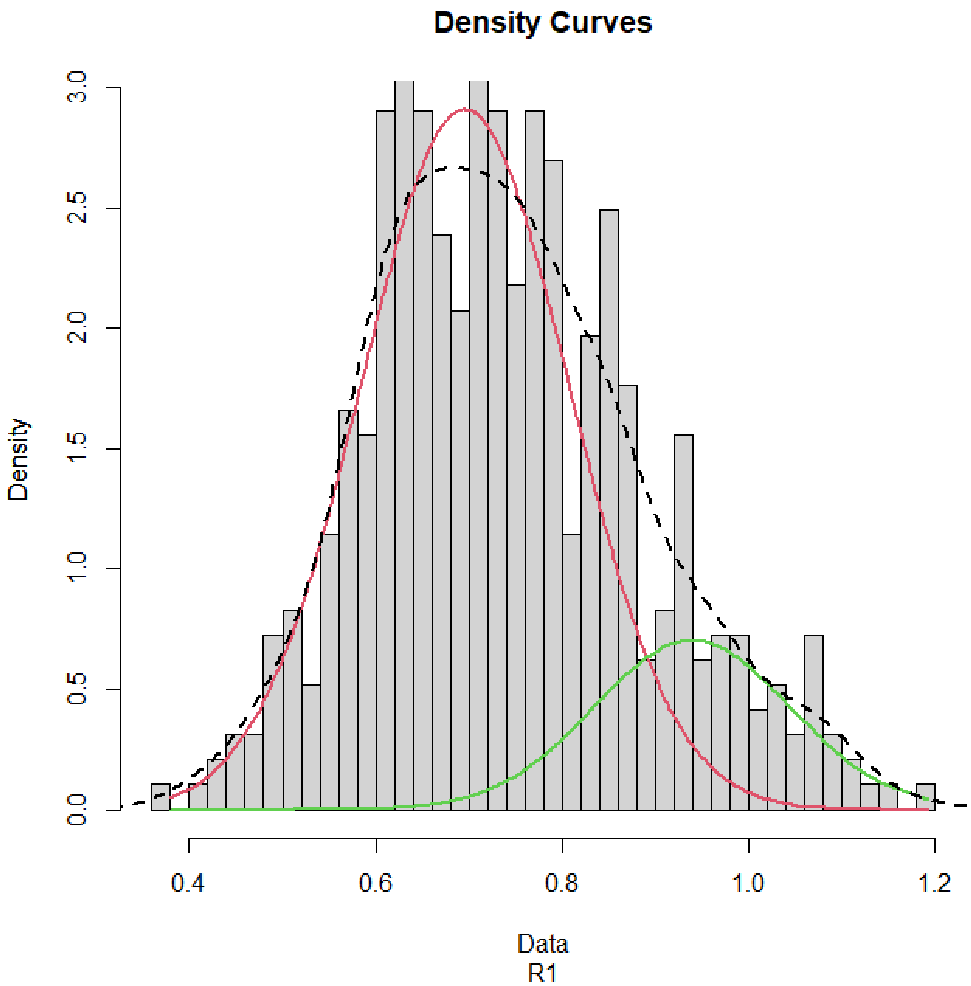
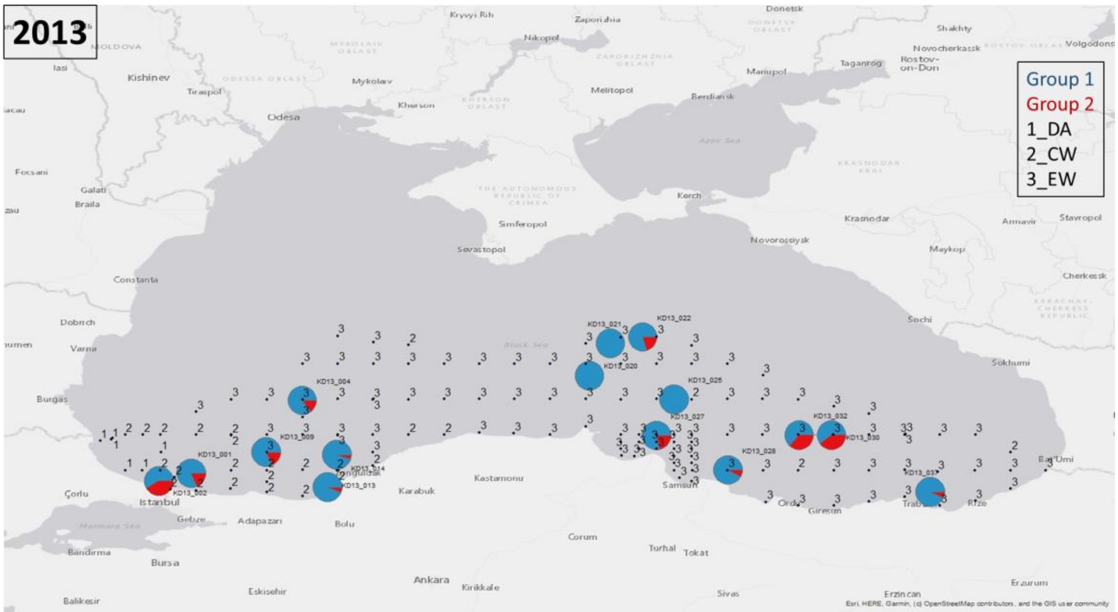


Figure 5.7: R1 density distribution of the defined anchovy groups. (Red line: G1, Green line: G2, dashed line: all data)

This reveals the hypothesis that the whereabouts of the fish in the summer are related to its growth. Thus, different growing groups may prefer different water masses. To test this, one-year-old fish, i.e. fish that had experienced only one winter, were used in both groups to eliminate the annual effect in anchovies, which abandoned their feeding grounds and hibernated largely in the same location during the winter months. For each group in either study year, there was no statistically significant difference in their first-year growth according to the water mass they caught ($ANOVA_{2013}$: F(1, 75) = [2.0812], p=0.1533; $ANOVA_{2015}$: F(1, 112) = [3.374], p=0.06888; $ANOVA_{2018}$: F(2, 84) = [0.5873], p=0.5581). Neither pattern was observed in the group distributions in the southeastern and southwestern Black Sea regions (Figure 5.8). Therefore, the tested hypothesis that adult anchovies leaving their wintering grounds do not spread randomly and prefer certain water bodies, which in turn affects their growth, was rejected.

On the other hand, the ORs of the young-of-year who were born in sampling years showed statistically significant differences over the factor of 2013, 2015, and 2018 (Kruskal Wallis rank sum test; Chi-square = 6.349, df = 2, p-value = 0.04182). The largest mean OR (growth of zero-aged anchovy) was measured in 2015 (M=0.818mm, SD= 0.184). This was followed by 2013 (M=0.789mm, SD= 0.172). However, according to the mean OR value (M= 0.767mm, SD= 0.159), the lowest growth was recorded in 2018 and it is significantly different from the 2015 (pairwise post-hoc Dunn test, 2015-2018 (p-value = 0.0366)). OR measurements in 2013 (ANOVA; F(1, 159) = [19.744], p-value < .001), in 2015 (ANOVA; F (1, 201) = [14.12], p-value < .001) and 2018 (ANOVA; F (2, 151) = [3.0905], p-value = 0.04837) also showed statistically significant differences between different water masses (Figure 5.9). The maximum increment of young-of-year was recorded in 2013 and 2015 in CW and DA water mass, respectively. While in 2018 the maximum growth was observed in EM.



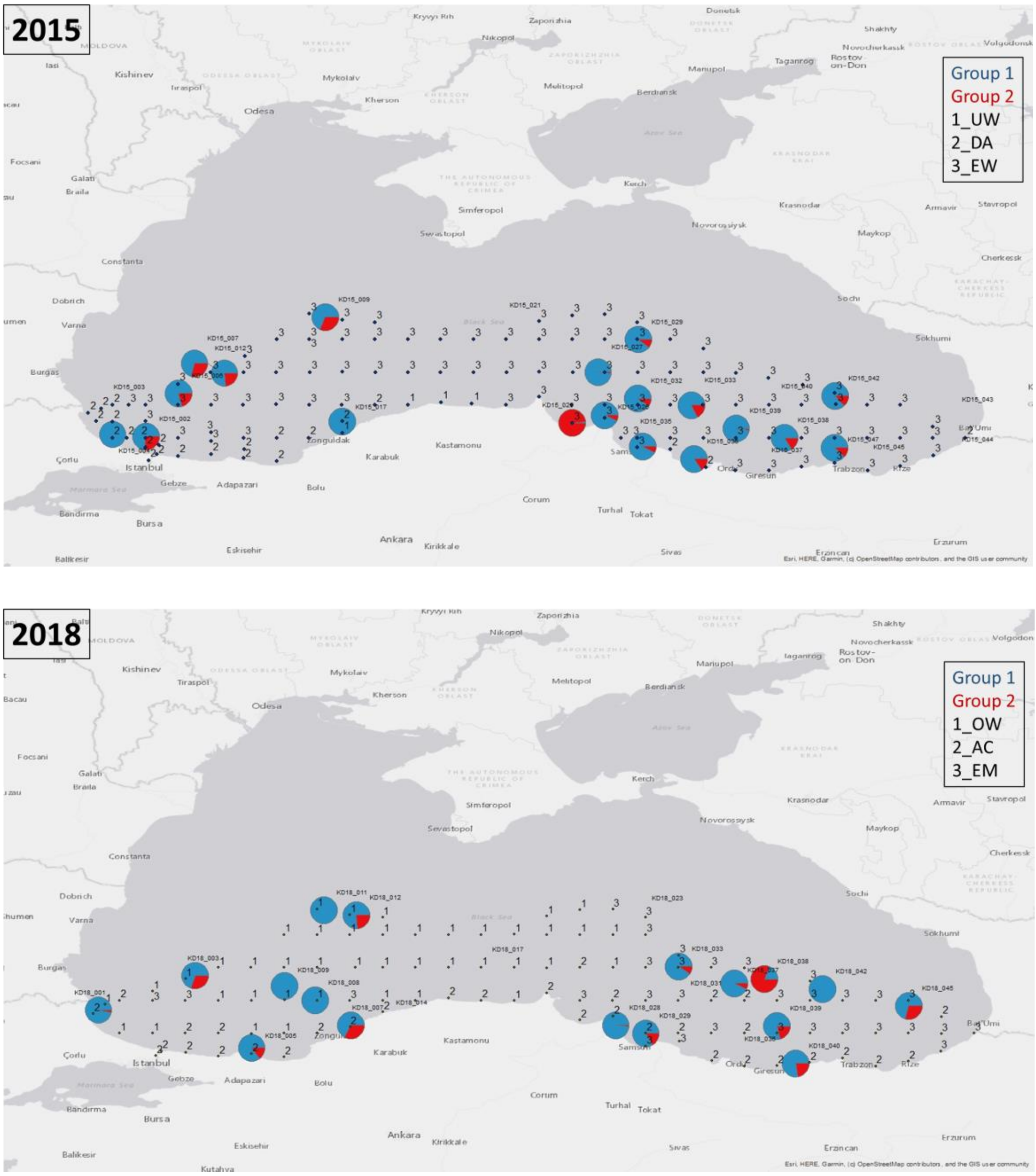


Figure 5.8: The percent distribution of the different growing anchovy groups over the sampling years

In 2013, the mean increment width of the 0-year-old anchovy in CW water mass was larger than those in EW (Figure 5.9). The main difference that separates this CW from EW was that CW had less saline characterization (Figures 5.1, Figure 5.4, and Table 5.3). EW, on the other hand, appeared to be warmer and poor in fluorescence. Similarly, in 2015, the mean OR in the DA was larger than those in EW (Figure 5.9). Since no anchovy samples could be taken from the DA in 2013 and UW in 2015, they could not be included in the evaluation. In addition, in 2018, the mean ORs of young-of-years in AC were narrower than that of OW. EM, on the other hand, was the warmest seawater mass and showed relatively higher fluorescence than other clusters.

Indeed, the highest OR was measured in this seawater mass this year. However, the highest mean OR recorded in 2018, the warmest year, was still lower than the highest mean OR of 2013 and 2015 (Figure 5.9).

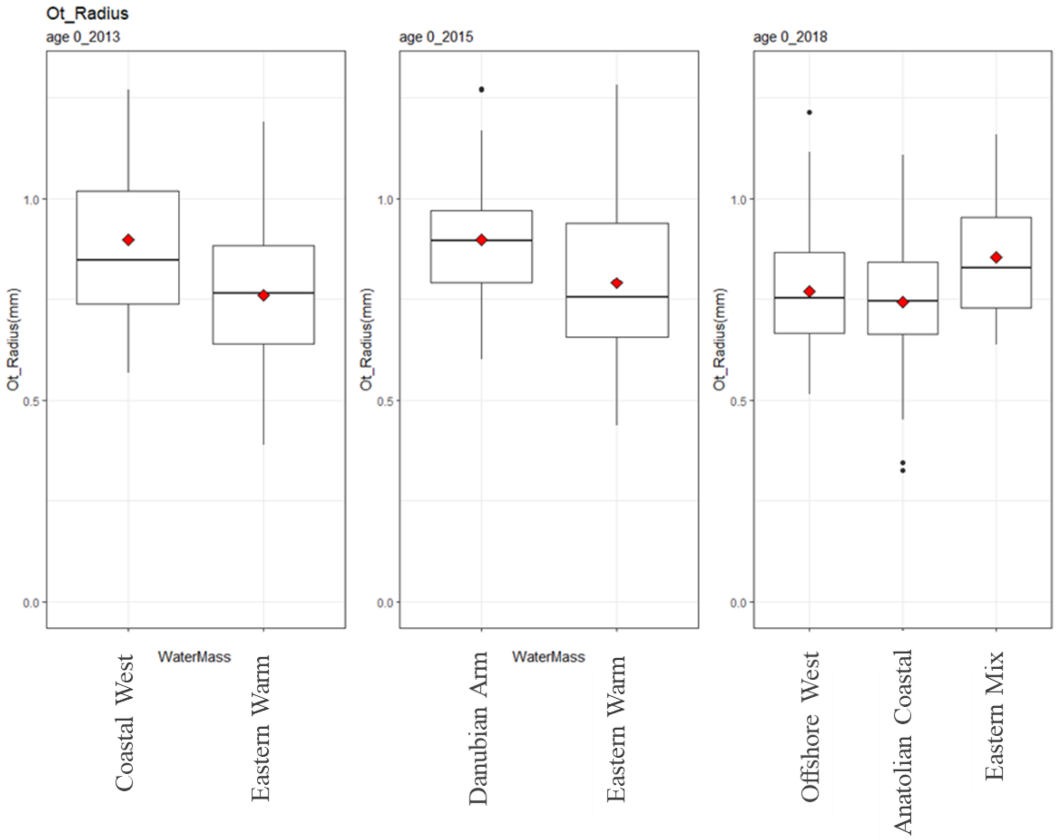


Figure 5.9: Otolith radius (OR) variances of the zero-aged anchovy to the water mass within years (red diamonds=mean OR)

Discussion

It is known in the literature that the two distinct higher hierarchical groups of the Black Sea and Azov anchovies, which have distinctive growth rates, may exhibit different preferences for seawater conditions during their feeding and reproduction (Mayorova, 1950; Chashchin, 1995;1996; Ilyin, 2009; Ivanov and Belokopytov, 2013; Zuyev, 2016; 2019; Yuneva et al., 2020). Moreover, it is emphasized that the distinction between these two groups is based on the isolation of their reproductive areas over successive generations (Zuyev, 2019; Chashchin, 1996). The recent genetic study results show that there are no genetic differences between the anchovy groups in the Black Sea and the spawning areas seem to be the main cause of the morphological differences, which are established at an early stage of development, in the first year of life (Vodyasova and Abramson, 2017; Nebesikhina and Lebedev, 2019; Vodiasova, 2020). Therefore, in this study, groups of anchovies were identified based on their first-year growth rate. According to the width of the first true annual increment on the otolith, it was found that there are two anchovy groups inhabit in the southern Black Sea which show different growth characteristics. However, our results indicate that these adult groups exhibit a mixed distribution, regardless of the water mass characteristics. On the other hand, a strong relationship was found between the growth of the young-of-year and the water mass in which they were caught and were probably born and grown.

The linear regression between OR and total fish length estimated in this study was relatively high compared to previous studies performed with European anchovy (Basilone et al., 2004; Zengin et al., 2015; Carbonara et al., 2023). Therefore, the assumption, which is a prerequisite for reliably conducting an otolith-based growth study (Campana and Neilson, 1985) was statistically validated for Black Sea anchovy. In this study, age readings were done and true annual rings were determined within the framework of the rules outlined in the latest anchovy age-reading protocol (GFCM, 2019), which are considered to largely eliminate inconsistencies in terms of repeatability and consistency.

Within the scope of the study, the water masses categorized within the years in terms of temperature, salinity, and fluorescence parameters obtained by in situ measurements can be temporal and belong to sampling time. However, in the Black Sea, where the current system is dynamic, the sampling season enables this large spatial categorization. Because, in summer, the rim current loses strength and the formation of the western and eastern gyres can be observed (Kubryakov and Stanichny, 2015; Oğuz, 1997). Many anticyclonic eddies are formed between the edge of the shelf and the Rim Current (Miladinova et al., 2020). It was found that the lifetime of some of these formations in the Black Sea changed from 6 months to a year (Zatsepin et al., 2003; Kubryakov and Stanichny, 2015). Thus, the slower-moving water bodies in the region may inevitably become trapped and exhibit distinctive features during the summer months.

Regarding the results obtained (Figure 5.1 and Figure 5.4), the water masses identified within the scope of the study can be interpreted as follows. The effect of the river in the Black Sea is known to strongly regulate salinity and primary production in particular (Oğuz et al., 1992; Özsoy and Ünlüata, 1997; Kideyş, 2002). Therefore, the DA and CW in 2013 and 2015 can be characterized as the water bodies formed by the effect of the shelf waters formed by the northern rivers, especially the Danube, Dniester, and Dnieper, to be carried to the south by the currents (Oğuz et al., 1994; Tolmazin, 1985). In this study, 2015 was defined with high fluorescence as highlighted in the literature (Kubryakov et al., 2019). This high fluorescence in the basin in this particular year is thought to be due to the transport of seawater from the coast to the open sea with above-normal wind patterns (Kubryakov et al., 2019). Moreover, the formation of the UW seawater mass in the known coastal upwelling region (Sur et al., 1994; Brink, 1983) and was recognized in other studies as well (Stanichnaya and Stanichny, 2021). On the other hand, 2018, the hottest in the sampling years of this study, was also recorded as the warmest year in recent years (Mohamed et al., 2022). This year, when the lowest young-of-year growth increment was detected, can be seen as a proxy to evaluate the effect of global warming on anchovy growth in the Black Sea.

It is known that anchovy prefer specific seawater bodies with optimal growing conditions (Palomera et al., 2007; Peck et al., 2013). Indeed, the Azov anchovy reproduces and grows in the Sea of Azov where the salinity is low, whereas the Black Sea anchovy can reproduce and grow in the basin which has higher salinity. Then, they experienced a winter in their overwintering areas (Chashchin, 1996). After winter, when the temperature begins to rise, they presumably begin to intrinsically spread into similar water features in which they were born and grown (Petitgas et al., 2006). According to the results of this study, contrary to expectation, for the anchovies that have different growth characteristics, no relationship was found between the first-year growth and the water mass preferences where they were caught. In addition, it was revealed that they were mixed in the sampling stations in the first summer they experienced after their first wintering, regardless of the seawater characteristics they were caught.

Considering the stock unit assumption made in the stock assessment of the Black Sea anchovy, this study has an important outcome. Because it is assumed that there are two different anchovy stocks with different growth rates in the region. It is assumed that these different groups do not overlap in their niches due to their different preferences for seawater properties and therefore never mix (STECF 17-14). This study confirmed the existence of different growing groups in the summer-in their reproductive season-, at least in the Southern Black Sea area. Contrary to this unit stock assumption, these groups were mixed. Accordingly, it should be determined whether the catch composition obtained from the southern Black Sea region consists of a single stock or the different growing groups mentioned. If there are different groups, based on this, it is recommended to re-evaluate the unit stock assumption made for Black Sea anchovy.

Chashchin (1995) indicated that the ranges of two anchovy races (the author's definition for Azov and Black Sea anchovy) annually vary with respect to their biomass. It affects the dispersion of the fish through the niche of other races and triggers the mixing of the spawning stocks during their reproductive periods. These overlapping zones, and the cross-fertilization between the Azov and the Black Sea anchovy and following abundant hybrid generations, strengthen the hybrid hypothesis (Kalnin& Kalnina, 1985; Kalnin et al., 1984, 1985, cf. Chashchin et al., 2015). Among the two groups defined as slow- and fast-growing in the southern Black Sea in this study, the fact that the slow-growing group may be the hybrid mentioned here or another non-migrating southern stock (Gücü et al., 2016) should not be disregarded, instead of being directly considered as Azov anchovy. Because, firstly, this group could not be characterized by a specific seawater mass in the study area. Second, the salinity in the southern Black Sea (Table 5.1) is relatively high for the optimal salinity for the Azov anchovy, namely 10.0–11.5 (Chashchin et al., 2015). In addition, the increased salinity of the Sea of Azov in recent years (Berdnikov et al., 2022; Yuneva et al., 2020) can also be considered an obstacle to the occurrence and abundance of the Azov anchovy.

The thermal window for the Black Sea anchovy reproduction is 16-28 °C and the salinity window is 7-18‰. They spawn in the warm upper layer in summer and mainly in coastal regions (Lisovenko and Andrianov, 1996). However, it is not explicitly known whether they have specific water body preferences for spawning or not. In other words, we do not know if adult anchovies with different growth characteristics in the southern Black Sea choose the water in which they spawn, but we can say that the water in which they spawn will determine the fate of their offspring (Planque et al., 2007). Indeed, according to the results of this study, a statistically significant relationship was found between the ORs of the young-of-year born in the sampling year and the water mass in which they were caught.

According to the hypothetical birth date of June 1st, the captured young-of-years were at most 40–60 days old in each sampling year (GFCM, 2019). It can therefore be assumed that the young juveniles examined were substantially of the same age. According to the results, the overall annual OR averages of young-of-years in 2015 were higher than in 2013 and 2018. Since 2015 is a year that stands out with high fluorescence, this gives us the conclusion that food may be the most crucial factor in summer juvenile growth.

Moreover, in 2013 and 2015, the young-of-year growth rate was higher in CW and DA, characterized by high fluorescence and low salinity, than in EW, a warmer water body with low fluorescence properties. Therefore, it would not be wrong to associate the high-growth fish in CW and DA with the low-salinity, high-nutrient river water coming from the northwest (Kıdeyş et al., 1999). In 2018, the highest growth was observed in EM, the water body associated with high temperature and relatively high fluorescence. As Bacha et al. (2010) underlined temperature and food have a major impact on anchovy growth in the first year of life. This study also showed that for the Black Sea anchovy, which reaches 73% of its total length in the first year of life, the amount of food may be the determinant of growth when all sample years are considered. However, to better comprehend the link between climate and growth, it can be added that understanding the impact of food type and quality on anchovy growth is another necessary step.

Temperature, another critical parameter, raises the question of what the upper limit of the optimal growth temperature for anchovies in the Black Sea region is. For instance, this temperature is above 21–22°C (SST) for young-of-year Japanese anchovy. It has been pointed out that above this temperature the growth rate begins to decrease (Takasuka and Aoki, 2006). As the lower temperature range may narrow with climate warming, knowing the upper thermal range of this species is important for optimal growth estimation (Nati et al., 2021). In the Black Sea, sea water temperature increase is more rapid in the eastern Black Sea than on the western side (Mohamed et al., 2022; Sakalli and Başusta, 2018). In the current study, warmer seawater was found in the eastern Black Sea for each sampling year. Except for 2018, the mean ORs were lower in 2013 and 2015 in the EW water masses. This may create different growing groups due to the temperature in this region. In addition, it can also affect the anchovy in the Black Sea as an indirect carry-over effect, which affects the vital stages of the fish (such as the first reproduction) (Moore and Martin, 2019; Pauly, 2021).

This study includes intermittent sampling over three years, which is the weakest aspect of this study. Anchovy is a short-lived fish. For this reason, it is greatly affected by annual environmental changes. Although the 3-year sample provides insight into the relationship between young-of-year growth and water mass, it is thought that otolith-based studies with more detailed and consecutive expanded sampling will provide more inclusive results. In addition, sampling could not be made among all the water masses detected, since no fish were caught in the nets at those stations.

Although the results provided important insight into the young-of-year anchovy growth and the water mass they caught, more extensive research is needed to better understand the species and its response to environmental changes. In marine ecosystems, small pelagic fish are excellent bioindicators of climate-driven changes (Peck et al., 2013). For this reason, the continued conduct of such studies is considered essential to assess the response of anchovy stocks to climate change. Stock management based on this knowledge is one of the important parameters to ensure the sustainability of this valuable stock.

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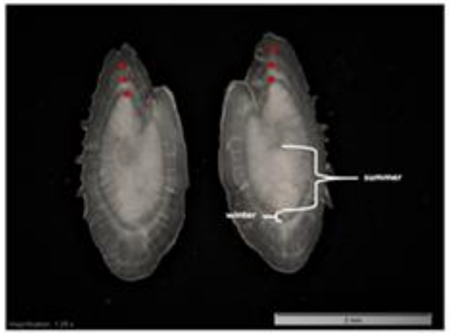
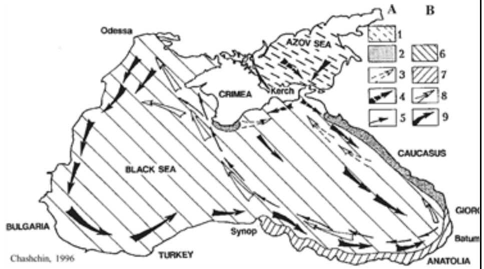
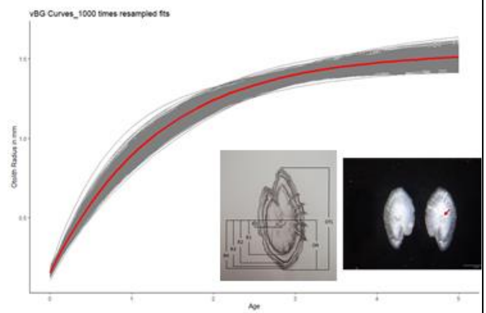
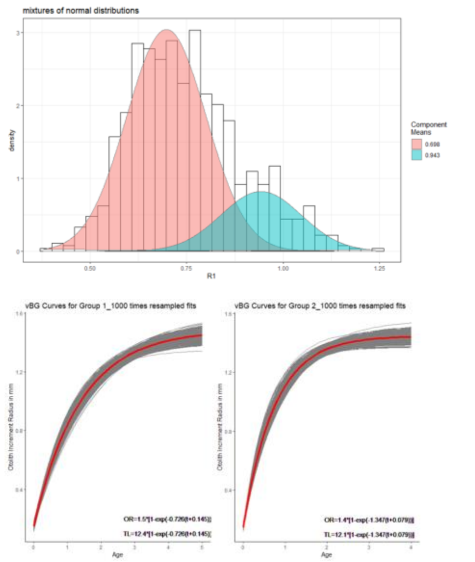
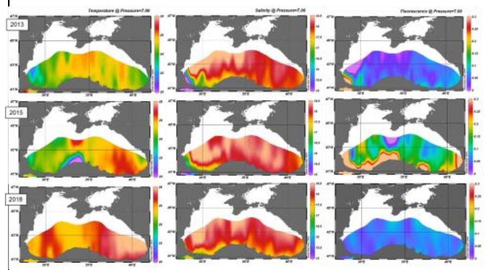
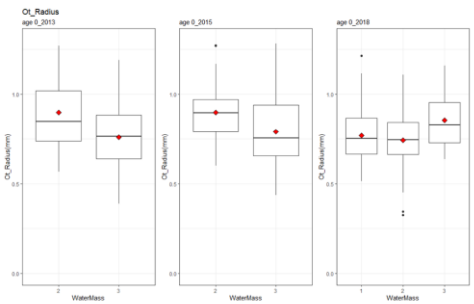
Thesis At a Glance

Chapters	Objective	Method	Infographic	Main Findings / Conclusions
Chapter 1 A Guide for The Black Sea Anchovy Age Reading	to demonstrate the developed age reading protocol for the anchovy in the Black Sea	Experts from the Black Sea contributed and were accepted by all		This common age reading protocol was prepared as part of this thesis. It was presented to the experts in the Black Sea under the leadership of the GFCM, their contributions were received and published as a technical report (GFCM, 2019). It is now actively applied by riparian countries for stock assessment purposes.
Chapter 2 Learning from the Past: A Review of Anchovy Classifications in the Black Sea	to comprehensively cover theoretical and conceptual frameworks on intraspecies/intrapopulation differences of the Black Sea and Azov anchovy groups in the Black Sea.	literature reviewed		Subspecies, whose morphological, behavioral, and biological differences in the past have been revealed and accepted, are not even genetically different from each other according to the results of the analysis made with modern genetic techniques. The most recent definition describing anchovy groups in the Black Sea is environmental ecotypes.

Chapter 3 An Otolith-Based Approach to Estimate the Growth Parameters: a Case Study for the anchovy in the Black Sea	_to find a way to solve the effects of aging, measurements, and sampling time as sources of bias in growth calculation _to propose a method for reliably calculating the growth parameters of that time from otoliths collected in the past	the 2919 otolith-based chronological increment widths were used in growth parametrization and the obtained results were compared with published growth parameters		Compared to the literature, growth parameters that biologically better reflect the stock were obtained with this otolith-based method. With this proposed method, growth can retrospectively be parameterized with information collected from the otoliths collected in the past.
Chapter 4 Detection of anchovy (*Engraulis encrasicolus*) groups with different growth characteristics in the southern Black Sea using otolith	to define possible distinct groups according to their first-year growth inferred from the otolith	first-year increment measurements of anchovies' right sagittal otolith samples collected from the entire southern Black Sea in 2012:2016, 2018, and 2020 were used. growth rate differences were used to separate the possible groups		Two groups of anchovy with growth performance indices of 2.05 and 2.29 and with different growth rates of K=0.726 and K=1.347 were identified. This method can be applied to otoliths from catch to distinguish the different growing groups within the annual catch. Over the born years, the proportion of Group 2 in the population has decreased significantly, while Group 1 has tended to increase. Based on this, it can be claimed that the sustainable management of this valuable fish can be ensured by knowing different growing groups in the

				catch and their proportional changes over the years and managing the stocks accordingly.
Chapter 5 Impact of Environment on the Anchovy Growth: A Hydrographic Perspective	To find out a relationship between the characteristics of the water body in which they are caught and the growth of anchovy	temperature, salinity, and fluorescence measurements were carried out at 363 stations and anchovy sampling at 75 stations in 2013, 2015, and 2018 (July-August) in the southern Black Sea (Turkish EZZ). In addition, measurements of otolith growth increment representing anchovy growth were used. For each sampling year, different water masses characterized by temperature, salinity, and fluorescence were defined.		Two different growing anchovy groups were detected in the southern Black Sea during their reproductive period. Results indicate that the different growing adult groups exhibit a mixed distribution, regardless of the water mass characteristics. However, a significant relationship was found between the growth of young-of-year and the water bodies in which they were caught. *The unit stock assumption made in the current stock assessments should be reconsidered. **The results of this last study will help to understand the effect of environmental factors on anchovy growth. Thence, environmental effects will be another factor to be taken into account while making stock management planning. Moreover, considering the environmental ecotype hypothesis, the outcomes of Chapter 5 could be used as proxy information in climate change studies in the Black Sea.

GENERAL CONCLUSION

This dissertation initially arose from the search for a solution to the problem of unit stock, age determination, and estimation of growth parameters for Black Sea anchovy. These are the main life history parameters used in age-based stock assessment approaches. Furthermore, it was proposed to separate different groups of anchovies by considering their growth in the first year of life, a hypothesis based on which different anchovy stocks, whose existence is debated in the Black Sea, may be separated. Finally, it is known that anchovy populations are strongly and differently affected by environmental factors at different stages of their lives (Fernández-Corredor et al., 2021). Furthermore, this has been accepted that there are two different growing anchovy stocks in the Black Sea, and these groups grow and reproduce in seawater that has different physical properties i.e., salinity (Mayorova, 1950; Chashchin, 1995;1996; Ilyin, 2009; Ivanov and Belokopytov, 2013; Zuyev, 2019; Yuneva et al., 2020). From this point of view, the hypothesis was tested that there could be a connection between the seawater properties in which anchovies are found and the instinctive water preferences of adults from different growing groups and the growth of the young-of-year.

Anchovy in the Black Sea has been managed according to the results of age-based stock assessment models. These models are statistically portrayed as a virtual reflection of the targeted stocks. If this reflection is to display the stock itself, the elements that define the stock used in the model must be correctly depicted. In other words, Box and Draper (1987) stated that all models are wrong, but some are useful. So, the usefulness of the statistical fish stock assessment model can only be achieved by improving input data quality, precision, and accuracy.

Uncertainties start far before the assessment models. There are many assumptions about the fishery-dependent and biological life histories data accepted before running the assessment models. The latter, which is the focus of this study, ultimately affects spawning stock biomass, recruitment, and fisheries mortality estimates. They are used in the model with the assumption that life history parameters such as age determinations, estimated growth parameters, and consequently natural mortalities-at-age are correct. Since we cannot observe and sample all fish in the basin, it is already hard to estimate all these parameters hundred percent correct. Therefore, an estimate that is as accurate as possible is essential to be able to give reliable advice for sustainable stock management.

For this purpose, this study provided two main outputs that would improve anchovy age determination and growth estimation. The first of these is that it acts as a catalyst in the preparation and implementation of a common anchovy age reading protocol in the Black Sea. This protocol will allow a more accurate determination of the demographic structure of the stock and the cohort tracking with the ages to be read separately for each country and each year. It will be able to do this by enabling the construction of compatible and comparable age information for use in stock assessment and other studies. Nevertheless, further marginal incremental analyses are recommended for future studies to improve the validation of the protocol. Another is to develop a method that uses the otolith increment information to estimate growth parameters. This method will help to eliminate cohort slicing bias and the errors introduced by sampling at different times of the year. In addition, the unrealistically high t0 value estimation is also removed with this method. By doing so, it is possible to estimate growth parameters with greater accuracy. Another advantage of this method is that the estimated growth parameters that are collected during the previous years can be retrospectively improved using the otoliths collected in relevant years.

The other main assumption of stock assessment studies is to accept Black Sea anchovies as unit stocks that are never intermixed. This situation makes the management advice based on the results of these models uncertain in terms of the targeted stock. Because it is estimated that there are at least two different anchovy stocks in the Black Sea, whose existence is examined by ongoing studies. Moreover, intermittent mingling of these anchovies has also been observed previously, resulting in the emergence of hybrid populations (see Chapter 2). Moreover, when managing targeted fish stocks, establishing stock boundaries according to biological characteristics (growth, mortality, first maturity, etc.) is a fundamental requirement to ensure sustainable management with plans based on more precise and accurate stock-based information. For instance, applying the same minimum catch size rule to different growing groups can be a disadvantage for fast-growing ones and may lead to the collapse of that stock. Considering that the minimum catch length for anchovies in the Black Sea is 9 cm, a fish that would have been longer than that length would be caught. Although this minimum landing size rule is based on the assumption that the fish spawn at least once in the sea, it does play a role in selecting different growing groups. According to the BOFFFF (big old fat fecund female fish- Hixon et al., 2014) and Maternal effects and egg size (Koenigbauer, 2020) hypotheses, the productivity of the stock will be greatly reduced, as it will be difficult for the fast-growing ones to stay in the system. This could result in the population shifting more in favor of smaller anchovies.

In fact, according to the results of the study presented in Chapter 4, two separate anchovy groups were identified in the southern Black Sea that grew differently during their first year of life. This proved the existence of distinct growth groups for the southern Black Sea as well, which was highlighted as unknown information in the studies conducted in the north (Maiorova, 1950;1954; Chashchin, 1996; Zuyev, 2019). As a result of the limited sampling area, the findings of this study could not be generalized. Because the studies in the north focus on the Azov anchovy and Black Sea anchovy according to their diverse reproduction habitat, while in our case, similarly, a non-migrating anchovy stock sign is claimed to exist in the southern Black Sea (Niermann et al., 1994; Gücü et al., 2016). In this regard, the lack of samples covering the entire Black Sea limits the conclusions for naming the detected distinct growing groups as Black Sea anchovy, Azov anchovy, or Southern anchovy.

Environmental parameters are factors that have a significant impact on anchovy recruitment in the Black Sea and the Sea of Azov. However, in such dynamic marine systems, measuring all environmental parameters and their interactions with each other is not possible. Whereas, in this study, the water masses in the sampling area were characterized by using the temperature, which is the most commonly measured and used parameter in ongoing oceanographic studies, as well as salinity and fluorescence data measured at the target depth by CTD. Accordingly, as presented in Chapter 5, different water masses with different properties were found for each sampling year. The determination of seawater mass properties is an important step in assessing the inherent water preferences of different growing groups. This importance arises from the well-established understanding that the Azov anchovy is known to spawn in the Sea of Azov, characterized by relatively diminished salinity compared to the more saline environment of the Black Sea, where the Black Sea anchovy reproduces. Within the scope of this study, it was postulated that even these anchovy stocks, which have different growth rates, intermixed in the basin during their reproductive period, it is hypothesized that these anchovy groups are likely to display preferences for water masses possessing similar attributes to those of their respective native habitats in which they were nurtured and matured.

According to the first-year growth of 1-year-old anchovy collected in the surveys, two distinct anchovy groups were found using the method proposed in Chapter 4. The growth of one-and-a-half-month-old juvenile anchovy since they were born was parameterized by measuring the radii of their otoliths. However, the results showed that there is no relationship between the water mass in which the adult anchovy is found and their growth in the first year. This does not support the hypothesis of returning to their native region after experiencing the winter migration for the anchovy in the Black Sea (Petitgas et al., 2006). The results support that different groups are intermixed. So, it can be concluded that the regional stock differences approach is not the best logical approach while assessing fish stocks. Because it appears that different growth groups are also mixed during the summer months without following any regional pattern or water mass.

Irrespective of their spatial dispersion dynamics, this selection remains a crucial determinant influencing the fate of the new generations. This can be seen in the significant relationship between the OR of young-of-year and the water bodies in which they are caught. This confirms the hypothesis that the water in which the anchovy was born or spent the first year of its life could be an important factor influencing the growth and eventually the overall lifespan of the anchovy (carry-over effect: Moore and Martin, 2019). It may be emphasized that this is a finding that should be considered when making stock management decisions. The water dynamics, which exhibit annual fluctuations, along with the notably pronounced impact of climate change, are factors that influence the growth of young-of-year and thus recruitment.

Based on the findings and outcomes of this study, it is therefore recommended that the age-length key be prepared regularly for each year from the samples from the commercial anchovy catch. This ensures better cohort tracking and age consistency. According to the prepared age reading protocol, the sagittal otolith pairs are used for the age determination of anchovy in the Black Sea. From these otoliths, while estimating the age, it is also possible to estimate growth parameters that reflect the annual catch using the otolith increment width measurement method proposed in Chapter 3.

Moreover, this study was carried out with anchovy otolith samples from fisheries-independent surveys. Therefore, as a solution to the unit stock uncertainty and/or definition, it is proposed to identify different growing anchovy stocks by their first-year growth by measuring the initial growth ring widths on otoliths collected from the annual catch. Besides, climate change and its impact on marine organisms is an important issue that needs to be comprehended. In order to understand the effect of characteristic changes in the environmental water masses on the growth of anchovy, the results presented in Chapter 5 are thought to be a proxy for future studies. Because water mass characteristics eventually affect the growth of the young-of-year. The question of how seawater bodies, which are warming and predicted to be warmer due to climate change affected and will affect fish growth include the answer that should be known for the future of the stock.

In conclusion, the results of the stock assessment models reflecting the targeted stock itself, the advice to be given in line with these results, and the fishery management strategy to be applied are all possible with reduced uncertainty input data. A good knowledge of the biological life history is the way to understand the resilience of the stock to fisheries and climate, thus enabling sustainable management. It is reasonable to say that this study arose from such a need. Thus, we believe that this study and its results represent the Black Sea anchovy in this regard. All these sub-findings in the chapters presented serve the main idea of better assessment goals for anchovy in the Black Sea.

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PUBLICATIONS

1. Akkus, G., & Gücü, A. C. (2023). First Maturity Size of the Black Sea Anchovy and Its Implications on the Age-Based Stock Assessment Models. Submitted to Acta Biologica Turcica on July 28, 2023.
2. (*Accepted oral presentation*) Akkus, G., & Gücü, A. C. (2023, October 9-13). Depiction of the different growing Black Sea anchovy groups using otolith annual growth widths [Life history, demography and connectivity studies]. 7th International Otolith Symposium. Viña del Mar, Chile (https://www.ios2023.cl/)
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10. Akkus G., & Gücü A.C., (2018). Behavioral changes observed in Black Sea anchovy and its possible causes. FAO. 2018. Fish Forum Book of abstracts. Rome. 338 pp. License: CC BY-NC-SA 3.0 IGO.
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HOBBIES

Reading, running, scuba diving, yoga, Latin aerobics, jewelry design, camping, gardening, and visiting art shows, exhibitions, museums, and ancient sites. Painting using techniques such as charcoal, watercolor, marbling, and oil painting was exhibited in eleven painting exhibitions.