THE EUROPEAN ANCHOVY AND ITS ENVIRONMENT, I. PALOMERA and P. RUBIÉS (eds.)

The Black Sea populations of anchovy*

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SUMMARY: A complex method of race determination based on the genetic, parasitological and morphological traits of the population was proposed to obtain a reliable scheme of distribution and migrations of the Azov and Black Sea anchovy stocks. The abundance of anchovy was determined using the data from surveys performed with standard trawl and hydroacoustic equipment. Experiments revealed considerable changes in the Azov anchovy's gene pool. The main reason was the increasing of water salinity as a result of river in-flow regulation. The excessive industrial catching also promoted the process. As a result, the proportion of hybrids increased. According to the data of the acoustic surveys in the waters of the former USSR in the period from 1980 to 1988, the average biomass of the Black Sea anchovy aggregations was 309,000 tons, and the biomass of the Azov anchovy, 169,000 tons. Since 1988 the situation of these stocks have dramatically changed. A great decrease of the populations has occurred. That has in all evidence been caused by excessive captures of anchovy by the USSR and Turkey. An additional important negative factor were the intrusions of a jellyfish, *Mnemiopsis leidyi*. The biomass of the anchovy near the Georgian coast increased up to 165,000 tons after the *Mnemiopsis* outbreak had passed its peak in winter 1991-92.

Key words: Anchovy, Engraulis encrasicolus maeoticus, E. e. ponticus, populations, Black Sea, Azov Sea.

RESUMEN: LAS POBLACIONES DE ANCHOA DEL MAR NEGRO. – Se propone un método complejo de determinación de razas, basado en caracteres genéticos, parasitológicos y morfológicos para obtener un esquema fiable de la distribución y migraciones de las poblaciones de anchoa del mar de Azov y del mar Negro. La abundancia de anchoa se determinó a partir de datos de campañas efectuadas con redes de arrastre estándar y equipo hidroacústico. Los experimentos revelaron cambios considerables en el *pool* génico de la anchoa de Azov. La causa principal era el aumento de salinidad debido a la regulación del flujo fluvial. El exceso de capturas industriales coadyuvó también al proceso. Como resultado, aumentó la proporción de híbridos. Según los datos de las agregaciones de anchoa del mar Negro fue de 309.000 toneladas, y la de anchoa del mar de Azov, de 169.000 toneladas. Desde 1988, la situación de dichas poblaciones ha cambiado drásticamente. Ello ha sido debido, según todas las evidencias, a un exceso de capturas pri parte de la URSS y Turquía. Un factor adicional importante fueron las invasiones de una medusa, *Mnemiopsis leidyi*. La biomasa de anchoa cerca de las costas de Georgia aumentó hasta 165.000 toneladas después de que la explosión de *Mnemiopsis* hubiera pasado su máximo en invierno de 1991-92. (Traducido por los Editores).

Palabras clave: Anchoa, Engraulis encrasicolus maeoticus, E. e. ponticus, poblaciones, mar Negro, mar de Azov.

INTRODUCTION

The rational utilization of the main fishery resource of the Black Sea –the anchovy, whose total catch more than doubled in the last decade and reached the max.

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level of 606,000 tons (FAO data) in 1984– is of utmost importance for the industrial fishery in the region. However, increases in anchovy catches alternated with sharp slumps, initiating the research presented in this paper. The main aim of the research was to reveal the range of each anchovy stock and to assess its biomass seasonally. To do this we explored methods of identifying races of fish and measuring stocks quantitatively.

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REVIEW

All authors acknowledge the validity of two anchovy subspecies -the Azov Engraulis encrasicolus maeoticus Pusanov and the Black Sea Engraulis encrasicolus ponticus Alexandrov (Alexandrov, 1927; Mayorova, 1934, 1951; Pusanov, 1936). The Azov anchovy is spawning and foraging all over the Sea of Azov from May through August. In September-October, with the advance of autumn cold the Azov anchovy migrates through the Kerch Strait to the Black Sea. Most authors have considered the sector Novorossiisk-Sochy (the Caucasus) and the region adjacent to the southern coast of the Crimea Peninsula to be the main areas of winter distribution of the Azov anchovy. The wintering area of the Azov anchovy spreads to Sukhumi where its southern border is assumed to lie. The Azov anchovy spring migration back into the Kerch Strait begins in about mid-April and is over by the end of May (Popova, 1954; Kornilova, 1960).

The summer distribution area of the Black Sea anchovy covers practically the whole sea. Under the influence of temperature decline the anchovy initiates migration to the southern Black Sea. According to the scheme suggested by Pusanov (1936), the migration sphere usually takes place along the Romanian and Bulgarian coastlines, followed by the approach of the wintering schools to Turkish Anatolia and even Georgia. According to Danilevsky (1964), the anchovy migration takes place from the northwestern Black Sea to the Southern Crimea. Black Sea anchovy in the eastern Black Sea spend the winter near the Georgian coast and can also form schools in Turkish waters.

In a number of preceding papers (Danilevsky, 1960; Althukov, 1974; Marty, 1980) an overlapping of the ranges of Azov and Black Sea anchovy was noted. In winter individuals of both races are frequently fished in the same areas. Even in summer they can both be caught in the Sea of Azov, where juveniles of Black Sea anchovy enter to feed. They also co-inhabit less saline waters in the northwest Black Sea accessible to Azov anchovy. Thus, the issue of determining race for anchovy in different areas of the sea is considered to be rather urgent.

The investigation carried out by several authors revealed significant transgression and variability in the morphometric characteristics suggested by Alexandrov (1927) as satisfactory features for race determination. Meanwhile, the difference in body length was generally adopted as a key trait: the Azov anchovy has a slower growth rate than the Black Sea anchovy (Gubanov and Limansky, 1968; Shevchenko, 1980). A consistent difference between anchovy races is revealed by the form of the otolith (Skazkina, 1965). The Black Sea anchovy's index of otoliths (length/width ratio) is 2.15, and that of the Azov anchovy, 1.96.

Due to the presence of nematode larvae in the Black Sea food plankton, the degree of infestation of the body cavity of the Black Sea anchovy by this parasite *Contracaecum aduncum* is always higher. In order to use this feature as a stock trait, however, it is necessary to take into account an increase in degree of infestation with age, as well as the possibility of nematode accumulation during wintering in the Black Sea (Terekhov, 1971; Chashchin, 1981).

In 1963 Altukhov et al.(1969) showed individual variability of antigenic properties in anchovy erythrocytes while conducting hemagglutination experiments with normal pig and horse blood sera. The blood cells of A₁ group (positive) triggered reaction with both types of the sera, the blood of A₂ group (intermediate) reacted with the pig serum only, and the blood of A₀ group (negative) interacted with neither of the sera. Furthermore, Azov anchovy is characterized by the presence of all three blood groups, whereas Black Sea anchovy possessed two groups only: A₁ and A₂. The frequencies of occurrence of the blood groups were as follows: Black Sea anchovy: $A_1 - 96\%$, $A_2 - 4\%$; the Azov anchovy: $A_1 - 63\%$, $A_2 - 16\%$, $A_0 - 21\%$. In 1980-1982 Kalnin and Kalnina (1985) obtained data showing a valid difference between anchovy populations in terms of genetic frequencies of isocitratedehydrogenase and esterase by the method of electrophoresis in PAAG. Obviously it would be advisable to compare past methods designed for the identification of anchovy races to reveal possible changes in features and to apply those methods in complex to complex differentiation of stocks.

MATERIALS AND METHODS

Summer distribution of anchovies was determined during May-June surveys performed with a pelagic trawl. Information on anchovy fry was gathered in July-August by a special small-mesh trawl. In the winter, once the anchovy had accumulated in dense schools, their biomass was evaluated by hydroacoustic surveys. Echosounder calibrations were made in accordance with Artemov's (1982)

 TABLE 1. – Estimating of ratios between Azov and Black Sea anchovy populations assuming their mechanical mixing in the Sea of Azov in 1978-1981.

Estimation formula	Estimation by allele A ₁ frequency	Estimation by allele A ₂ frequency	Estimation by allele A ₀ frequency			
$\frac{C}{C+D} = \frac{Qn - Qd}{Qc - Qd}$	$\frac{0.660 - 0.450}{0.783 - 0.450} = 0.630$	$\frac{0.157 - 0.087}{0.217 - 0.450} = 0.583$	$\frac{0.450 - 0.183}{0.450} = 0.593$			

where Qn is the final frequency of gene in the mixed population Qc is gene frequency in Black Sea population

Qd is gene frequency in Azov population

C and D are corresponding shares in the mixed population.

method: measuring the reflectivity of a fish passing under an external antenna fastened to the upper plate of a pelagic trawl.

In the period 1977 through 1987, during trawling and acoustic surveys, anchovy samples were gathered, which were used for racial identification of fish. We tested 10,000 individuals. In order to obtain comparable results in the determination of populations features, we followed the methodology of previous researchers. A statistical analysis of a number of stock traits showed a negative correlation between frequency of the negative blood group and body length on the one hand and the otolith index and degree of infestation on the other. Therefore, we were able to identify a fish sample with regard to variability of all the traits.In addition, some samples were studied using electrophoresis in PAAG with labelled isoenzymes.

MODERN FEATURES OF ANCHOVY POPULATIONS

To obtain a reliable scheme of distribution and migration of each anchovy stock, it was of great importance to choose the most valid and distinctive features of fishes of the Azov and Black Sea populations. Repeated experiments with serological reactions carried out in the 1970s revealed considerable changes in the Azov anchovy's gene pool. The frequency of occurrence of A₀ group reduced to 4%, and that of A_2 to 8%. For a possible explaination to the differences between our data and previous data we checked the hypophesis of dominance of Black Sea anchovy in the Azov Sea, which could have migrated there at the time of sampling. Using the data obtained for the variables of the previously established formula for the determination of the proportions of each population in the mixed pool (Althukov, 1974) show that in the case of pure mechanical mingling of populations in the Azov Sea, the share of the Black Sea fish should exceed 50% (Table 1). In oder to check this assumption we performed analyses of the difference between anchovy populations in growth rate and nematode infestation. This revealed a considerable negative correlation (r = 0.60-0.88) between body length in



anchovy: a) Black Sea; b) Azov; c) mixed concentrations in winter fishing area. Designations: ■ invasion intensity, number of specimens; □ invasion extensity, %; Age groups: — 0+ and 1; ---- 1+ and 2; •••• 2+ and 3; xxx 3+ and 4

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the one-age groups and frequency of allele a (negative blood group). A less considerable but also reliable negative correlation (r = 0.40-0.70) was found between this allele frequency and the level of nematode infestation. In summer, as was noted previously, fishes in all samples from the Sea of Azov and the Kerch Strait were smaller and had fewer accumulated nematodes compared with anchovy spawned in the Black Sea (Figs. 1a, 1b). At the same time, winter samples from the zones of the usual mechanical mixing off the northern Caucasus were notable for intermediate values of both body length and nematode accumulation in one-age groups. Usually we observed here a double-peaked distribution (Fig. 1c), which was absent in the Sea of Azov samples.

For two great samples from the Sea of Azov we have taken into account the dose effect thus having determined the share of heterozygous specimens. At this we have established the reliable conformity of the experimentally determined frequencies of genotypes expected by Hardy-Weinberg equation, which indicated the balanced ratio of genotypes. Thus the hypothesis about the mechanical mixing of spawning populations in the Sea of Azov has been finally discarded.

The most probable explanation of our results can be given after analyzing the ecological situation in the marine environment. In the 1970s a significant increase in salinity occurred in the Sea of Azov as a result of a river in-flow regulation. Obviously, under these circumstances, the typically Azov form of the anchovy suffered definitive elimination. Evidently the sign by which elimination of specimens goes is the form of eggs determining their buoyancy. Typical specimens of Azov anchovy always differed from Black Sea fish by a more rounded form of eggs, which ceased to ensure the normal development of the organism after increase in water density. Excessive industrial exploitation in the wintering places in the Black Sea also promoted this process. As a result, the share of hybrids in the population increased. This conclusion as to the increase in hybridization is not in conflict with earlier data obtained by us on the statistically reliable reduction in differences between the two populations by otolith index from 0.19 to 0.064, as well as on the increased juvenile growth rates in the Sea of Azov. The hybrid origin of the contemporary anchovy was also pointed out in the results obtained by Kalnin and Kalnina (1984, 1985) while determining the frequencies of the isocitratdehydrogenase and esterase

alleles performed by the method of electrophoresis in PAAG. Nevertheless we found that differences in frequency of a recessive allele a determining the negative blood group, in body length, in a degree of infestation, and in otolith form, still exist between the anchovy populations. In our research we started using only new values of distinctive features of the anchovy populations.

DETERMINATION OF ANCHOVY STOCK RANGES

A series of new characteristics of migration and spatial distribution of anchovy stocks has been discovered. In the wintering approach of the anchovy to the southern Crimea it was impossible to find a considerable number of individuals of the Black Sea population in schools. From the growth rate, degree of infestation, frequencies of the blood groups, as well as the occurrence of the alleles of isocitratdehydrogenase and esterase, the anchovy shoals near the Crimea may be referred to the Azov race. Thus, the previous information on presumed wintering and fishing of Black Sea anchovy in this region was the result of an erroneous identification of the races.

Azov anchovy migration southwards to Poti-Batumi being most evident in 1976-77, 1979, and 1983-85 proved to be rather usual. The fish of the Azov population which mingled with the Black Sea anchovy most frequently reached Batumi region, but in certain years a general shift of the fish southwards along the coastline down to Turkish waters was registered, whereas the Azov anchovy schools approached the Turkey-Georgia border. This was followed by an amplification of the Azov anchovy's spring migration to the northwest Black Sea, trailing behind fish of the Black Sea stock. The most extended migrations of Azov anchovy along the Caucasus coast and their coming to the north-western part of the Black Sea were related to the formation of very abundant yearling classes of the stock in 1979, 1981, and 1985. The presence of Azov anchovy on the Black Sea spawning grounds reached its maximum level in summer 1987, when it contributed up to two thirds of all the biomass of the spawning stock in the western half of the sea.

In October, the greater part of the Black Sea anchovy stock shifted to the southern coast of the basin. Meanwhile, Azov anchovy, appeared in trawl catches over a large area of the north-eastern Black Sea. As the process of an autumnal water cooling in

the Sea of Azov is more intensive the anchovy of the Azov race school much earlier then the Black Sea anchovy. Usually, the Azov anchovy's dense accumulations, coming through the Kerch Strait, can be observed near the northern Caucasus and the Crimea at the beginning of November. On reaching the warmer Black Sea, a part of the Azov anchovy, mostly juveniles, disperses and continues foraging while moving southwards to the wintering grounds. As was evident from population and genetic analysis, these fish approach the Georgian coast and are frequently fished by ring nets, being confused with Black Sea anchovy. It is significant that the first anchovy accumulations of the Black Sea population itself were never registered in the south-eastern part of the sea before mid-November and they usually appeared there only at the end of November or the first week of December.

Observations on the formation of Black Sea anchovy wintering schools demonstrated that numerous but small schools always moved from the west or south-west to the Georgian coast. The first approach of Black Sea anchovy to the shores of Georgia and the formation of dense schools invariably took place in the Poti-Batumi region. Such a direction of migration of Black Sea anchovy leads us to suppose that fish from the highly productive western part of the sea, where the majority of the stock occurs in summer, are shoaling near Georgia in winter. The coastal zone of Turkey, from the Georgian border to Sinop, is also the main region of the anchovy's distribution in winter (Johannesson and Losse, 1973). Only once during winter was a small school of anchovy observed near Bulgaria. The reasons for Black Sea anchovy's migration from the western and southern parts of the sea to the coasts of eastern Anatolia and Georgia is more obvious if one takes into consideration that this region is protected by the Main Caucasus Ridge from northern winds, and not influenced by the cold currents predominant in the western Black Sea. The general scheme of the anchovy's migrations in the Azov-Black Sea area based on our and the confirmed literature data is shown in Fig. 2. The migration routes in Turkish waters remain to be clarified by the surveys in that region.

Following a long and snowy winter off Georgia, the spring migration in the direction of Sinop, and then to the northwest sea, is predominant. Under less extreme winter conditions, fish also move along the Caucasus coast reaching the spawning grounds in the eastern part of the sea and remaining there for the summer.

STOCK ESTIMATIONS

Important results pertaining to anchovy resources were obtained in the course of the hydroacoustic surveys carried out regularly in the winters 1980 through 1992. Hydroacoustic surveys were conducted in both the Crimean and northern Caucasus regions, where



FIG. 2. – The general scheme of anchovy migration. (A) Azov anchovy: 1 – spawning and foraging region; 2 – wintering region; 3 – spring migration; 4 – autumnal migrations; 5 – periodic migrations of a mingled population. (B) Black Sea anchovy: 6 – spawning and foraging region; 8 – spring migration; 9 – autumnal migrations.

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Population	1980	1981			1982			1983					
	XII	Ι	II	III	XII	Ι	II	III	XII	Ι	II	III	XII
Black Sea anchovy Azov anchovy	110 218	261 211	140 180	40	40 175	320 150	220	190	70 200	550 180	100 140	70 120	270 160
		1984			1985			1986					
Black Sea anchovy Azov anchovy		160 140	150 120	100	135 80	45 70	20 70	10 60	235 260	90 260	40 240	10 200	350 150
		1987			1988								
Black Sea anchovy Azov anchovy		40 190	0 120	0	250 110	350 100	100 70	150					

TABLE 2. – Biomass of anchovy accumulations in the winter 1980-1988 according to hydroacoustic data (t x 10^3)

accumulations of Azov anchovy occurred, and off Georgia, where Black Sea were found. As the ranges of the two anchovy stocks varied from year to year, and the formation of mingled populations was rather frequent, all biomass evaluations were differentiated according to the data of race determination of the fish in catch samples (Table 2).

The fact that fish of the Azov stock are distinguished by good body condition in the autumn, when their fat content reaches 20-25%, makes it expedient to permit an intensive fishery until January 1. A rapid decline in the amount of the Azov anchovy requires, however, the prohibition of fishing in the winter in the areas where the proportion of Azov anchovy in mingled schools exceeds 25%. Reliable estimations of stock biomass and the determination of the race of the fish allowed us to install reasonable limits to the fishing areas. Fishing vessels were prohibited to operate beyond such limits. Thus, the reduction in Azov anchovy stock observed annually in the winter may be chiefly referred to natural mortality.

According to the data of acoustic surveys in the waters of the USSR from 1980 through 1988, the average biomass of the Black Sea anchovy accumulations (in the early winter) was 309,000 tons, and that of the Azov anchovy 169,000 tons.

However, in 1989-1991 the measures for protection of Azov anchovy failed because of the invasion of an exotic species of jellyfish, *Mnemiopsis leidyi* Agassiz, from the Atlantic Ocean into the Sea of Azov. This species depletes the food supply in the shallow sea and consumes fish eggs. In consequence the Azov anchovy population dropped sharply, and its autumn fishery in the Kerch Strait was suspended in 1989-1991.

Since 1988 the relatively satisfactory situation with respect to the Black Sea anchovy stock has drastically changed with a major decline of the population. The USSR catch in 1989 and 1990 reached only 64,000 and 29,000 tons respectively, and the size of the spawning stock slumped to 30,000-50,000 tons. It is significant that a Black Sea anchovy stock decline occurred twice within the last several years (Table 2). This was evidently caused by an excessive harvest of anchovy near the former USSR and Turkey in 1984 and 1988, when the total annual catch exceeded 500,000 tons. The last decline in the Black Sea anchovy population was due largely to the Mnemiopsis leidyi intrusion. The winter of 1990/1991 was the worst period for Black Sea anchovy: emaciated fish did not form dense schools and stayed dispersed in the water column so that the industrial fishery on anchovy was impossible.

In the summer of 1991 near the Georgian coast conditions for nourishment and fattening of anchovy were much better. Hence, in the winter, the total biomass of anchovy schools in this region increased to 165,000 tons. In 1992-1994 the level of biomass of the Azov anchovy population also rose to about 100,000-150,000 tons. New data lead us to think that the *Mnemiopsis* outbreak in the Azov and Black Sea areas has now passed its peak, and that its negative impact on the anchovy populations is diminished.

CONCLUSION

Two geographical populations of anchovy (the Azov and Black Sea anchovies) adapted to reproduction in water with different salinity are still found in the Azov-Black Sea area. However, since the begin-

ning of the seventies an impact of the anthropogenic factors and excessive fishing activity have induced hybridization of the two subspecies of the anchovy which has more acutely interfered with the Azov anchovy's gene pool. It has almost effaced some differences, serving as traits, between the races.

The new data on anchovy migrations presented here reveal that frequent and tangible overlapping of the two subspecies' areas has occurred. The divergence of the subspecies is supported by natural selection. The main factor of selection is the water salinity. Because the intraspecific structure of the anchovy has an adaptational importance and needs protection, it would be expedient to regulate the industrial fishery harvest in relation to the size of stock for each race. Fishery regulations may be based upon the data of hydroacoustic surveys of the anchovy stock. The surveys should be conducted both in the Georgian and Turkish zones to permit evaluations of the stock throughout the Black Sea.

The acoustic and trawling surveys performed in the Black Sea have shown a considerable reduction in fishable and spawning stocks over the last years. The anchovy catch has also decreased. Introduction of catch quotas and measures aimed at avoiding further degradation of the ecosystem (pollution, curtailment of fresh water inflow) is quite evidently necessary. This calls for urgent unified action by all States in the area.

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