BIOLOGICAL MONITORING OF LANDINGS OF COMMERCIALLY IMPORTANT SPECIES

Scientific report under contract N157/13/03/2023 covering the results for I-VI 2024

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MINISTRY OF AGRICULTURE AND FOOD

MARITIME, FISHERIES AND
AQUACULTURE PROGRAMME

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BULGARIAN ACADEMY OF SCIENCES
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In connection with the implementation of Contract No. 157 of 13.03.2023, sampling was done for inclusion in the biological monitoring from the northern and southern coasts. The data of this analysis are collected from the landings in the ports of the Bulgarian Black Sea coast of the Black Sea.

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I. Biological monitoring of sprat (*Sprattus sprattus*) landings

I.1 Objectives

The purpose of biological monitoring is to collect data that will be used to analyze sprat catches, as well as to form a database. The collection of biological samples of sprat catches in I-VI 2024 includes the following tasks:

1. *To collect and analyze the dynamics of length, weight and age distribution.*
2. *To determine the state of the sprat using the so-called state factor (Ricker, 1975).*
3. *Characteristics of the reproductive biology of sprat.*
4. *Collection of data on ports of landing, sampling vessels, number of samples collected, number of specimens tested, geographical catch data.*

I.2 Sampling

The sampling was carried out by the actively operating fishing fleet in Bulgaria.

I.2.1 Geographic area coverage

The data from the current analysis is collected directly from the landings in the ports of the Bulgarian Black Sea coast. During the **period I-VI, 2024** were collected **5 samples with 592 specimens.** Information on the size of the catches was also collected.

I.2.2 Sampling period

The biological data on sprat were collected from a total of **5 landings at the ports of Sozopol and Nessebar**. Information on the size of the catches was also collected. Ports and ships from which monitoring was carried out to collect biological data from landings are presented in Table I.2.2.1.

Table I.2.2.1 Ports and ships from which monitoring was carried out to collect biological data from sprat discharges.

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№	Date	Harbour	Species code SPR	Fishing vessel	External marking	Fishing gear	Catch	Coordinates
1	**17.1.2024**	Nessebar	**SPR**	Р/К 40	BS288	OTM	1100	42.6604614258 27.7332077026
2	**9.2.2024**	Sozopol	**SPR**	РК 27	BS 290	OTM	960	42.3398 27.9051
3	**11.4.2024**	Nessebar	**SPR**	СВЕТИ НИКОЛА I	BS 175	OTM	660	42.6604309082 27.7332210541
4	**29.5.2024**	Nessebar	**SPR**	ИЩАР	NS 1182	OTM	120	42.4930000305 27.9638881683
5	**25.6.2024**	Nessebar	**SPR**	НИКО	BS152	OTM	1000	42.6702690125, 27.8198375702

I.2.3 Statistical analysis of data

All samples were collected according to variation statistics from significant catches where possible. Samples were collected randomly. Samples are processed in laboratory conditions. Length is measured to the nearest 0.5 cm, and only the total length is taken into account. Weight is measured to the nearest gram (0.1 gram). Age determination was performed under an Olympus CX 31RTSF-6 microscope. Thus, the annual rings stand out as transparent zones, followed by darker zones (opaque) - zones of stagnation (stagnation) in growth. The Fulton index is estimated according to Ricker equation, (1975):

$$K = \frac{W}{L^3} * 100$$

"Length-age keys" are created for all samples. In this way, the average values of length, weight and condition factor are determined. The share (in %) of individuals from the respective age groups is determined.

Fecundity: All fish were measured to the nearest 1 mm in total length (TL) and weighed to the nearest 1 gram. The gonads of the fish are examined under a microscope for external characteristics such as hardness and color to determine the stage of maturity.

The sex ratio was also calculated in this study (i.e, number of males ♂/ number of females♀ (Simon *et al*., 2012). Females were determined by macroscopic observation of a mature ovary (Laevastu, 1965a).

Batch fecundity rates can vary greatly during the short spawning season, being low at the beginning, peaking during the heaviest spawning and declining again towards the end.
The fecundity rate of sprat was determined by the "hydrated oocyte method" (Hunter *et al* 1985). Oilly hydrated females were used. After sampling their body cavity was opened and they ovary were 'preserved in a buffered formalin solution (Hunter *et al* 1985). The ovary free female

weight and the ovary weight were determined. Three tissue samples of -50 mg were removed from different parts of the ovary and their exact weights were determined. Under a binocular microscope, the number of hydrated oocytes in each of the three subsamples was determined. Hydrated oocytes can be easily distinguished from all other types of oocytes due to their large size and translucent appearance and their wrinkled surface due to formalin preservation. Batch fecundity was estimated based on the average number of hydrated oocytes per unit weight of the three samples. The gonadosomatic index (GSI) was calculated as:

$$GSI = \frac{GW}{SW} X\ 100$$

where, GW is gonad weight and SW is somatic weight (represents BW without GW).

The length – weight relationship is obtained by the following equation: $W_t = qL_t^n$
where: q – parameter, n – parameter of the relationship between length and weight.

I.3 Results

I.3.1 Landings statistics

In March and June 2024, the discharges from the sprat were the highest, while the decrease was recorded in January (Fig.I.3.1.1).

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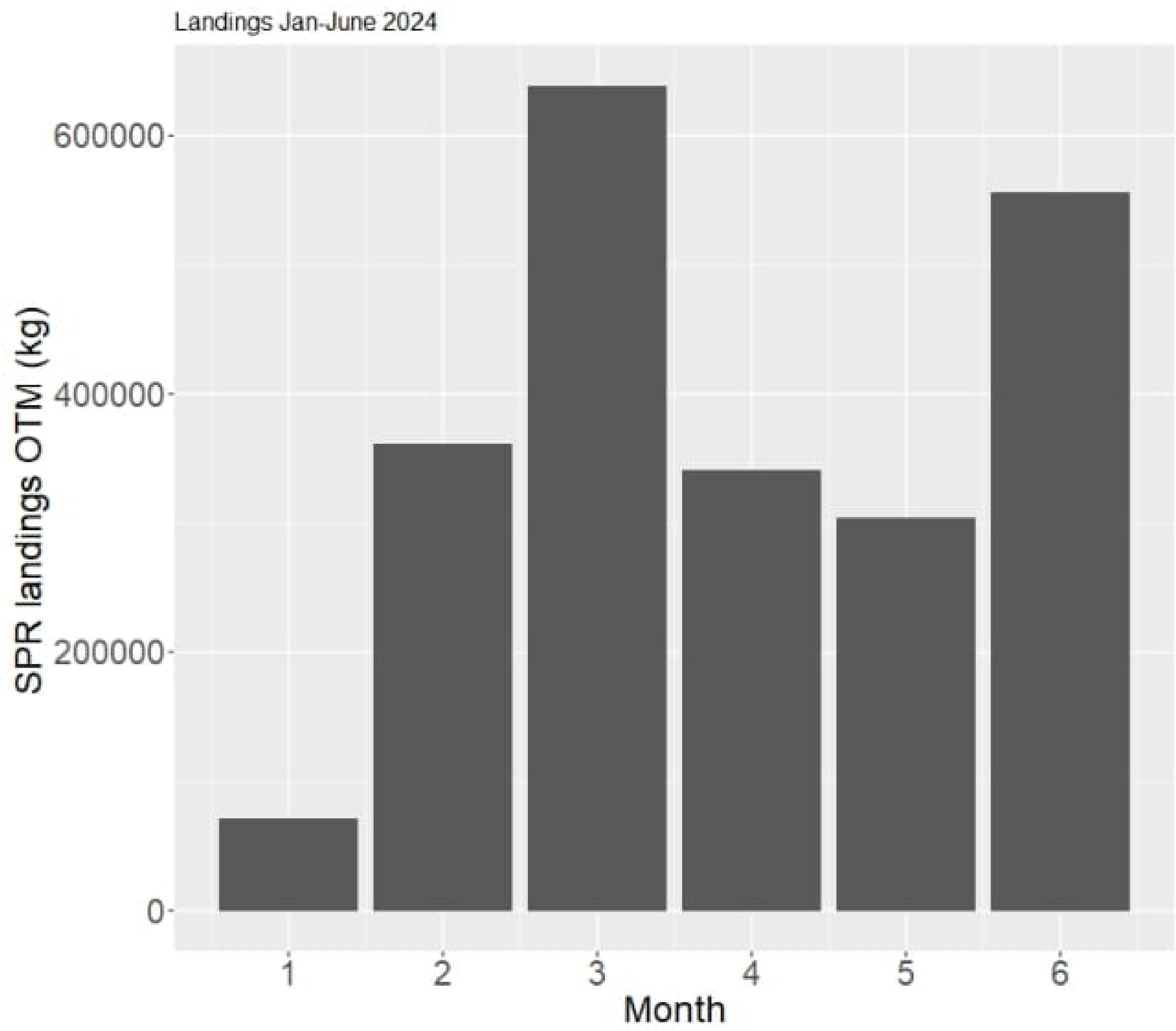

Fig I.3.1.1 Official statistics records for sprat landings by month in the first half of 2024.

I.3.2 Length structure of landings

The size structure of the sprat during the period I-III of 2024, shows a bi modal distribution with a peak of size group 8.0 and 8.5 cm participating with one share - 21.36% and prevailing significantly above the others. An increase in the percentage (20%) of individuals from the 9.00 cm size group is also noted. In the first quarter, equal participation of size groups 7.5cm, 9.5cm and 10.0 cm was established in the catches, which participated with 10.45% and 10%, respectively. A sharp drop in attendance marks the 10.5 cm and 11 cm groups with 3.64% and 2.73%, respectively. In the second quarter, the size groups 7.5 and 8 cm participated with the highest percentage with 13.44% and 12.90% respectively. The lowest prevalence in the the second quarter is observed in size groups 11 - 11.5cm, which are represented by 4.3% and 3.23% in the catch (Fig.I.3.2.1).

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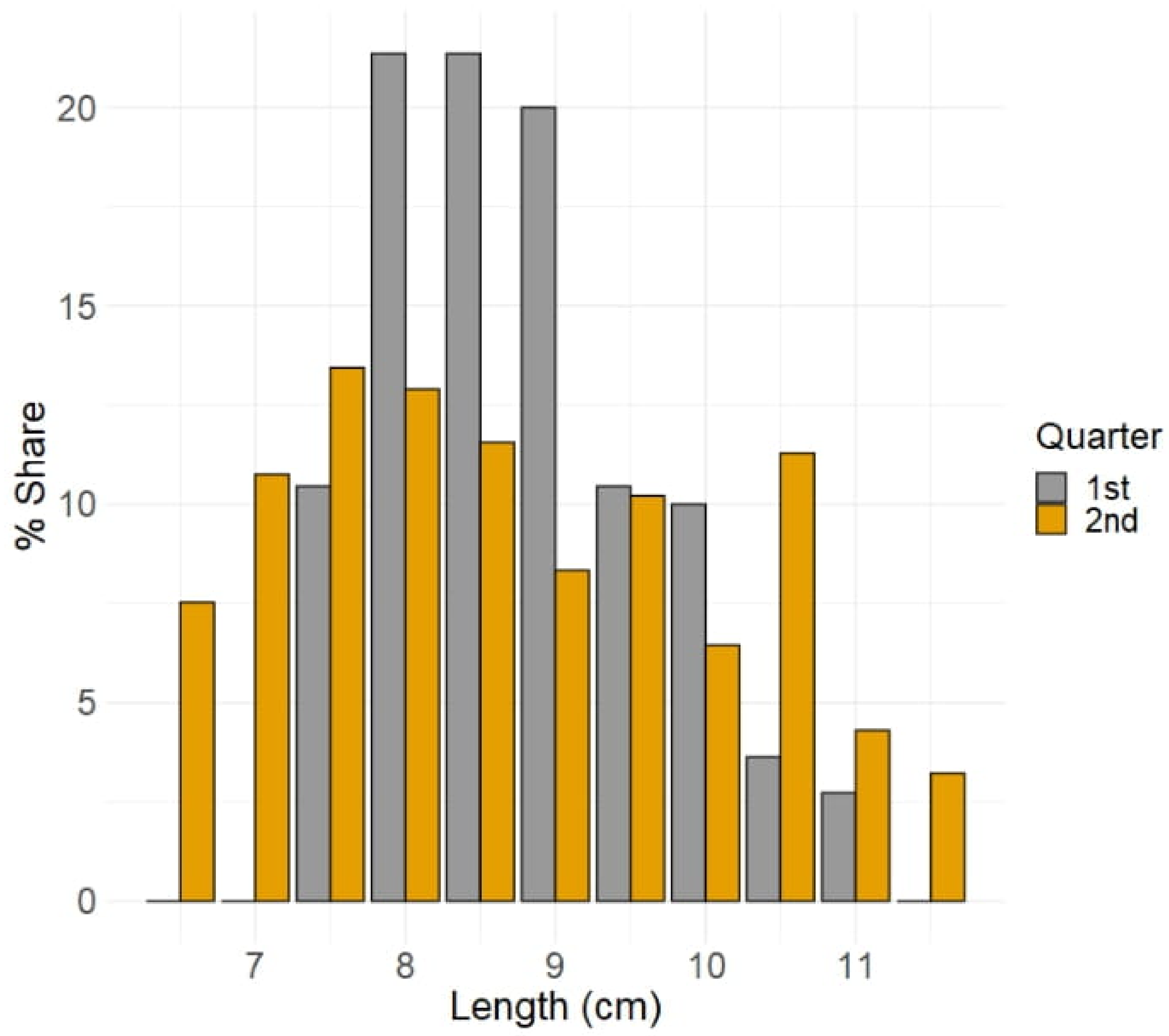

Fig I.3.2.1 Size structure and percentage share of length classes in the catch composition.

I.3.3 Age structure of landings

The three experts determined the age of the otoliths, and assessor 1 looked at all otoliths twice **(n = 592)**. In the first and second quarters, the highest percentage share is occupied by individuals in the age group 2-2+, followed by 3-3+. In the first quarter of 2024, in terms of the age of the sprat, the age group 2-2 + reached 51.82 %. The age group of 1-1+ and 3-3+ year olds are represented by 19.09% and 27.72%, respectively. The oldest age groups 4-4+ are represented by 1.36%. In the second quarter, the participation of 2-2 + age groups reached 36.83%, followed by 3-3 + with 29.57%. The presence of 0 +, 4-4+ and 5-5 + age groups is small with 7.53%, 2.69% and 3.23% respectively.

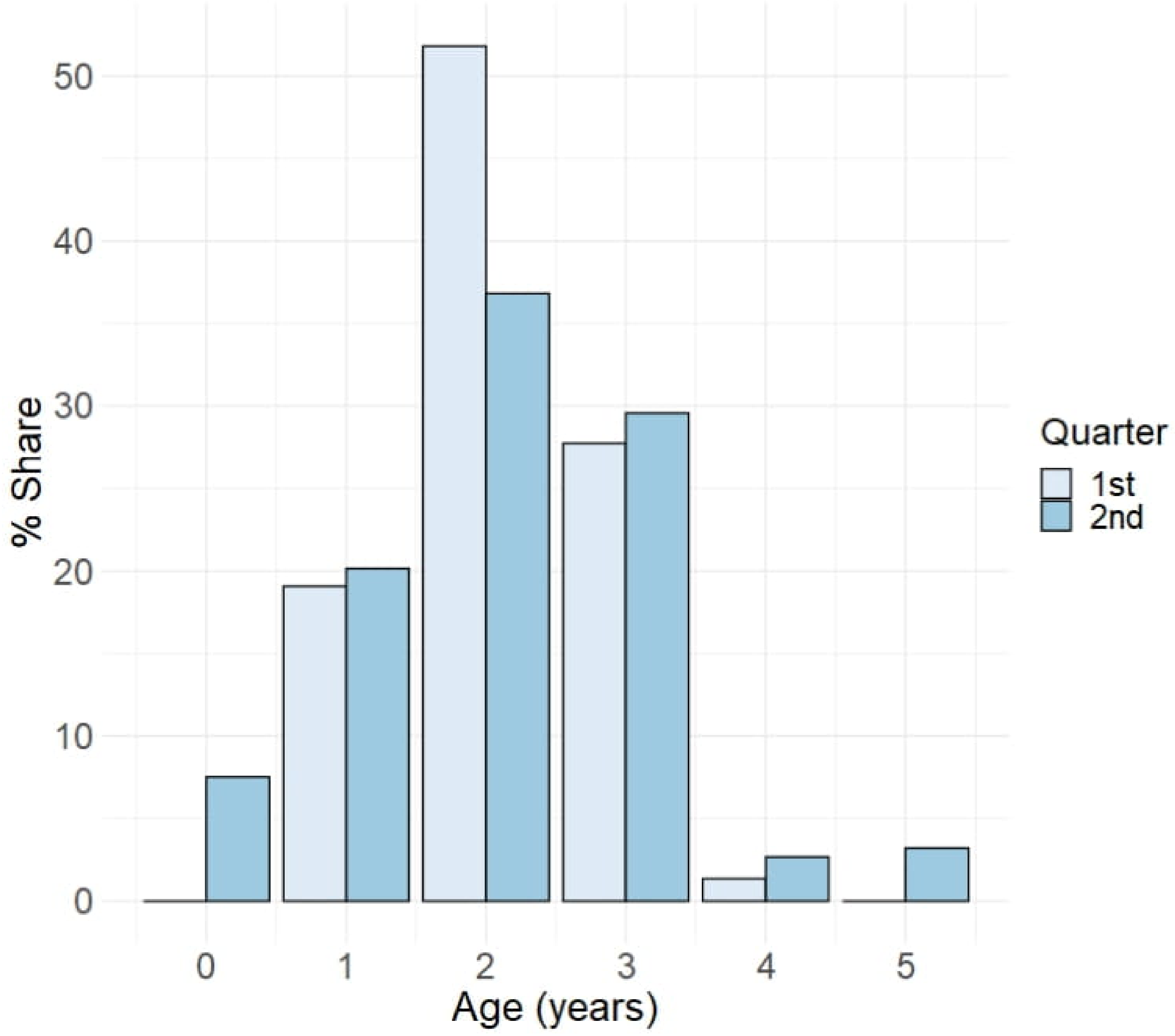

Fig. I.3.3.1 Age structure of the sprat and % presentation of the size classes in the catches.

Results of age slicing methods – analyzes were performed in the R programming environment (Program Language for Statistical Modeling and Analysis) using ELEFAN functions (ELectronic LEngth Frequency Analysis).
ELEFAN is a size-frequency sampling analysis method that is used in fish stock research to estimate growth and other biological parameters of research species based on measured lengths of individuals of different age groups.
The samples are restructured for analysis purposes to follow the evolution of the cohorts over time (Fig. I.3.3.2 - the first frequency diagram shows the size structure of the collected specimens in the respective months, and the second - the restructured data with a moving average MA = 7).

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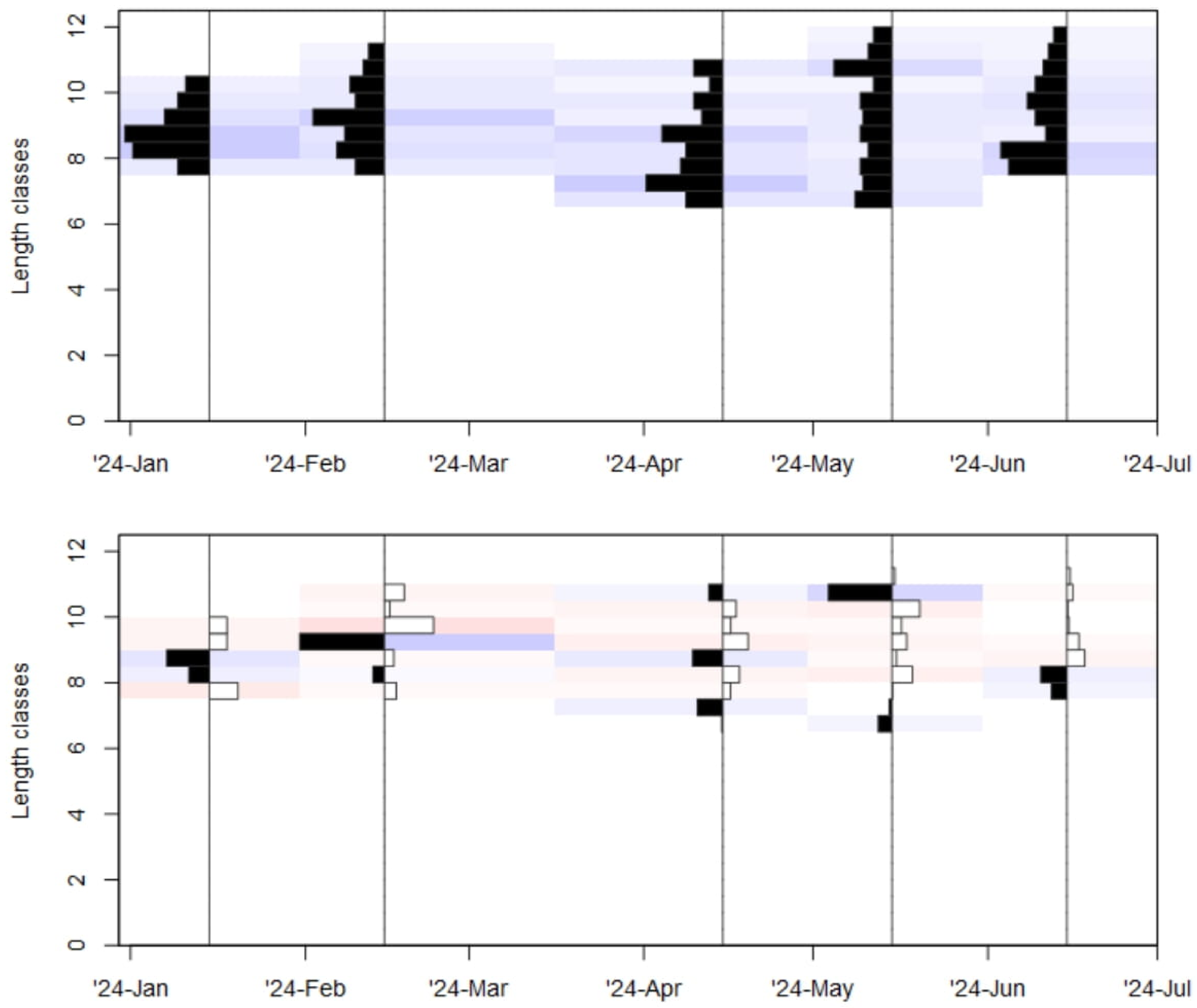

Fig.I.3.3.2 Size-frequency samples visualized as (diagram above) number of individuals in a size class and (diagram below) the restructured data with moving average MA = 7 for the purposes of frequency analysis and determination of growth parameters.

I.1.3.3.1 Frequency analysis RSA – response surface analysis

Growth parameters determined by RSA with initial conditions: conditional interval of for the asymptotic length L_∞ in the sample [11;13.5cm] and the parameter that determines the rate of growth to L_∞ - $K = \exp\ (seq(from\ log(0.1), to\ \log(2))$ at an upper limit for L_∞ = 13.5 cm
The parameters in the von Bertalanffy model are calculated as follows:

L_∞=12.35 cm; K = 0.5 and t_0=-0.53. According to the latter, the ages with their corresponding lengths were calculated (Table I.1.3.3.3.1). A disadvantage of the method is the difficult optimization of L_∞ and K and the need for a priori expertise to define the interval for the expected asymptotic length, which is expected to be overcome by applying genetic algorithms for age slicing.

I.1.3.3.2 Frequency analysis ELEFAN with simulated annealing (ELEFAN SA)

The simulated annealing method in the context of ELEFAN (Electronic Length Frequency Analysis for estimating fish stock parameters) is a technique used for optimization. The advantages over RSA are: avoidance of local optima: the algorithm explores a wider range of possible solutions and thus avoids local minima that may not be optimal. Accordingly, this approach increases the chances of finding a globally optimal solution, which is especially important when analyzing complex ecological data such as those on fish stocks.

The growth parameters determined under initial conditions: a conditional interval of for the asymptotic length 〚L_∞〛 $_{guess}$=12.25 cm and a search for the value of L_∞ in the interval [$L_{(\infty guess}$)*0.8 ÷$L_{(\infty guess}$)*1.2], and for K in the interval [0÷1].

The parameters in the von Bertalanffy model were calculated as follows: L_∞=12.73 cm; K = 0.48 and t_0=-0.51. The ages with their corresponding lengths were calculated using the latter (Table I.1.3.3.3.1).

I.1.3.3.3 Frequency analysis ELEFAN – Genetic algorithm (ELEFAN GA)

Growth parameters determined under initial conditions: conditional interval of for the asymptotic length 〚L_∞〛 guess=12.25 cm and search for value L_∞ in the interval [$L(_{\infty guess}$)*0.8 ÷$L_{(\infty guess}$)*1.2], and for K in the interval [0÷1]. The parameters in the von Bertalanffy model were calculated as follows: L_∞=12.68 cm; K = 0.49 and t_0=-0.44. The latter were used to calculate the ages with their corresponding lengths (Table I.1.3.3.3.1).

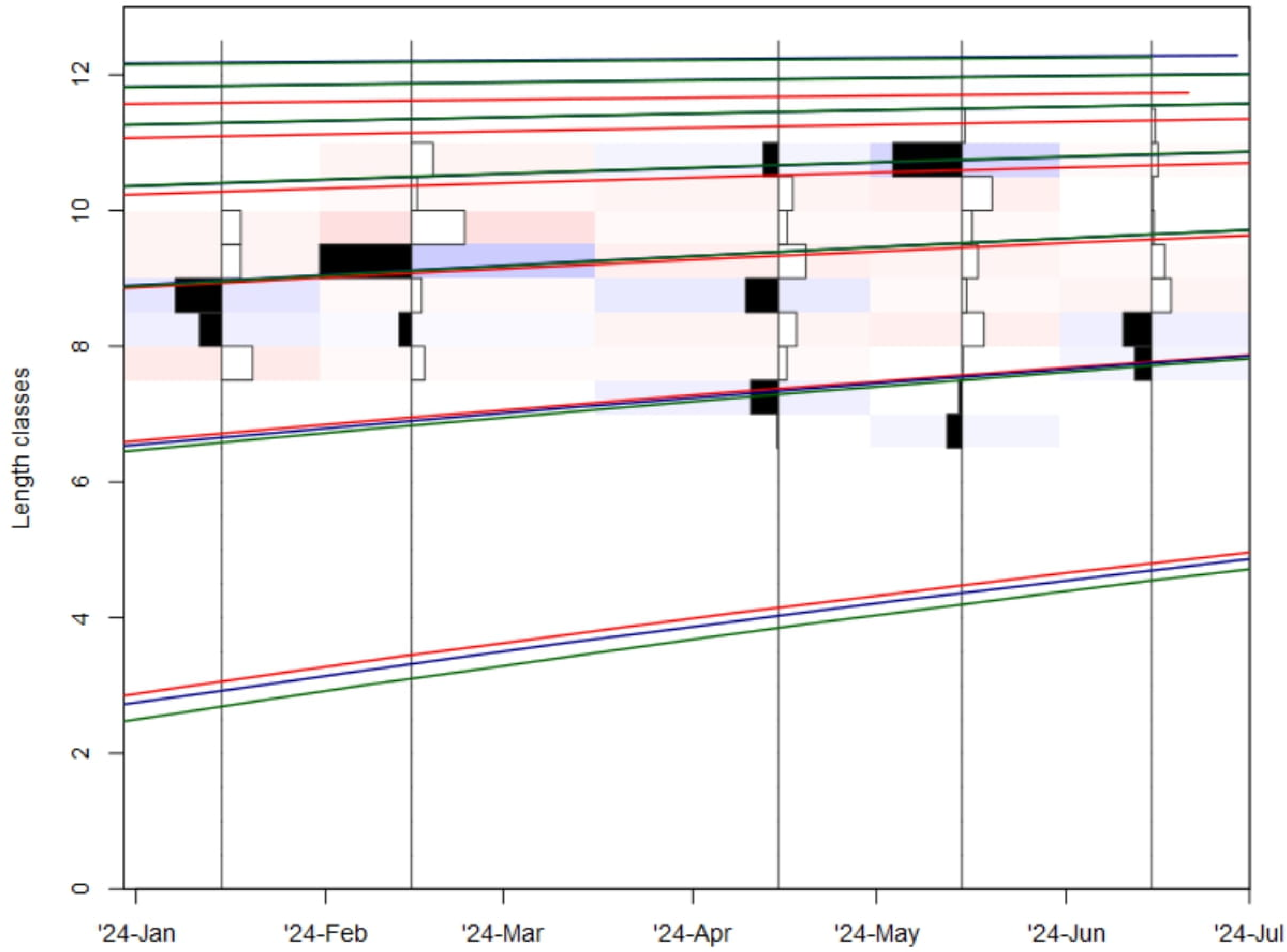

Fig. I.1.3.3.3.1 Growth curves (in red for growth parameters determined with ELEFAN RSA, in blue for ELEFAN SA, and in green for ELEFAN GA), visualized on the restructured data for the purpose of visualizing the tracking of cohorts over time.

Table I.1.3.3.3.1 Ages with their corresponding lengths for the studied species calculated using von Bertalanffy parameters, obtained with RSA, ELEFAN with simulated annealing, ELEFAN with genetic algorithm and compared with experimentally determined ages from otoliths.

	ELEFAN RSA	ELEFAN SA	ELEFAN GA	Experimentally determined
age	La	La	La	Lmean
0	2.9	2.7	2.9	n/a
0.5	5	4.9	4.9	6.5

1	6.6	6.5	6.5	7.5
1.5	7.9	7.9	7.8	
2	8.9	8.9	8.8	8.25
2.5	9.6	9.7	9.7	
3	10.2	10.4	10.3	10
3.5	10.7	10.9	10.9	
4	11.1	11.3	11.3	11
4.5	11.3	11.6	11.7	
5	11.6	11.8	12	11.5

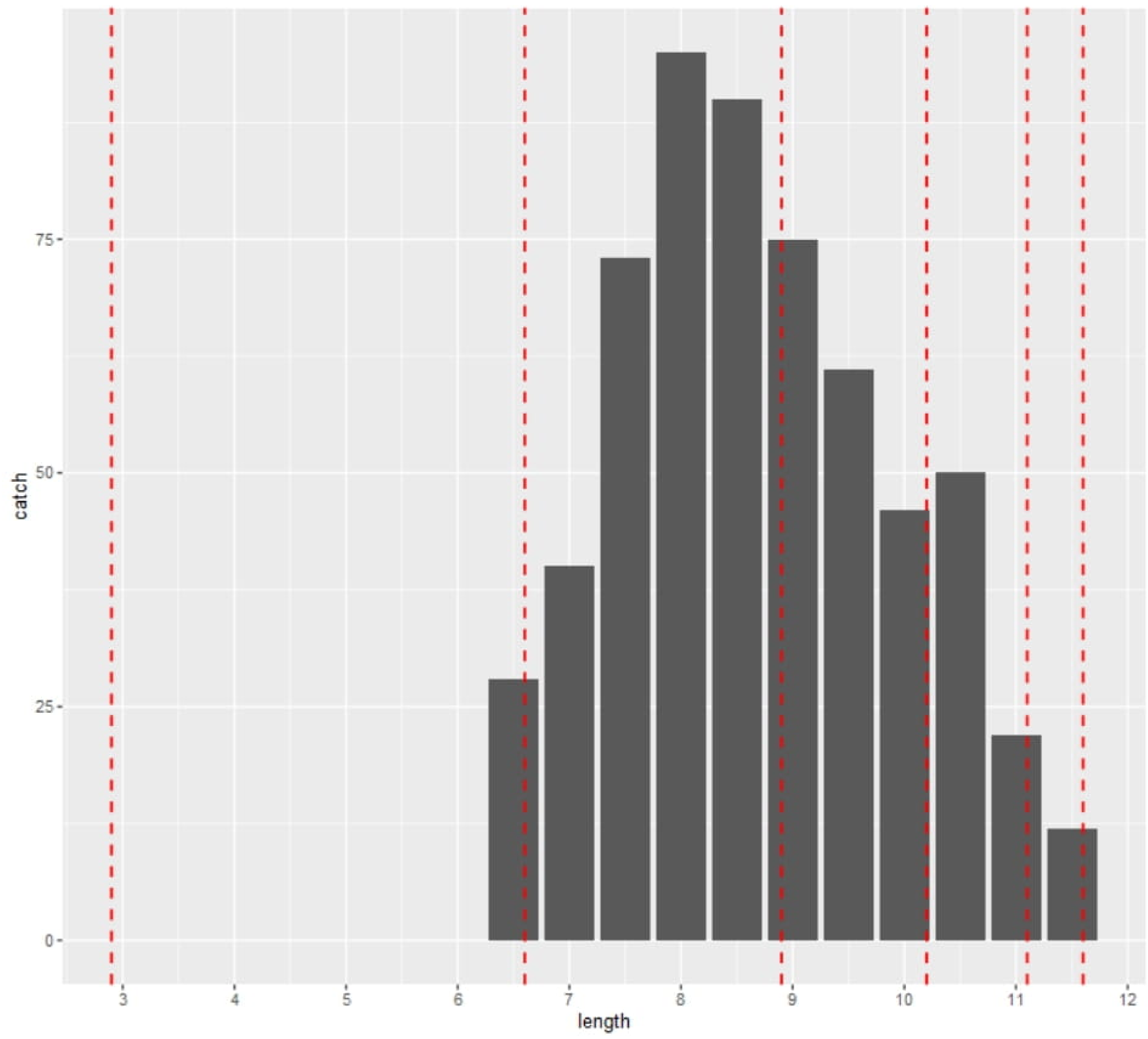

Figure I.1.3.3.3.2 The ages represented with dashed lines, calculated based on the growth parameters determined by ELEFAN RSA, are overlaid on the cumulative sample of the composition of commercial catches of sprat, aiming to visualize the range of size classes represented in a specific age group (0-5 years) for the first half of 2024.

The results from ELEFAN with the three applied methods are consistent and applicable also to the experimentally determined ages with small discrepancies for the age group 1-1+, the probable reason for this being limiting factors such as the sample size and the consideration of growth parameters for an inflow of less than a year. Another serious problem is the selectivity, which leads to an incomplete representation of the size composition of the stock in the samples, which in turn affects the calculation of biological potentials.

Based on the analysis conducted, ELEFAN RSA gives results closest to those experimentally determined during the aging of the individuals in the samples and, accordingly, the growth parameters of the S. sprattus stock for the first half of 2024 can be determined as follows: L_∞=12.35 cm; K = 0.5 and t_0=-0.53.

I.3.4 Condition factor

The graph (Fig.I.3.4.1) shows that the size groups are in good condition, which indicates food supply and good physiological condition.

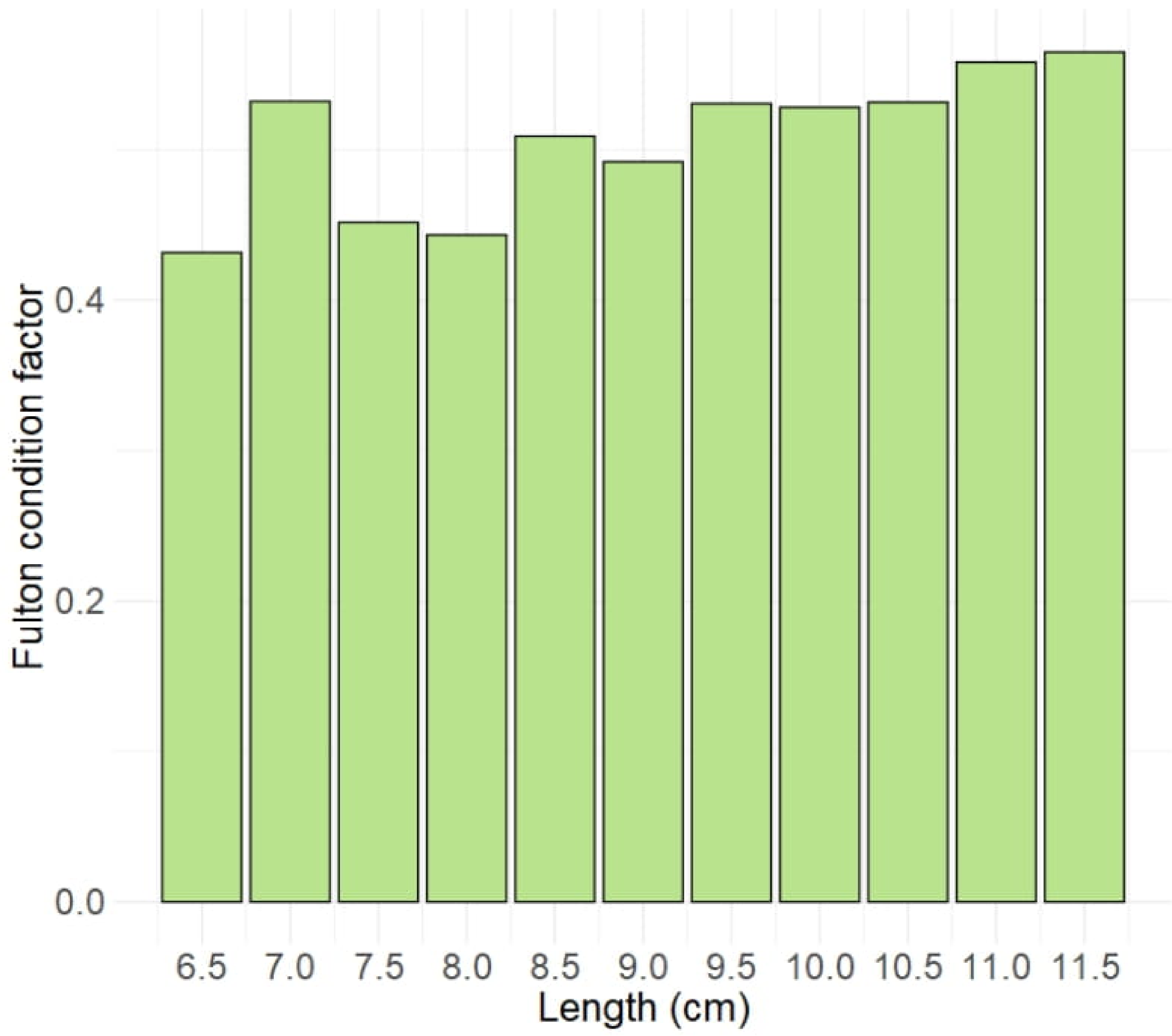

Fig. I.3.4.1 Fulton condition coefficient values of sprat by length classes.

The condition of the sprat by age shows a predominance of 4-4+ and 5-5+ year olds, and the condition varies in different age groups.

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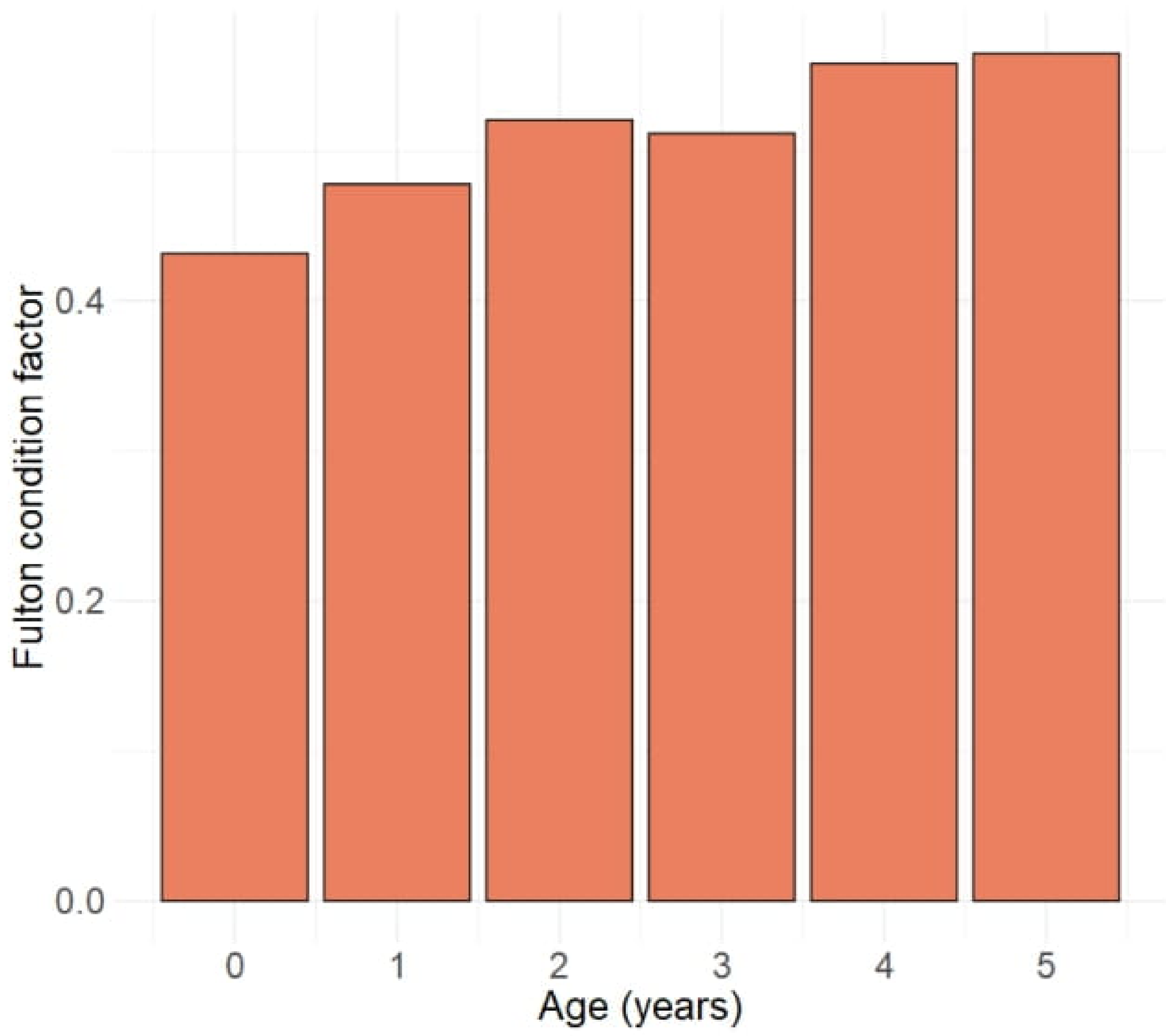

Fig. I.3.4.2 Fulton condition coefficient values of sprat by age groups.

I.3.5 Weight structure of sprat

The weight of **592** specimens was measured. For age group 0-0+, the lowest average weight is observed, and for group 5-5+, the highest average weight is observed (Fig. I. 3.5.1). The graph shows the distribution of the average weight of the sprat by age groups for two quarters. A gradual increase in the average weight is observed in relation to the age groups for both quarters. For the age group 0-0+ the lowest average weight is -1.18g (28 number), and for the group 5-5+ the highest average weight is observed -8.59g (12 number). For the other age groups the weights are as follows: 1-1+ -2.02g (110 number); 2-2+ -2.92g (250 number); 3-3+ -5.12g (177 number); 4-4+ -7.43g (15 number).

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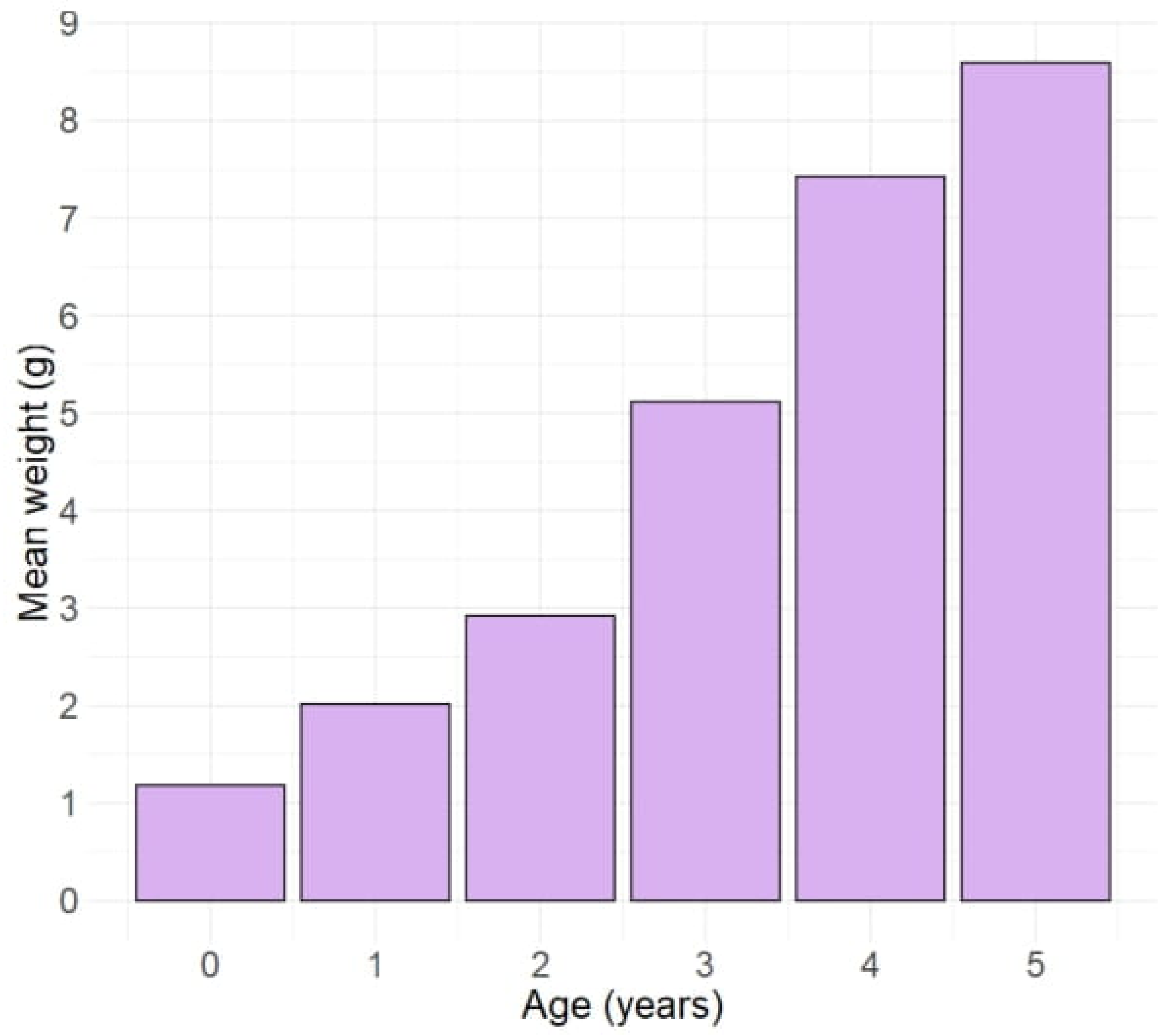

Fig.I.3.5.1 Average weights by age groups.

The most widely distributed size groups 8-8.5cm represent 16.05% and 15.20% and for them average weights of 2.26g and 3.12g were measured.

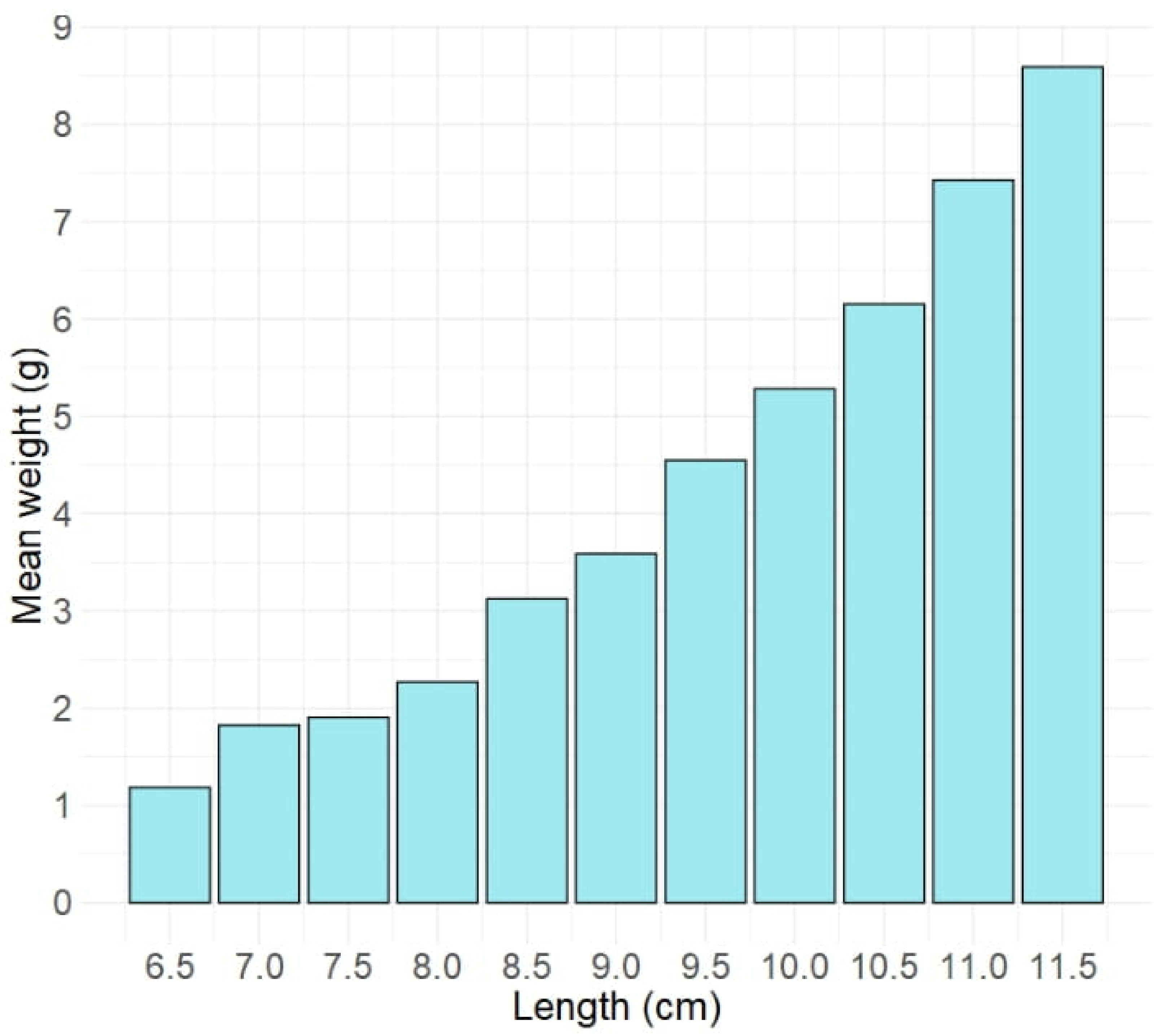

Fig. I. 3.5.2 Distribution of the average weight of the sprat by size groups for the first half of 2024.

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I.3.6 Size structure of sprat by age group

The length of **592 specimens** was measured.

Table I.3.6.1 Size structure of the sprat by age groups.

Lmean/cm	Age
6,50	0-0+
7,55	1-1+
8,37	2-2+
9,87	3-3+
11,00	4-4+
13,75	5-5+

I.3.7 Length- weight relationship

To calculate the length-weight relationship, a linear model was used on the logarithmically transformed values for the average weights and lengths for the first half of 2024. The modeling results are presented in Table I.3.7.1.

Table I.3.7.1 Results from modeling the length-weight relationship of sprat.

```
Call:
lm(formula = logW ~ logL, data = weight_l)

Residuals:
     Min        1Q    Median       3Q       Max
-0.090598 -0.034778 -0.000891  0.020566  0.143258

Coefficients:
            Estimate Std. Error t value      Pr(>|t|)
(Intercept)  -6.1253     0.2297  -26.66 0.000000000711 ***
logL          3.3833     0.1050   32.23 0.000000000131 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.06252 on 9 degrees of freedom
Multiple R-squared:  0.9914,    Adjusted R-squared:  0.9905
F-statistic:  1039 on 1 and 9 DF,  p-value: 0.0000000001309
```


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The resulting model is statistically significant, the value of the scaling coefficient a in the length-weight relationship model is: $\boldsymbol{W_i = aL_i^b}$ **e:** $\boldsymbol{a = 0.0022}$, and the allometry coefficient b=3.38, which indicates positive / allometric growth of the species in the first half of the year, or the increase in weight is proportional to or greater than the increase in length.

I.3.8 Sex ratio

The gender ratio was determined **for 250 individuals**. Females♀ predominate over males♂.

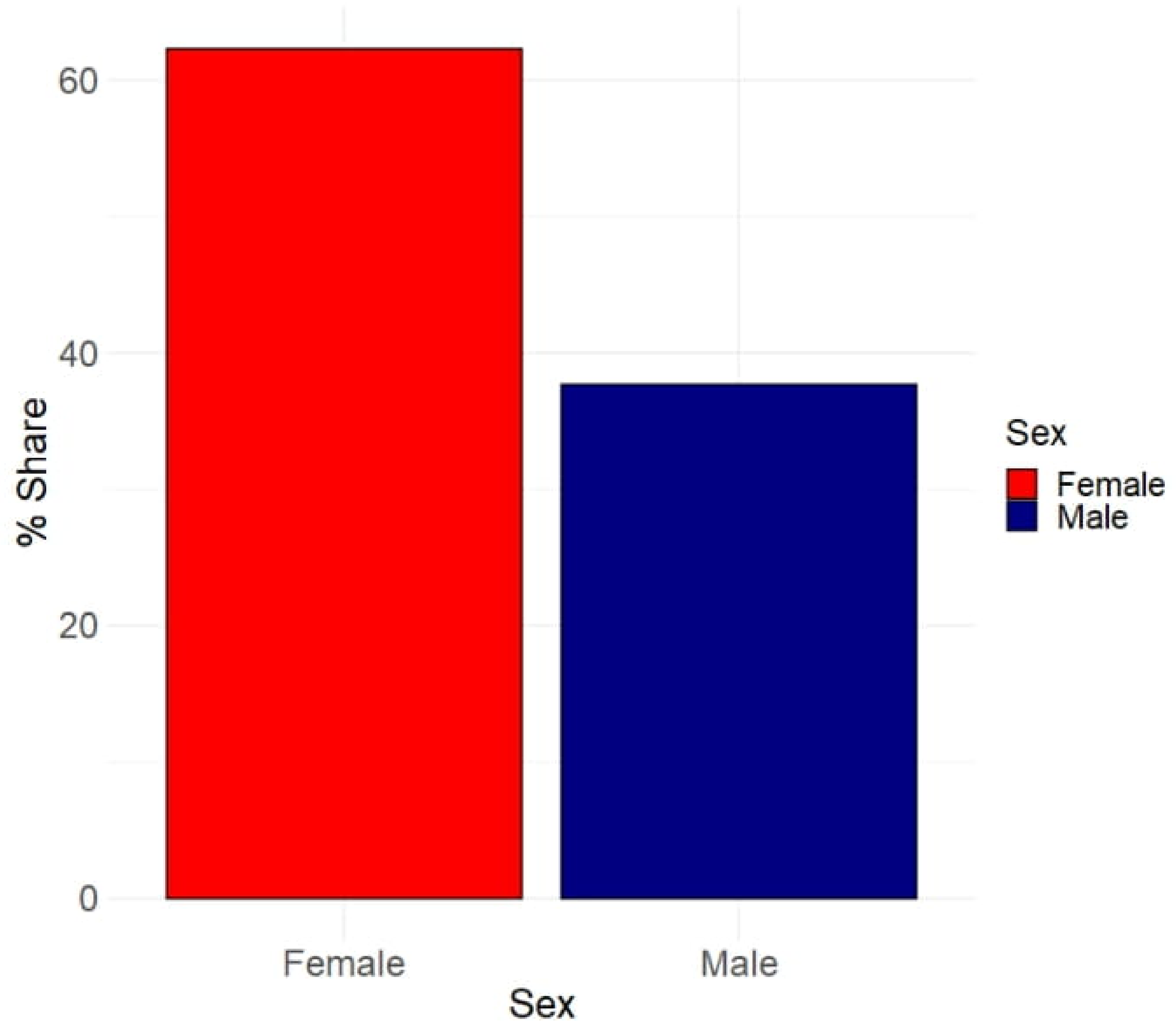

Fig.I.3.8.1 Sex-ratio distribution of sprat.

I.3.9 Fertility

Maturity is determined at **250specimens**. Portion fecundity (Log F) versus sprat weight (Log W) showed a strong relationship (R^2 =0.942), which proved a strong dependence of fecundity on individual weights.

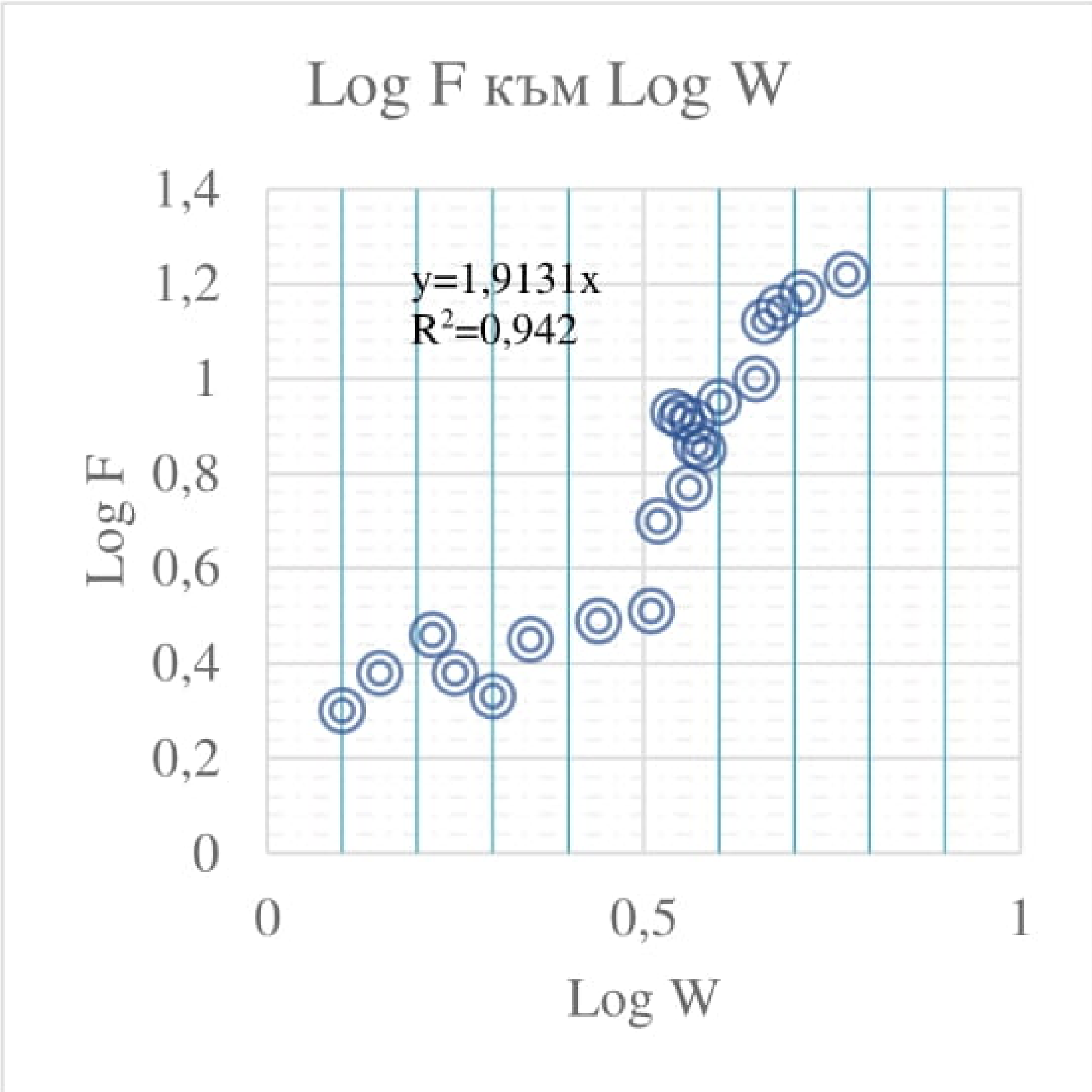

Fig. I.3.9.1 LOG dependence of fertility on individual weights, first semester 2024.

The gonadosomatic index depends strongly on individual weights in the first six months of 2024 ($R^2 = 0.912$).

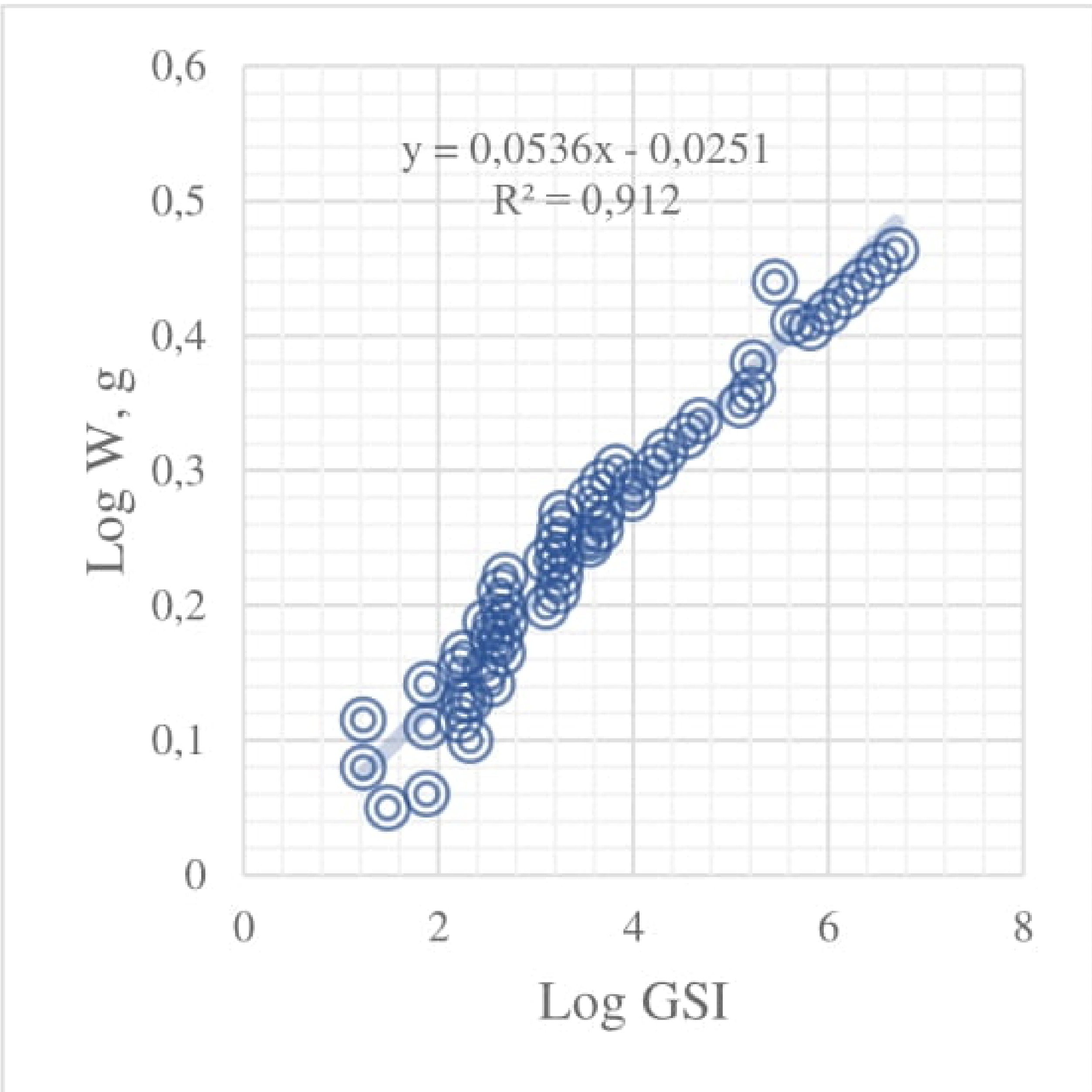

Fig. I.3.9.3 LOG dependence between GSI and individual weights (g) first semester 2024.

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Table I.3.9.1 describes the absolute and relative fecundity of sprats with average lengths and weights. The average value of absolute fecundity was estimated at 33101,55 spawn grains. The average value of relative fertility is 9473,67.

Table I.3.9.1 Absolute and relative fecundity of the sprat for the fist semester, 2024 .

Lengths class, cm	Mean weight,w,g)	Absolute fecundity F, (cav.grains)	Relative fecundity	No. ♀
6,5	1,11	16878,00	15205,41	23
7	1,39	20498,00	14746,76	28
7,5	2,11	23609,50	11189,34	45
8	2,81	29935,50	10653,20	29
8,5	3,23	34407,25	10652,40	18
9	3,67	36174,25	9856,74	28
9,5	4,99	37507,50	7516,53	34
10	5,65	41184,25	7289,25	23
10,5	6,11	41408,25	6777,13	9
11	7,55	41228,00	5460,66	7
11,5	8,49	41286,50	4862,96	6
		33101,55	**9473,67**	**250**

I.3.10 Sexual maturity

250 specimens were examined to determine sexual maturity. The degree of sexual maturity of the sprat by size groups for the period January-March is presented in Fig. I.3.10.1. The predominant maturity stage is IV-V (actively breeding), as the oldest groups have VI-I (resting) stages of sexual maturity (3 and 4 years old).

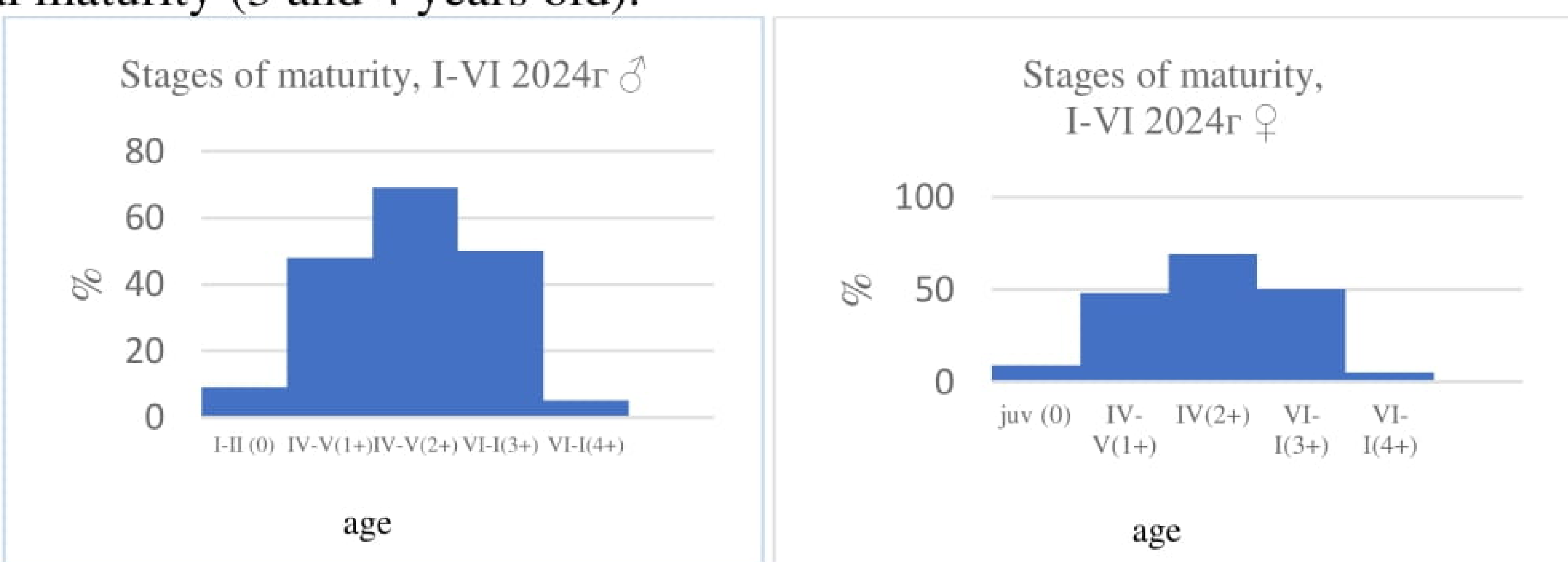

Figure I.3.10.1 Degrees of maturity by age of sprat.

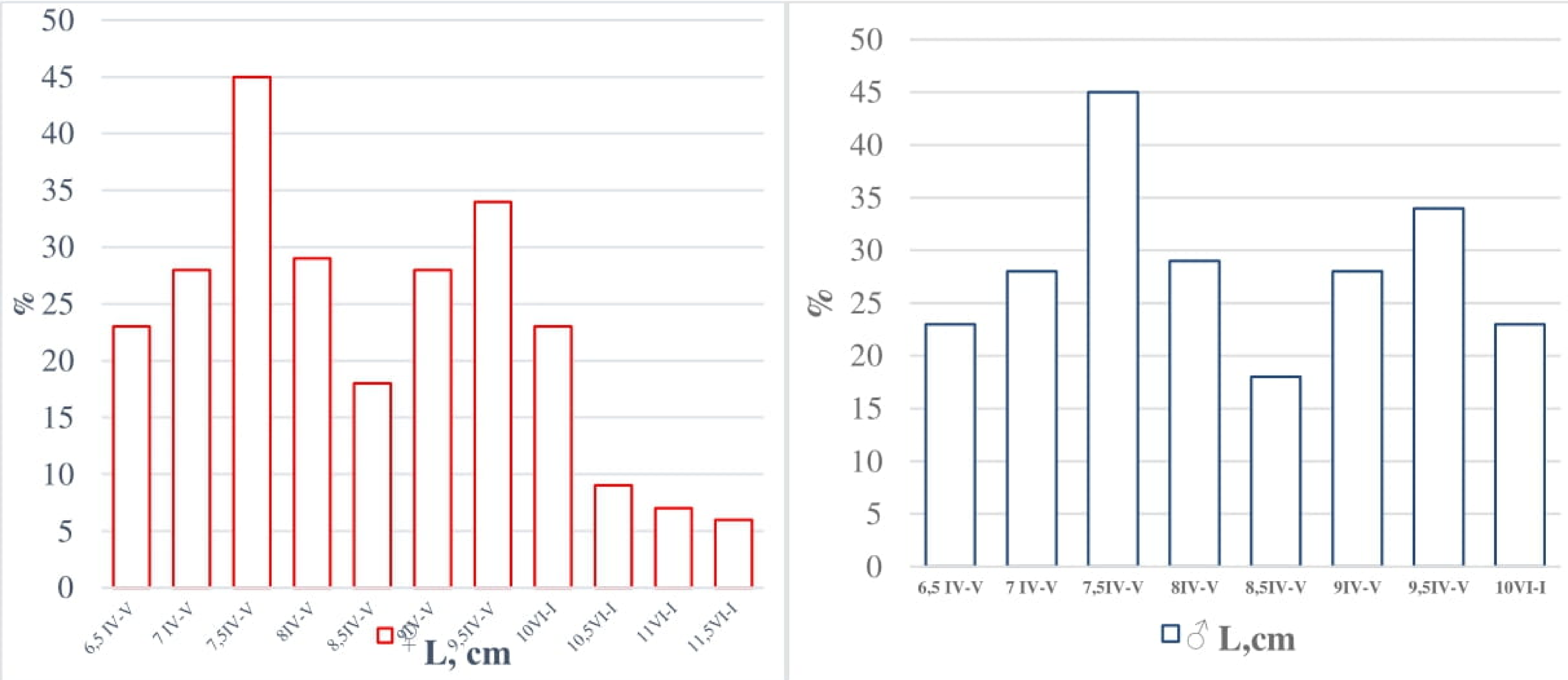

Fig.I.3.10.3 Degrees of maturity of the sprat by length.

I.3.11 Catch numbers and biomass by age and length

Monthly catches with OTM (in tonnes) together with mean weights of sprat were used to derive the monthly catch numbers. The share (%) by age groups and catch numbers were used to create catch-at-age matrix for selected months by age groups (Table I.3.11.1).

Table I.3.11.1 Catch at length (10^{-6}) and catch at age (10^{-6}) matrix and biomass (kg) of sprat.

Catch in numbers*10^{-3}		
Age groups (yr	*Ist quarter*	*IInd quarter*
0-0+	14242,41	15975,31
1-1+	55952,31	62760,15
2-2+	127164,3	142636,7
3-3+	90032,35	100986,8
4-4+	7629,86	8558,202
5-5+	6103,888	6846,561
∑	**301125,2**	**337763,7**
	Biomass (kg)	
Age groups (yr	*Ist quarter*	*IInd quarter*
0-0+	16877,25	18930,74
1-1+	112786,1	126509
2-2+	371684,5	416908,1
3-3+	460574,3	516613,4

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4-4+	56679,46	63575,77
5-5+	52432,4	58811,96
∑	**1071034**	**1201349**

The monthly catch with OTM(in tonnes) together with the average weights are used to obtain the monthly catch numbers. The proportion (%) by age group and catch abundance is used to create a ***catch-by-length matrix*** (Table I.3.11.2).

Table I.3.11.2 Catch length (10^{-6}) realized with OTM, matrix and biomass (kg) of the sprat.

Catch in numbers*10^{-3}		
Length classes (cm)	*Ist quarter*	*IInd quarter*
6,5	14242,41	15975,31
7,0	20346,29	22821,87
7,5	37131,99	41649,91
8	48322,45	54201,94
8,5	45779,16	51349,21
9	38149,3	42791,01
9,5	31028,1	34803,35
10	23398,24	26245,15
10,5	25432,87	28527,34
11	11190,46	12552,03
11,5	6103,888	6846,561
∑	**301125,2**	**337763,7**
Biomass (kg)		
Length classes (cm)	*Ist quarter*	*IInd quarter*
6,5	16877,25	18930,74
7,0	37131,99	41649,91
7,5	70744,06	79351,65
8	109661,4	123004,2
8,5	143080,2	160489,1
9	136783	153425,7
9,5	141137,2	158309,6
10	123573,2	138608,6
10,5	156483,3	175523
11	83129,87	93244,46
11,5	52432,4	58811,96
∑	**1071034**	**1201349**

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I.3.12 Conclusions

The analysis of the biological parameters of the sprat makes it possible to draw the following **conclusions:**

1) The size structure of the sprat during the period I-III of 2024, showed a modal distribution with a peak at size group 8.00 - 9.0 cm.

2) In the first and second quarters, the highest percentage share is occupied by individuals in the age group 2-2+, followed by 3-3+.

3) The results from ELEFAN with the three applied methods are consistent and applicable also to the experimentally determined ages with small discrepancies for the age group 1-1+, the probable reason for this being limiting factors such as the sample size and the consideration of growth parameters for an inflow of less than a year. Another serious problem is the selectivity, which leads to an incomplete representation of the size composition of the stock in the samples, which in turn affects the calculation of biological potentials.

4) The condition of the three-zone by age shows a predominance of 4-4+ and 5-5+ year olds, with the condition varying in different age groups.

5) The resulting model is statistically significant, the value of the scaling coefficient a in the length-weight relationship model is $\boldsymbol{W_i = aL_i^b}$: a=0.0022, and the allometry coefficient b=3.38, which indicates positive / allometric growth of the species in the first half of the year, or the increase in weight is proportional to or greater than the increase in length.
6) Portion fertility (Log F) versus litter weight (Log W) show a strong relationship (R^2 =0.942), which proves a strong dependence of fertility on individual weights.

7) The predominant maturity stage of the sprat is IV-V (actively breeding), as the oldest groups have VI-I stages (resting) of sexual maturity (3 and 4 years old).

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II. Biological monitoring of horse mackerel (*Trachurus mediterraneus*) landings

II.1 Objectives

The purpose of biological monitoring is to collect data that will be used to analyze horse mackerel catches, as well as to form a database. The collection of biological samples of horse mackerel catches in **I-VI 2024** includes the following tasks:

1. *To collect and analyze the dynamics of length, weight and age distribution.*
2. *To determine the state of the of horse mackerel using the so-called state factor (Ricker, 1975).*
3. *Characteristics of the reproductive biology of horse mackerel.*
4. *Collection of data on ports of landing, sampling vessels, number of samples collected, number of specimens tested, geographical catch data.*

I.2 Sampling

The sampling was carried out by the actively operating fishing fleet in Bulgaria.

II.2.1 Geographic area coverage

Data of present analysis were collected from landing ports of Bulgarian Black Sea coast**. In I-VI 2024, 3 samples with 649 specimens** were collected and processed. Information on the size of the catches was also collected.

II.2.2 Sampling period

In 2024, the biological data on sprat were collected from a total of **3 landings at the ports of Nesebar, Sozopol and Tsarevo**. Information on the size of the catches was also collected. Ports and ships from which monitoring was carried out to collect biological data from landings are presented in Table II. 2.1.1.

Table II. 2.2.1 Ports and ships from which monitoring was carried out to collect biological data from horse mackerel discharges.

№	Date	Sampling ports	HMM	Fishing vessel	External marking	Fishing gear	Coordinate	Catch,kg
1	3.1.2024	Sozopol	HMM	MEDUZA 3	BS 288	OTM	42.3064 27.9862	1335

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2	25.4.2024	Nesebar	HMM	RK 29	BS 222	OTM	42.6604309082 27.7332210541	140
3	8.6.2024	Tsarevo	HMM	CIKLAMA V	AH215	OTM	42.2445335388 28.0178565979	30

II.2.3 Statistical analysis of data

See section statistical analysis of sprat

II.3 Results

II.3.1 Landings statistics

The highest catch of Black Sea horse mackerel with OTM was achieved in May in the Bulgarian waters of the Black Sea Figure II. 3.1.1.

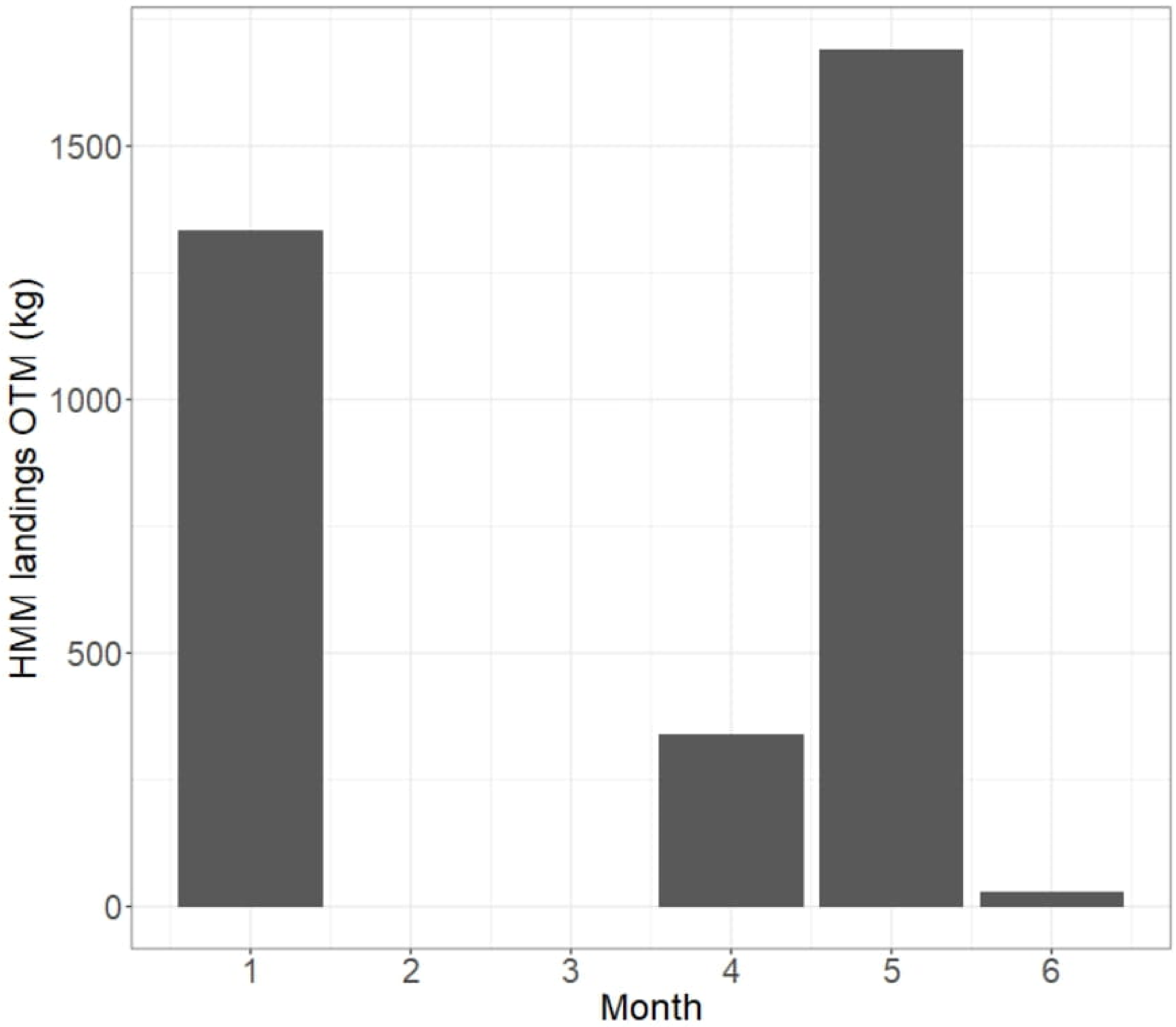

Figure II. 3.1.1 Landings statistics of horse mackerel.

II.3.2 Length structure of landings

In the first quarter, the size structure showed a normal distribution, with a predominance of the 12cm size group by 17%. Length classes 13-14 cm are represented by an equal percentage - 9.09%, with smaller (11-11.5cm) and larger lengths (15 cm) being insignificantly represented in the catch by 6.29%, 7.69% and 6.29%. In the second quarter, the percentage share for horse mackerel is in favor of the 13.5 cm length class, which accounts for 14.47% of all groups. The size classes 10.5-11-11.5cm are represented by a small share of participation in the catches. The participation of 13cm (11.44%)-14 (11.45%) and 14.5 cm (11.87%) is almost equal.

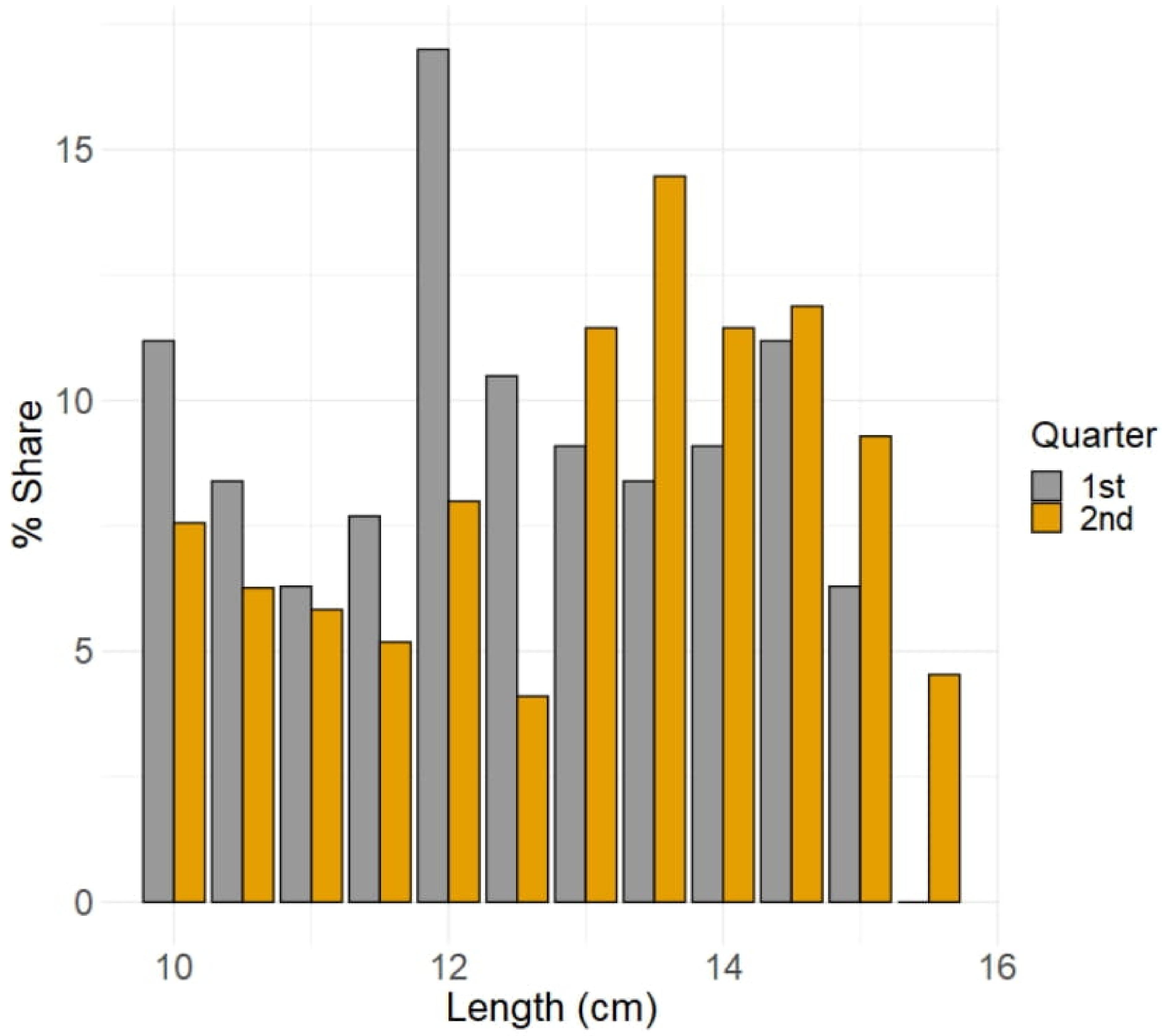

Figure II. 3.2.1 Percentage representation in the composition of catches.

II.3.3 Age structure of landings

The three readers determined the age of horse mackerel otoliths, and reader 1 read all otoliths twice. Specimens **(n = 649)** were used for age determination. Three-year-olds dominate the age structure in the first quarter of 2024 with 36.26%. Two- and four-year-olds are represented with 25.17% and 26.57%. The least represented in the catch are five-year-olds with 11.89%. Three- and four-year-old fish participate with the highest percentage in the catches of the second half of the year with 25.92% and 39.09% respectively, followed by the lower representation of two (17.93%) and five-year-old fish (17.06%).

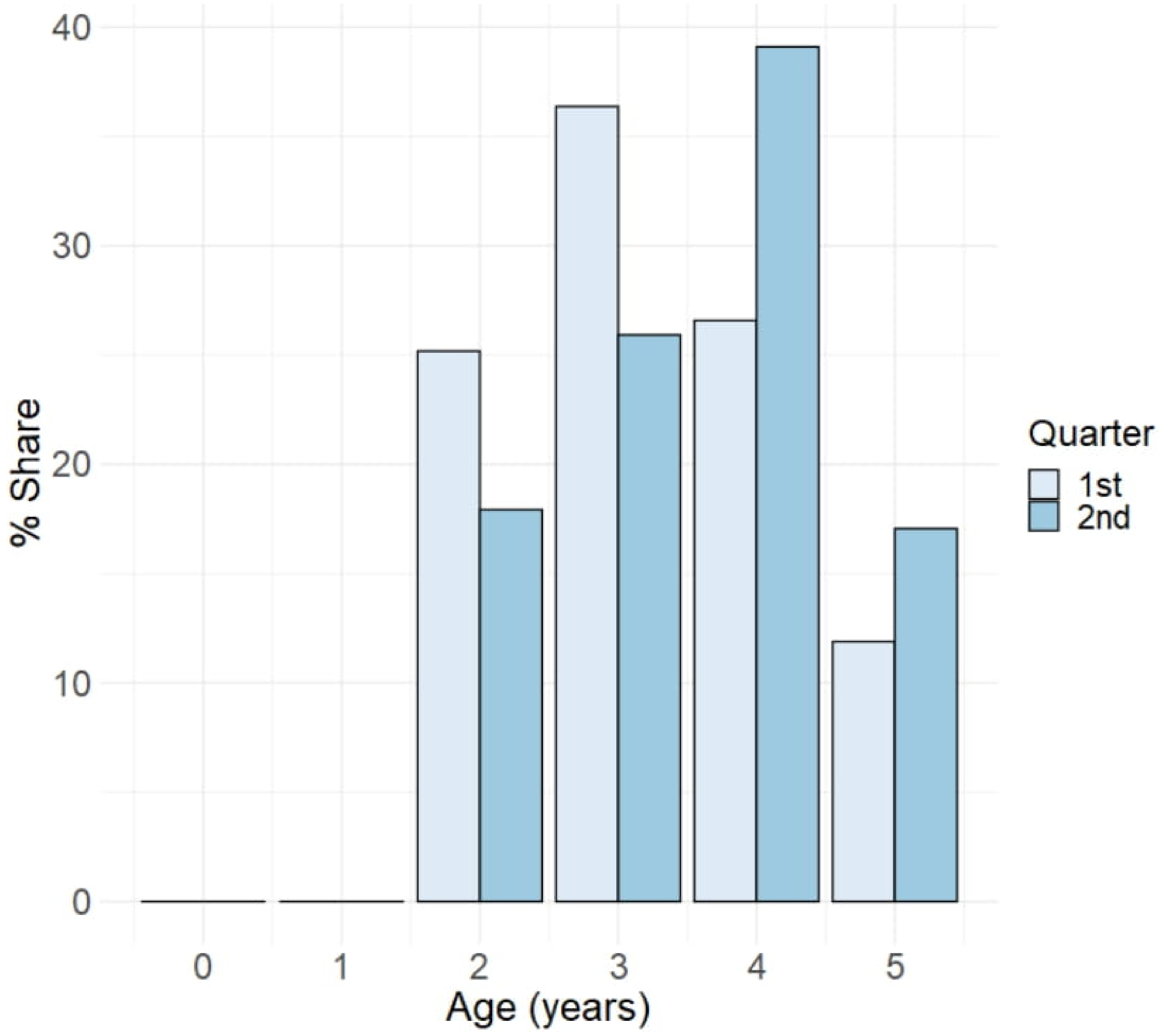

Figure II.3.3.1 Age distribution of horse mackerel.

Results of analytical age slicing methods – similar to the analysis of the age structure of the sprat, the analyzes were made in the R programming environment (Program Language for Statistical Modeling and Analysis) using the ELEFAN functions (ELectronic LEngth Frequency Analysis) and the growth and other biological parameters of the studied species were evaluated based on the measured lengths of individuals of different age groups. The samples are restructured for the purposes of the analysis in order to track the evolution of the cohorts over time (Figure II.3.3.2 - the first frequency diagram shows the size structure of the collected specimens in the corresponding months, and the second - the restructured data with a moving average MA = 7).

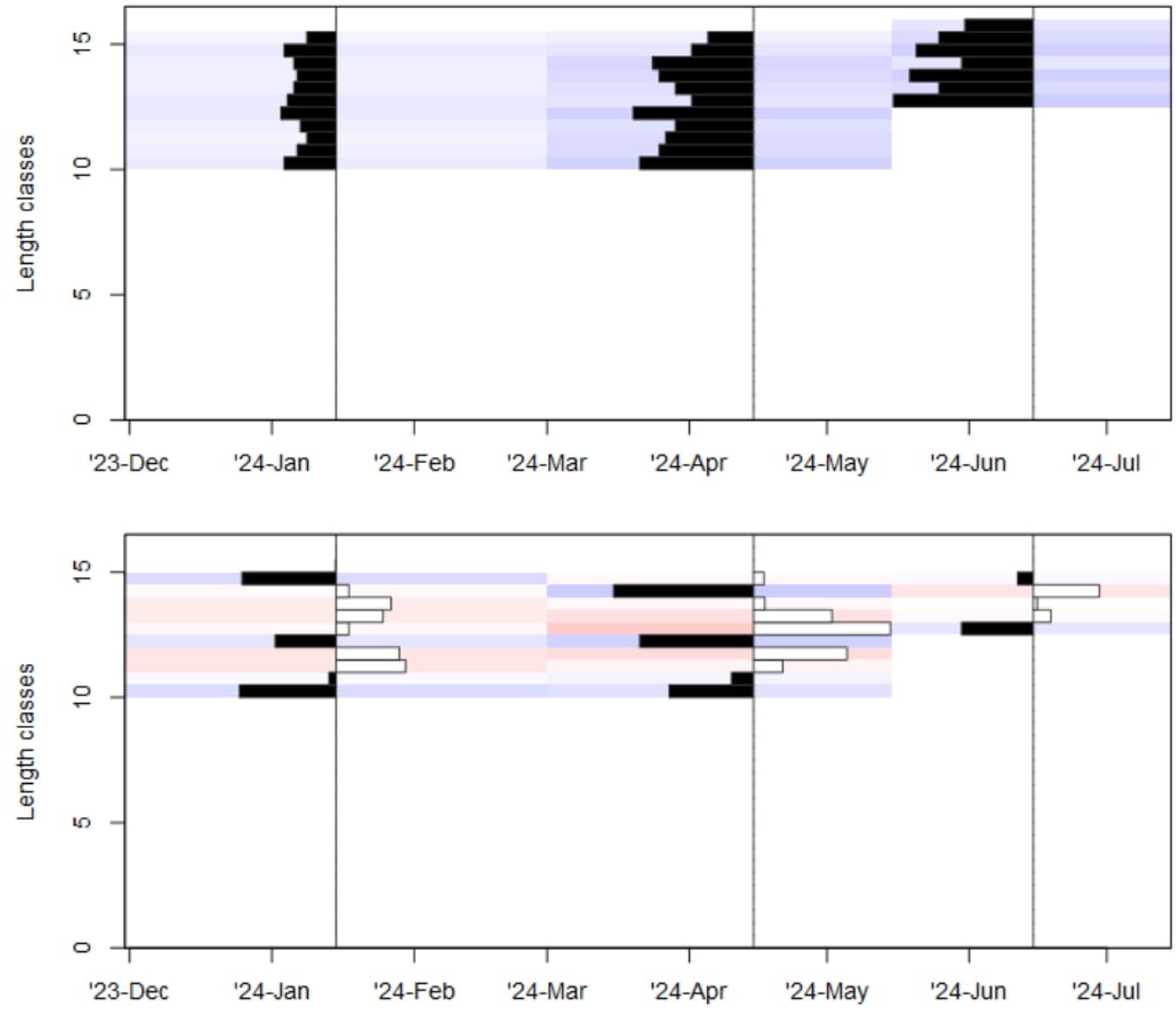

Figure II.3.3.2 Size-frequency samples, visualized as (top diagram) number of individuals in size class and (bottom diagram) the restructured data with moving average MA = 7 for the purposes of frequency analysis and determination of growth parameters.

II.1.3.3.1 Frequency analysis RSA – response surface analysis

Growth parameters determined by RSA with initial conditions: conditional interval of for the asymptotic length L_∞ in the sample [14;19 cm] (based on a literature review on the characteristics and biological potentials of the species for the Black Sea) and the parameter that determines the growth rate to L_∞ -$K = \exp\,(seq(from\ log(0.1), to\ \log(1))$ - with an upper limit for L_∞ = 19 cm. The parameters in the von Bertalanffy model were calculated as follows: L_∞=16.22 cm; K = 0.36 and t_0=-0.71. The ages with their corresponding lengths were calculated using the latter (Table II.1.3.3.3.1). A disadvantage of the method is the difficult optimization of L_∞ and K and the need for a priori expertise to define the interval for the expected asymptotic length, which is expected to be overcome by applying genetic algorithms for age slicing.

II.1.3.3.2 Frequency analysis ELEFAN with simulated annealing (ELEFAN SA)

The growth parameters determined under initial conditions: a conditional interval for the asymptotic length 〚L_∞〛 guess=17.10 cm and a search for the value of L_∞ in the interval [L($_{\infty guess}$)*0.8 ÷L($_{\infty guess}$)*1.2], and for K in the interval [0÷1]. The parameters in the von Bertalanffy model were calculated as follows: L_∞=16.09 cm; K = 0.4 and t_0=-0.47. The ages with their corresponding lengths were calculated using the latter (Table II.1.3.3.3.1).

II.1.3.3.3 Frequency analysis ELEFAN – Genetic algorithm (ELEFAN GA)

The growth parameters determined under initial conditions: a conditional interval of for the asymptotic length 〚L_∞〛 $_{guess}$=17.10 cm and a search for the value of L_∞ in the interval [L($_{\infty guess}$)*0.8 ÷L($_{(\infty guess}$)*1.2], and for K in the interval [0÷1]. The parameters in the von Bertalanffy model were calculated as follows: L_∞=16.48 cm; K = 0.35 and t_0=-0.72. The ages with their corresponding lengths were calculated using the latter (Table II.1.3.3.3.1).

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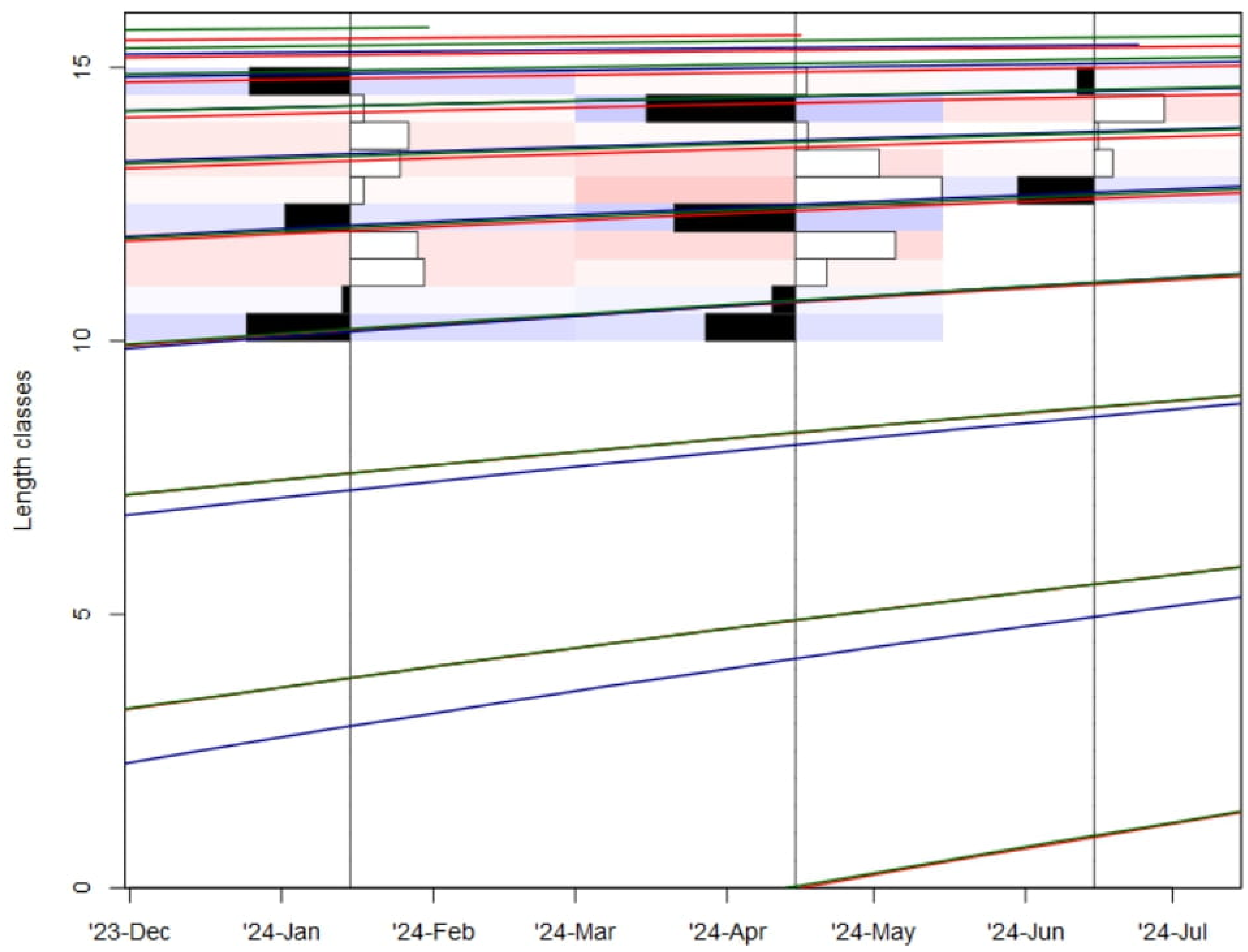

Figure II.1.3.3.3.1 Growth curves (in red for growth parameters determined with ELEFAN RSA, in blue for ELEFAN SA, and in green for ELEFAN GA), visualized on the restructured data for the purpose of visualizing the tracking of cohorts over time.

Table II.1.3.3.3.1 Ages with their corresponding lengths for the studied species calculated using von Bertalanffy parameters, obtained with RSA, ELEFAN with simulated annealing, ELEFAN with genetic algorithm and compared with experimentally determined ages from otoliths.

	ELEFAN RSA	**ELEFAN SA**	**ELEFAN GA**	**Experimentally determined**
age	La	La	La	L_{mean}
0	3.7	2.7	3.7	n/a
0.5	5.7	5.2	5.7	
1	7.5	7.1	7.5	n/a
1.5	8.9	8.7	8.9	
2	10.1	10.1	10.1	10.75
2.5	11.1	11.2	11.2	
3	12.0	12.0	12.0	12

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3.5	12.7	12.8	12.7	
4	13.2	13.4	13.3	13.75
4.5	13.7	13.9	13.8	
5	14.1	14.3	14.3	15

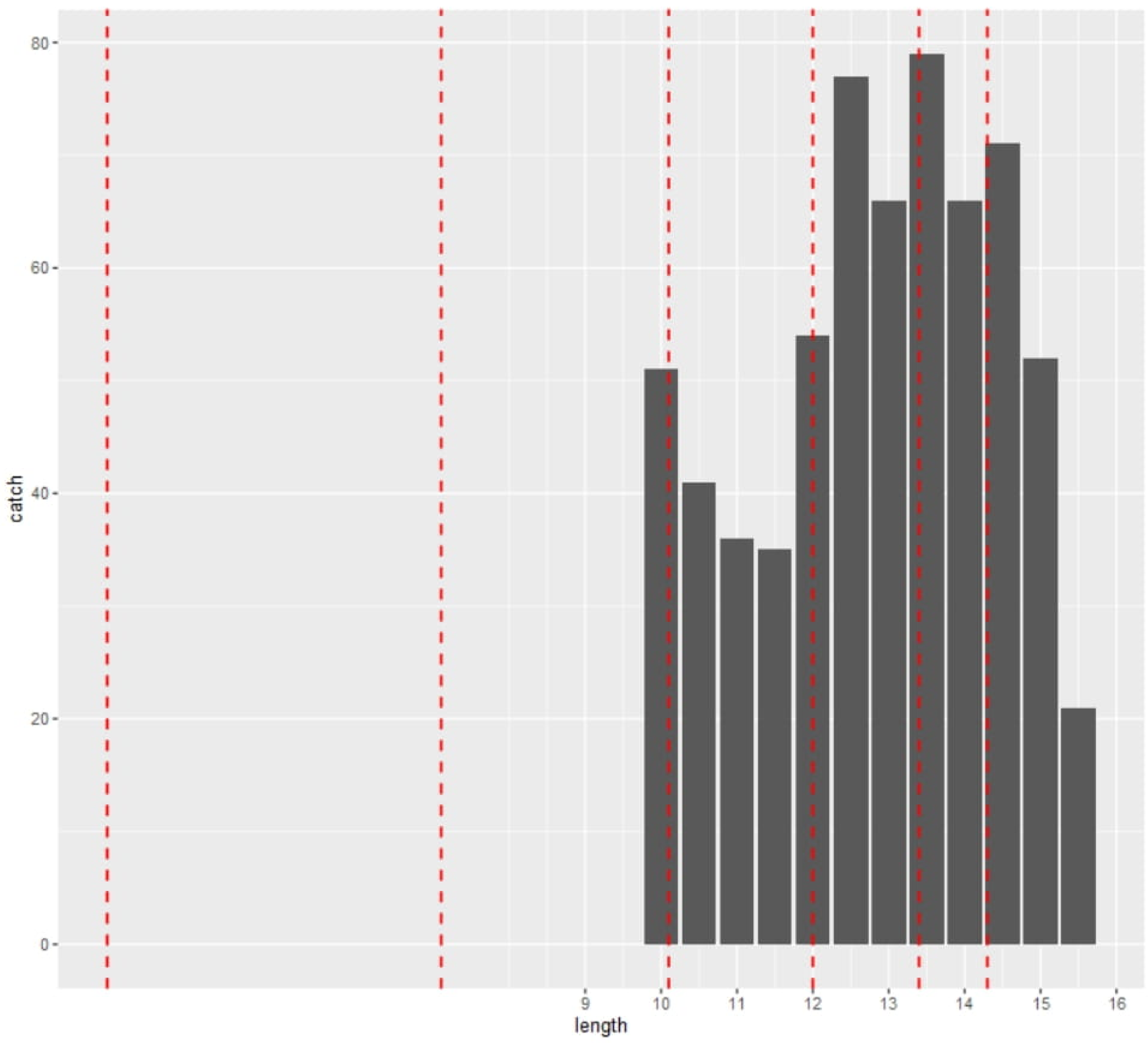

Figure II.1.3.3.3.2 The ages represented with dashed lines, calculated based on the growth parameters determined by ELEFAN SA, are overlaid on the cumulative sample of the composition of commercial catches of horse mackerel, aiming to visualize the range of size classes represented in a specific age group (0-5 years) for the first half of 2024.

The results of ELEFAN with the three applied methods are consistent and applicable to the experimentally determined ages. The sample size and the consideration of growth parameters for a period of less than a year generally have an impact on the accuracy of determination. ELEFAN SA is very close to the experimentally determined ages and, accordingly, the biological potentials of horse mackerel for the first half of 2024 are best described by the following analytically determined parameters: L_∞=16.09 cm; K = 0.4 and t_0=-0.47.

Since the calculated asymptotic length is smaller than that reported in the literature, it is likely that some or all of the samples represent the small-sized population. The latter is difficult to identify without genetic findings and therefore the application of such for the sustainable management of horse mackerel stocks is necessary.

II.3.4 Condition factor

The condition of the horse mackerel by size shows a predominance of 11.5-12.0 cm. specimens (Fig. II.3.4.1).The highest value of the condition factor was shown by three-year-old fish, and the lowest by two- and five-year-old fish, respectively.

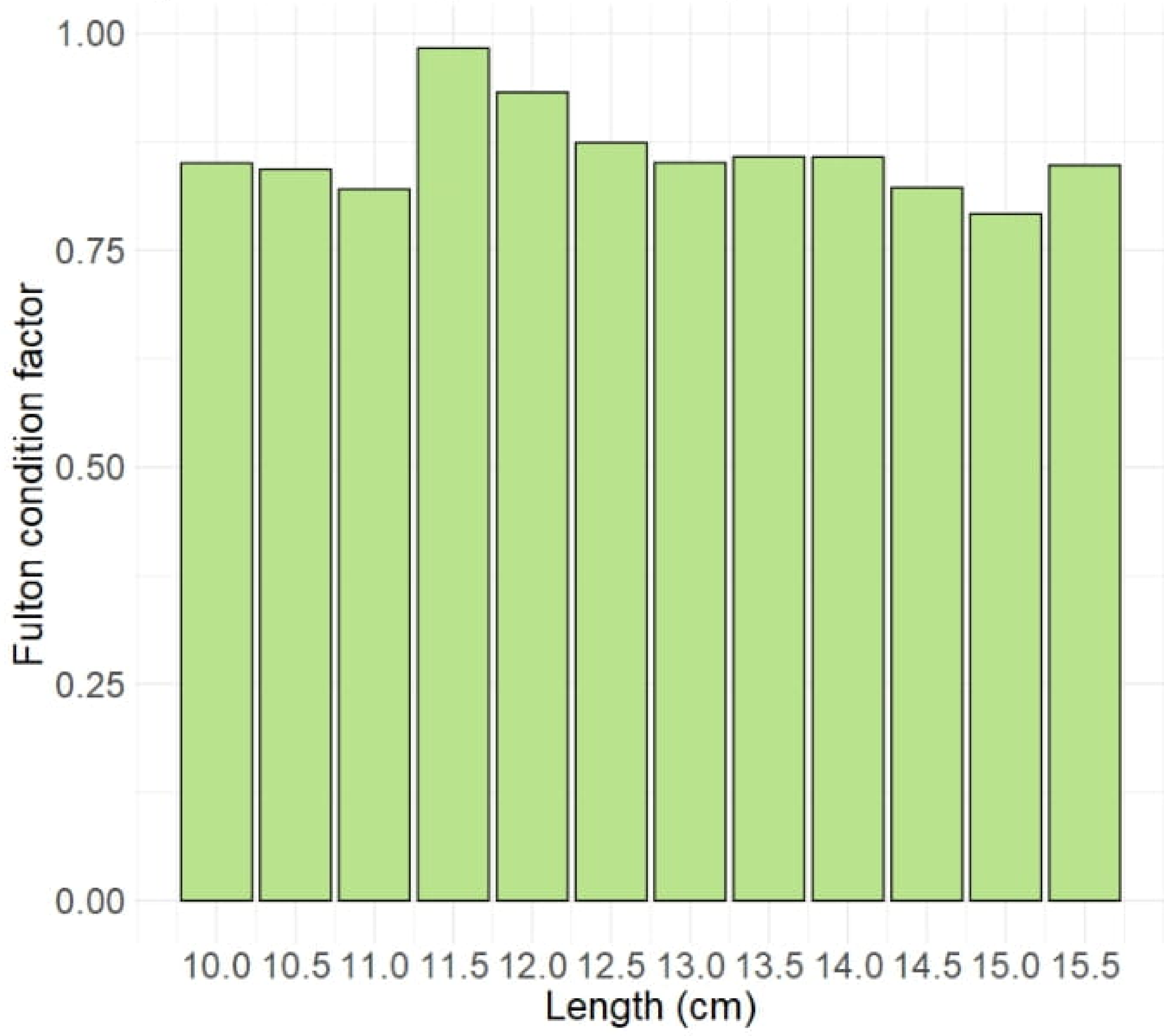

Fig. II.3.4.1 Fulton condition coefficient values of horse mackerel by length classes.

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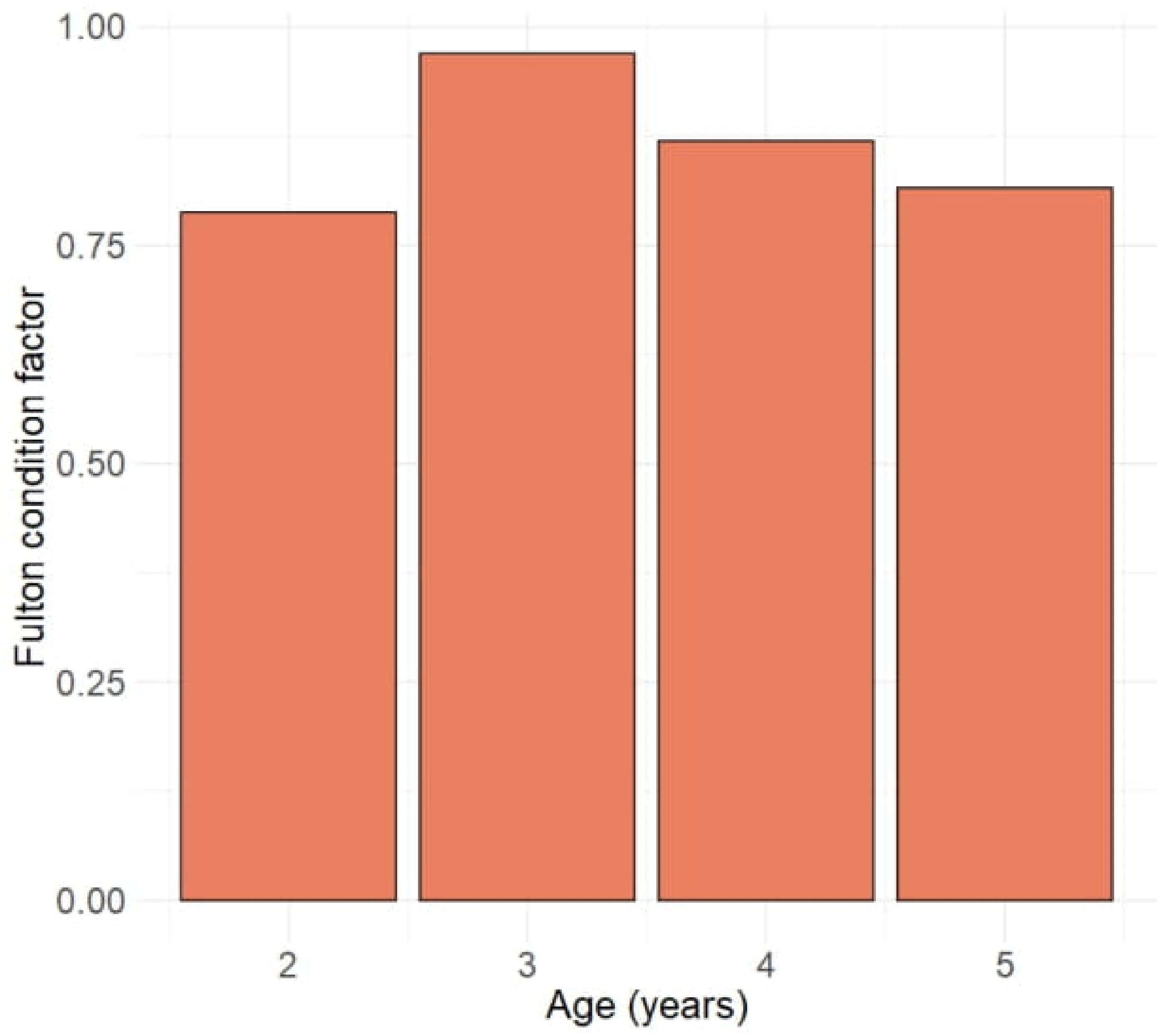

Fig. II.3.4.2 Fulton condition coefficient values of horse mackerel by age groups.

II.3.5 Weight structure of horse mackerel

The weight was measured on **649 specimens**. A gradual increase in the average weight is observed by age group for both the first and second trimesters. The average weight is lowest for age group 2+, and the highest average weight is observed for group 5+ (Fig. II. 3.5.1).
The graph shows the distribution of the average weight of horse mackerel by age group for two quarters. A gradual increase in the average weight is observed in relation to the age groups for both quarters. For the age group 2-2+ the lowest average weight is -9.78g (129 number of horse mackerel), and for the group 5-5+ the highest average weight is observed -27.53g (91 number of horse mackerel). For the other age groups the weights are as follows: 3-3+-16.76g (212 number of horse mackerel); 4-4+-22.61g (217 number of horse mackerel).

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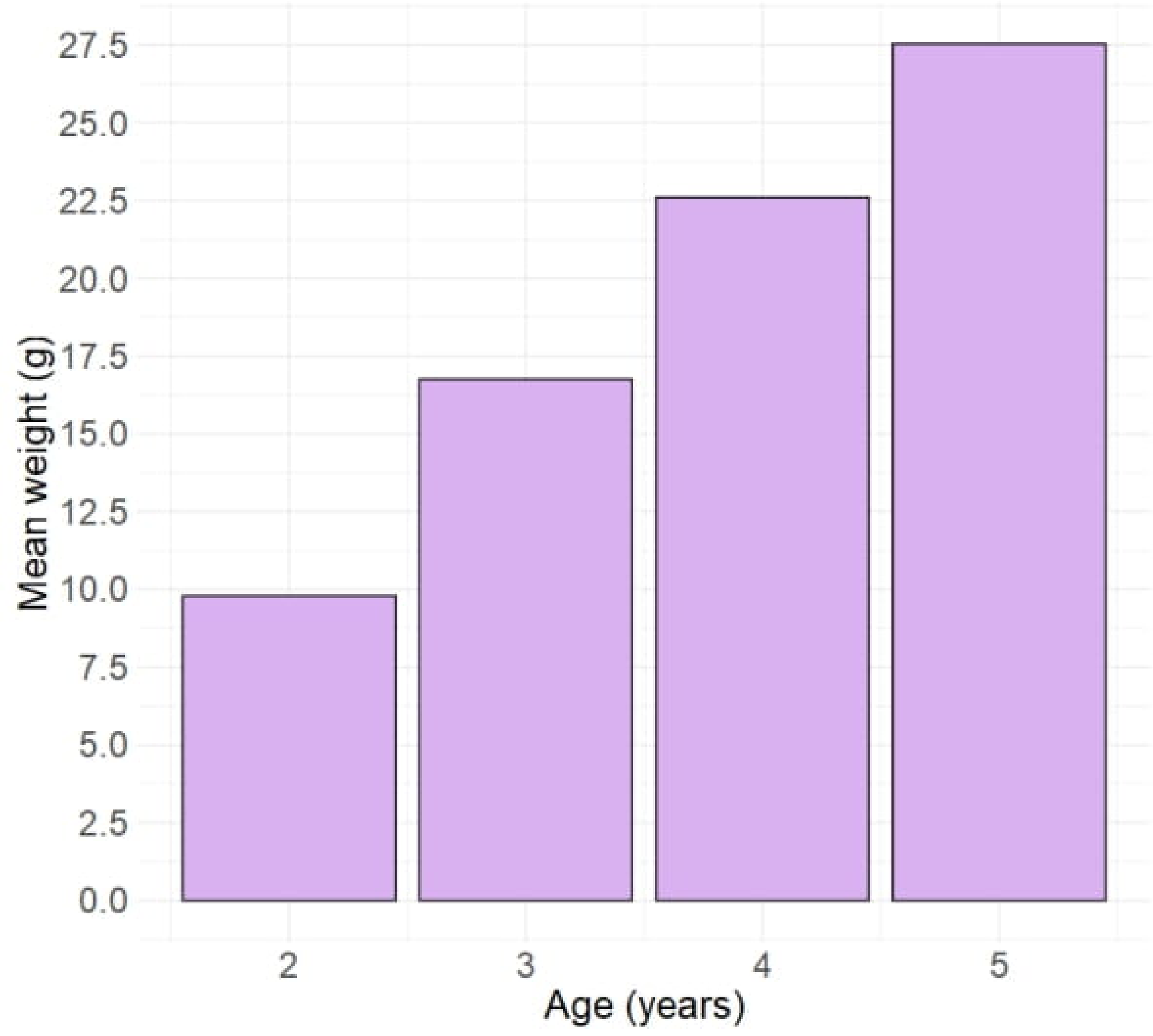


Fig. II. 3.5.1 Distribution of average weight of horse mackerel by age groups, first half of 2024.

The most widely distributed size groups 13.5-14.5cm represent 12.17% and 10.94% and for them average weights of 21.10g and 25.07g were measured.

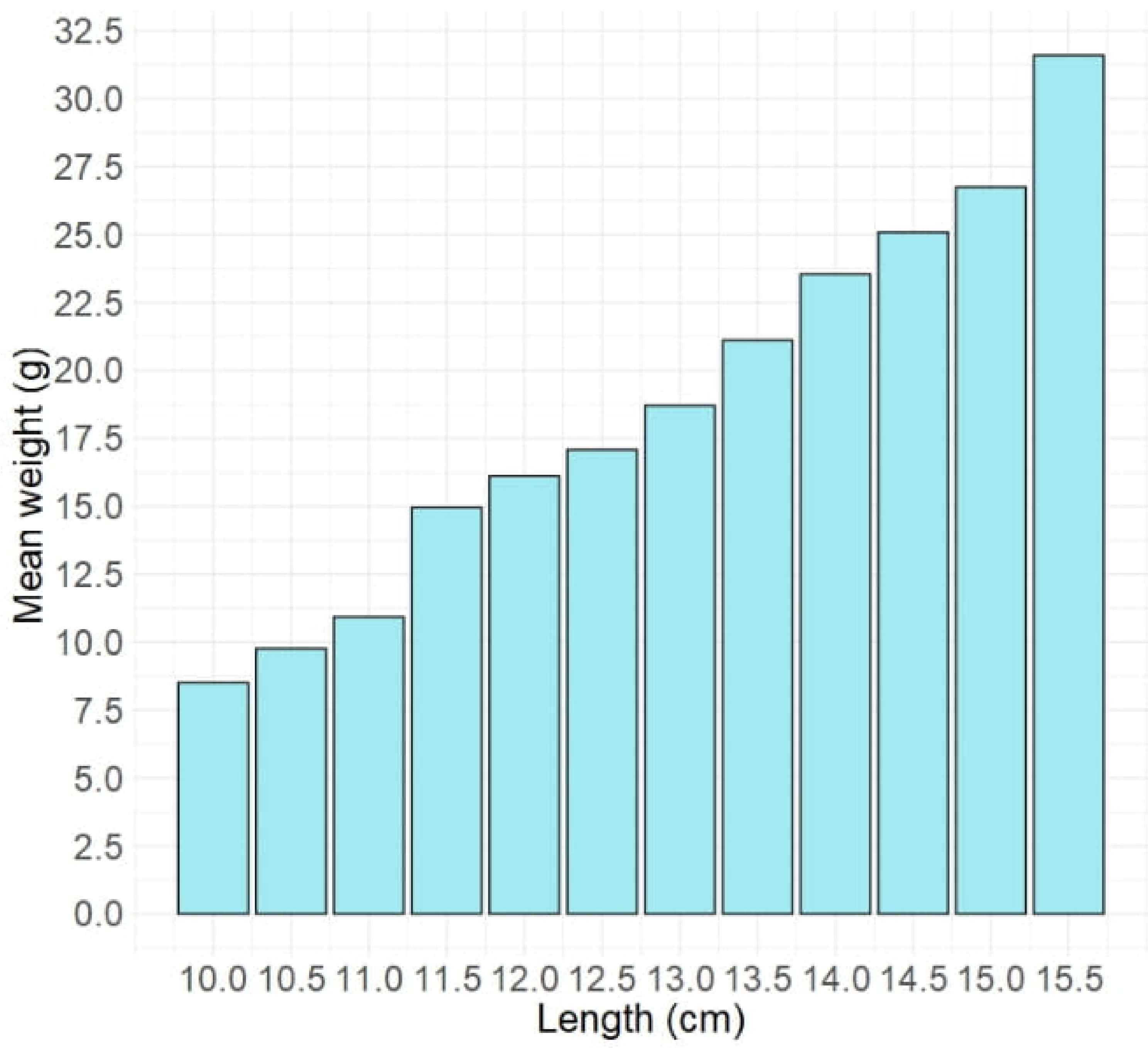


Fig. II. 3.5.2 Distribution of average weight of horse mackerel by size groups for the first half of 2024.

II.3.6 Size structure of horse mackerel by age group

The fish length was measured of **649 specimens**.

Table III.3.6.1 Size structure of horse mackerel by age groups.

L_{mean}/cm	Age
10,89	1-1+
11,69	2-2+
13,05	3-3+
14,35	4-4+
15,14	5-5+

II.3.7 Length- weight relationship

To calculate the length-weight relationship, a linear model was used on the logarithmically transformed values for the average weights and lengths for the first half of 2024. The modeling results are presented in Table II.3.7.1.

Table II.3.7.1 Results of modeling the length-weight relationship.

```
Call:
lm(formula = logW ~ logL, data = weight_l)

Residuals:
      Min        1Q    Median        3Q       Max
-0.063806 -0.040394 -0.000302  0.012688  0.122337

Coefficients:
            Estimate Std. Error t value       Pr(>|t|)
(Intercept)  -4.4402     0.3069  -14.47 0.00000004947 ***
logL          2.8755     0.1208   23.80 0.00000000039 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.0574 on 10 degrees of freedom
Multiple R-squared:  0.9826,	Adjusted R-squared:  0.9809
F-statistic: 566.3 on 1 and 10 DF,  p-value: 0.0000000003901
```

The resulting model is statistically significant, the value of the scaling coefficient a in the length-weight relationship model, $W_i = aL_i^b$ e: $a = 0.012$, and the allometry coefficient

b=2.88, which indicates negative allometric growth of the species in the first half of the year, or the increase in weight is not proportional to the increase in length.

II.3.8 Sex structure

The sex ratio in the first six months was determined on **150 specimens**. Males ♂ predominate over females♀.

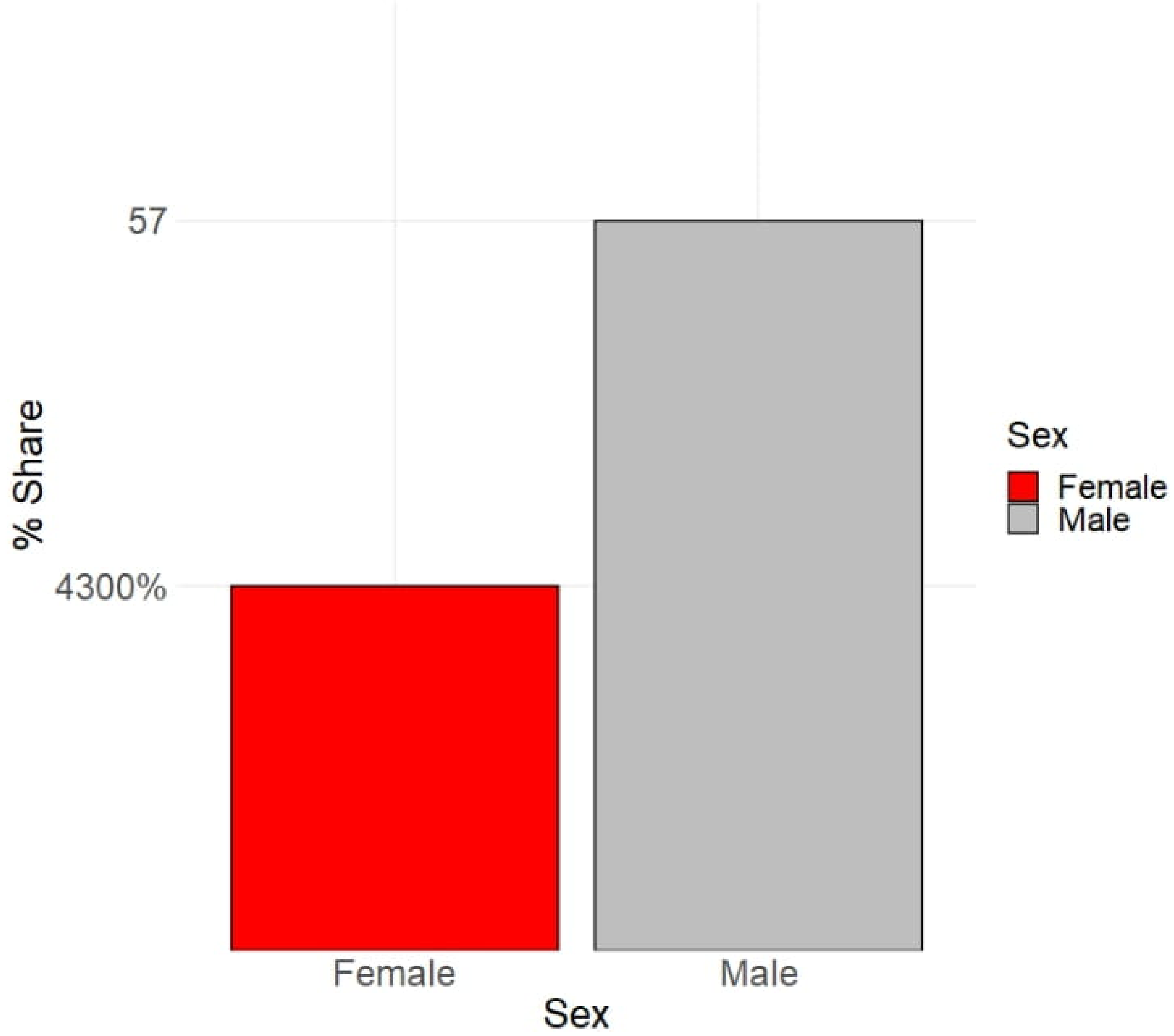


Figure II. 3.8.1 Sex ratio of horse mackerel.

Average lengths of female ♀ are higher in all year old fish (Figure II.3.8.2).

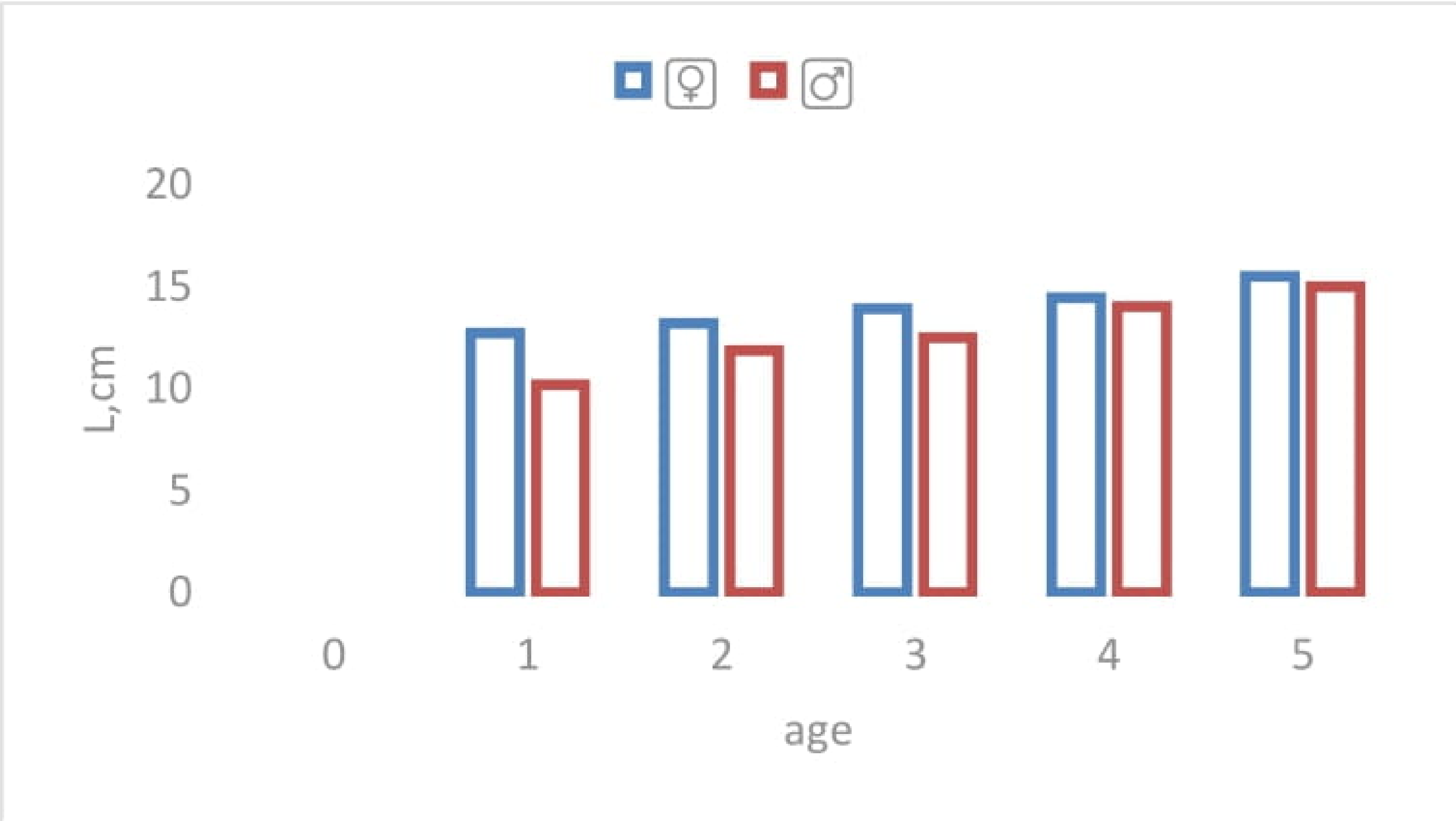


Figure II.3.8.2 Sex ratio(♂♀) by size and age of horse mackerel.

II.3.9 Fertility

Fertility was determined on **100 specimens**. Portion fecundity (Log F) versus litter weight (Log W) show a strong relationship (R^2 =0.89), which proves a strong dependence of fecundity on individual weights.

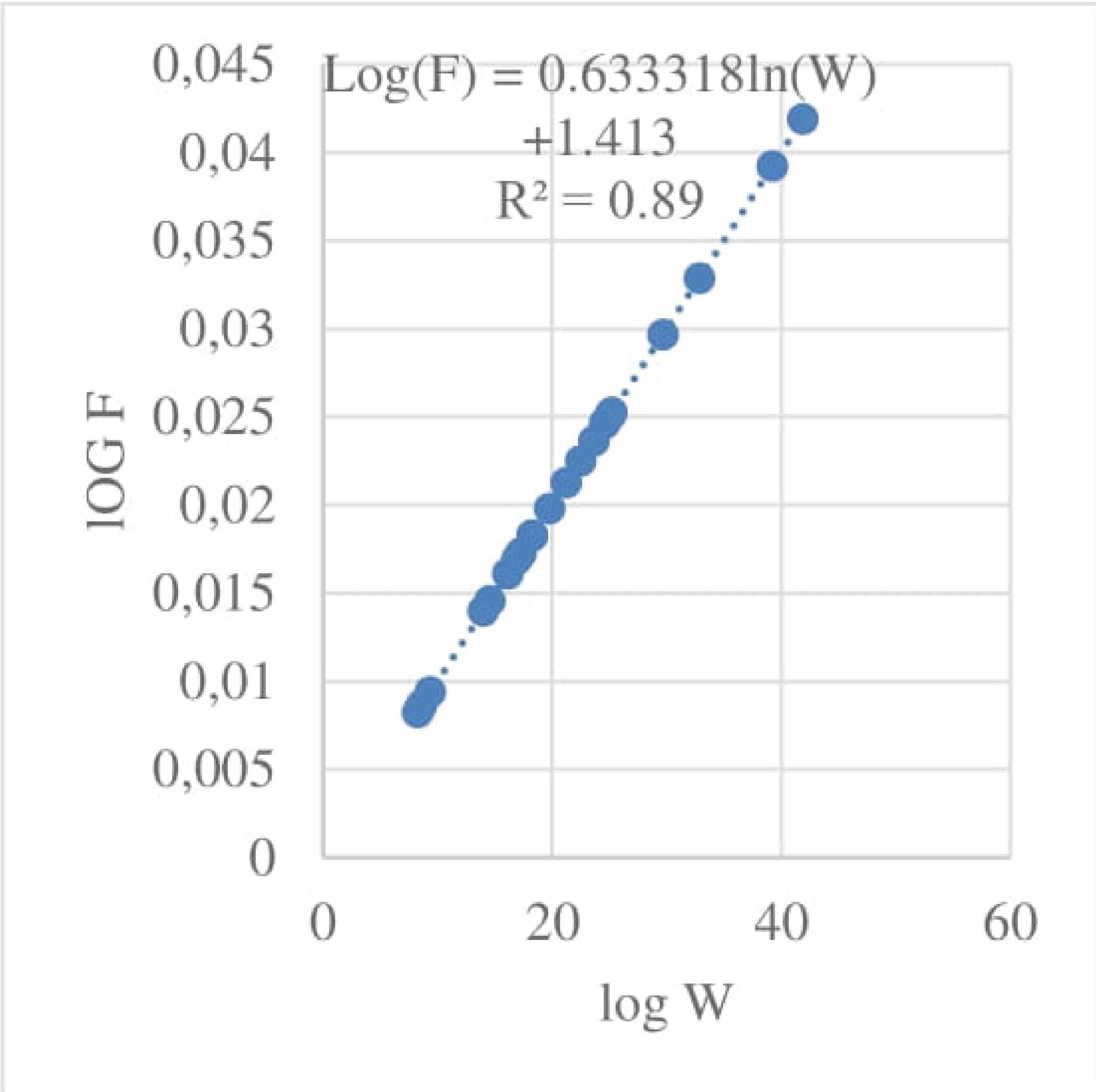


Figure. II.3.9.1 Dependence of portion fertility on horse mackerel weight.

The relationship between the weight and fertility of the mackerel in the I-VI 2024 showed a relatively low dependence (R^2=0.5802), on the Gonado-somatic index and the weight of the gland (ovary) (Figure II.3.9.2).

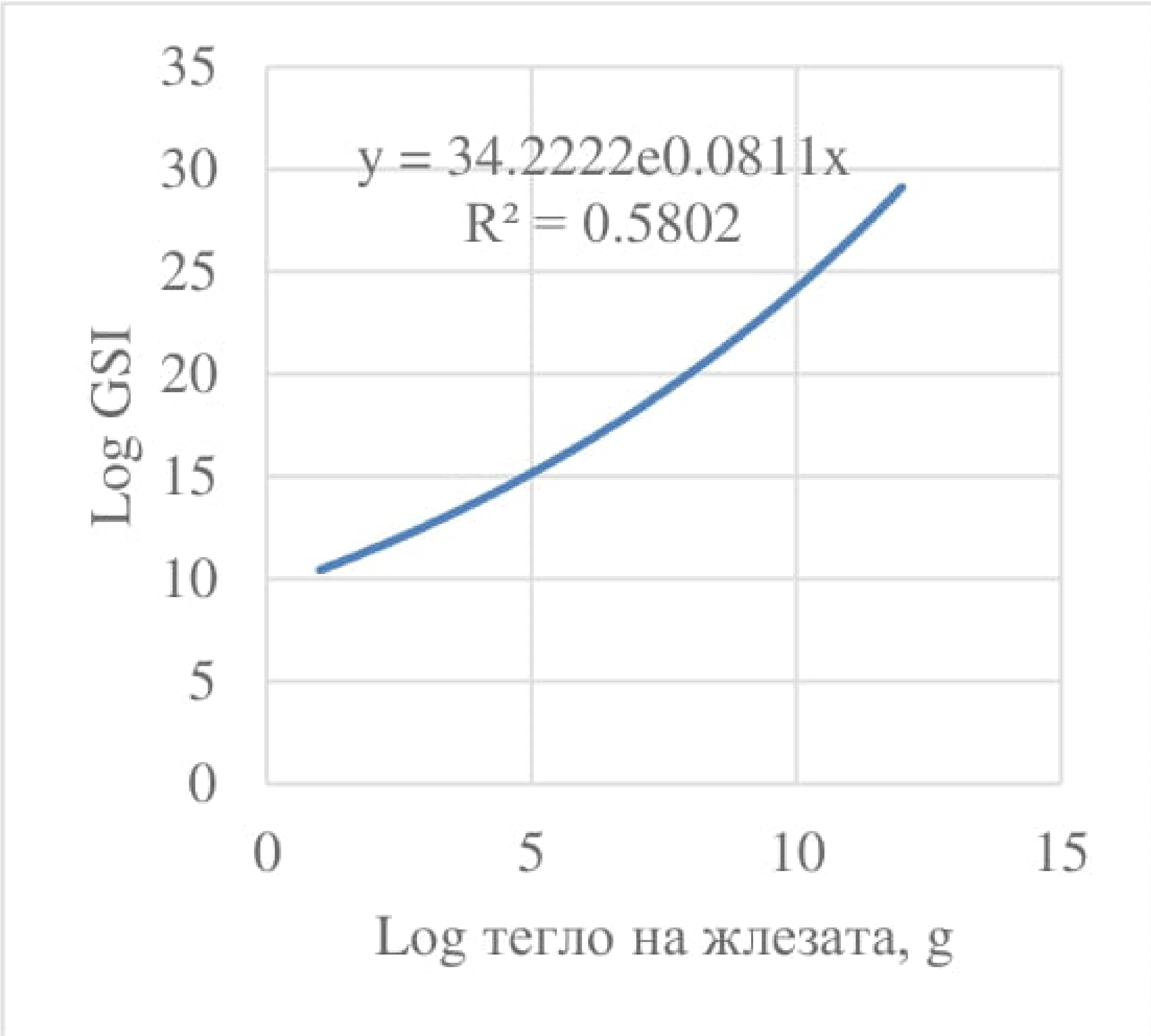


Figure II.3.9.2 Dependence of gland weight on gonadosomatic index (GSI).

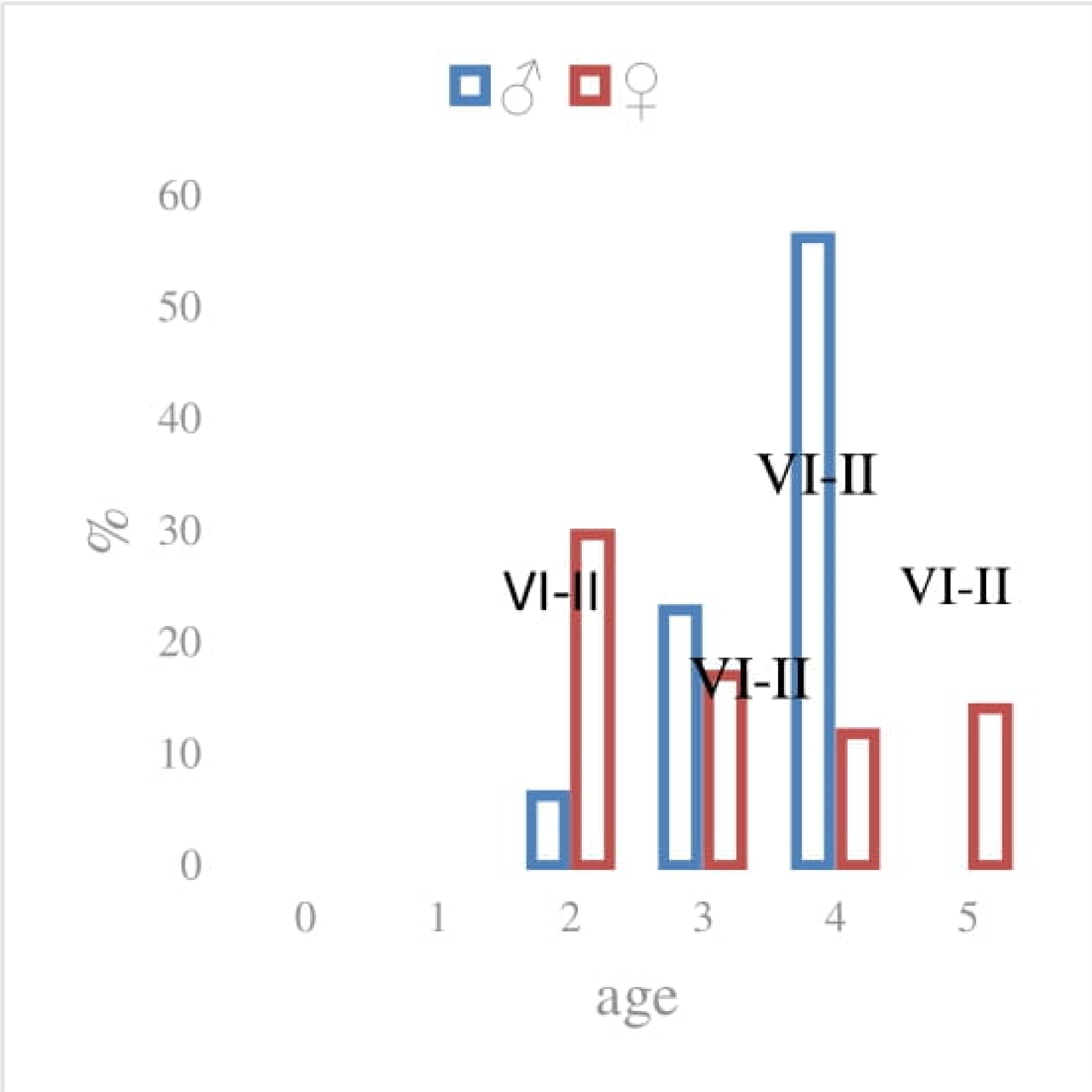
Absolute fertility fluctuates with average weighted fertility 7135,29 caviar grains. Absolute fertility increases with increasing length, weight and age. The weighted average relative fertility was 3384,23 (Table II.3.9.1).

Table II.3.9.1 Absolute and relative fertility.

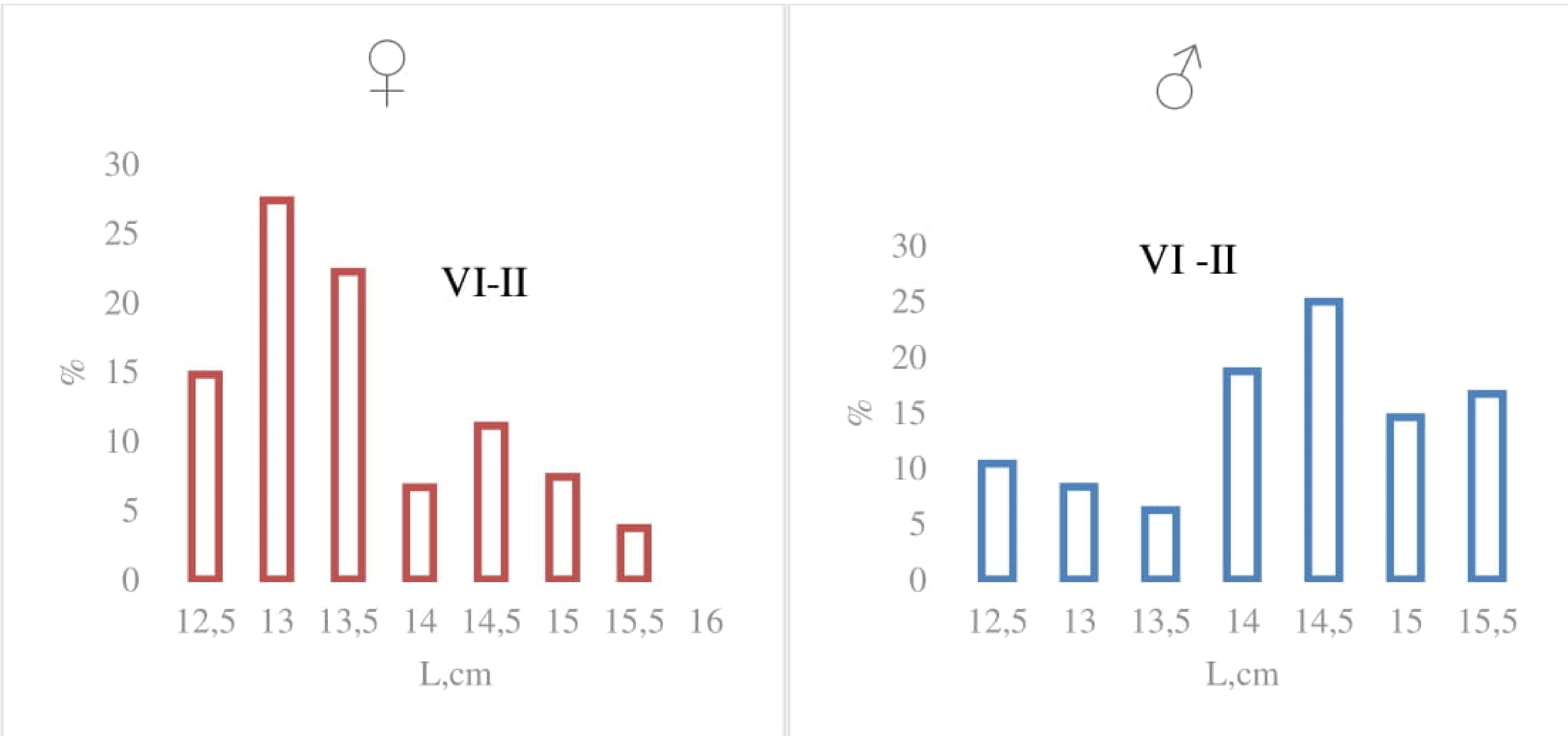
Size class	Average body weight (W, g)	Absolute fertility F, caviar grains)	Relative fertility	N ♀
12,5	16,11	4422,20	2744,21	20
13	17,08	6311,40	3696,18	17
13,5	18,70	9542,00	5101,76	24
14	21,11	9668,00	4580,23	9
14,5	23,54	6336,30	2691,94	15
15	25,08	6675,10	2661,88	10
15,5	31,59	6992,00	2213,36	5
		7135,29	3384,23	100

II.3.10 Sexual maturity

150 specimens have been assigned sexual maturity. Study specimens showed a degree of running gonads (VI-II).



Figute II.3.10.1 Sexual maturity by age of horse mackerel - female ♀ and male ♂.



Figute II. 3.10.2 Sexual maturity by length(cm) of horse mackerel - female ♀ and male ♂.

II.3.11 Catch numbers and biomass by age and length

The monthly catch with OTM (in tons) together with the average weights of horse mackerel were used to derive the monthly catch numbers. The proportion (%) by age group and catch numbers are used to create a ***catch-at-age matrix*** (Table II.3.11.1).

Table II.3.11.1 Catch at age (10^{-6}) with OTM, matrix and biomass (kg) of horse mackerel.

Catch in numbers*10^-3		
Age groups (yr	*I^st quarter*	*II^nd quarter*
2-2+	14,0862	21,7361
3-3+	23,1495	35,7213
4-4+	23,6954	36,5638
5-5+	9,9368	15,3332
∑	**70,8679**	**109,354**
	Biomass (kg)	
Age groups (yr	*I^st quarter*	*II^nd quarter*
2-2+	137,876	212,753
3-3+	387,902	598,561
4-4+	535,656	826,555
5-5+	273,565	422,131
∑	**1335**	**2060**

The monthly catch with OTM (in tons) together with the average weights of the horse mackerel were used to obtain the monthly catch numbers. Proportion (%) by age group and catch abundance were used to create a ***catch-by-length matrix*** (Table II.3.11.2).

Table II.3.11.2 Catch at length (10^{-6}) with OTM, matrix and biomass (kg) of horse mackerel.

Catch-at-length *10^-3 (in thousands)		
Length groups (cm)	*I^st quarter*	*II^nd quarter*
10	5,56897	8,59332
10,5	4,47702	6,90836
11	3,93104	6,06588
11,5	3,82185	5,89738
12	5,89656	9,09881
12,5	8,40806	12,9742
13	7,20691	11,1208
13,5	8,62645	13,3112
14	7,20691	11,1208
14,5	7,75289	11,9633
15	5,67817	8,76182
15,5	2,29311	3,53843
∑	**70,8679**	**109,354**
Biomass (kg)		

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Length groups (cm)	*I st quarter*	*II nd quarter*
10	47,3843	73,1174
10,5	43,7121	67,4509
11	42,9466	66,2697
11,5	57,1541	88,1928
12	95,0209	146,624
12,5	143,571	221,541
13	134,793	207,996
13,5	182,088	280,975
14	169,636	261,761
14,5	194,416	299,998
15	151,838	234,296
15,5	72,4393	111,779
∑	**1335**	**2060**

II.3.12 Conclusions

The analysis of the biological parameters of the horse mackerel makes it possible to draw the following **conclusions:**

1) In the first semester, the size class with the highest percentage is 12 cm, and in the second semester it is 13.5 cm.

2) In the first and second quarters, the highest percentage share is occupied by individuals in the age group 3-3+, followed by 2-2+.

3) The results of ELEFAN with the three applied methods are consistent and applicable to the experimentally determined ages. The sample size and the consideration of growth parameters for a period of less than a year generally have an impact on the accuracy of determination. ELEFAN SA is very close to the experimentally determined ages and, accordingly, the biological potentials of horse mackerel for the first half of 2024 are best described by the following analytically determined parameters: L_∞=16.09 cm; K = 0.4 and t_0=-0.47.

4) Since the calculated asymptotic length is smaller than that reported in the literature, it is likely that some or all of the samples represent the small-sized population. The latter is difficult to identify without genetic findings and therefore the application of such for the sustainable management of horse mackerel stocks is necessary.

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5) A gradual increase in average weight is observed across age groups for both the first and second trimesters.

6) The resulting model is statistically significant, the value of the scaling coefficient a in the length-weight relationship model, $\boldsymbol{W_i = aL_i^b}$ **e:** $\boldsymbol{a = 0.012}$, and the allometry coefficient ***b=2.88,*** which indicates negative allometric growth of the species in the first half of the year, or the increase in weight is not proportional to the increase in length.

7) Males ♂ predominate over females ♀.

8) Portion fecundity (Log F) versus horse mackerel weight (Log W) show a strong relationship (R^2 =0.89), which proves a strong dependence of fecundity on individual weights.

9) Absolute fecundity is with an average weighted fecundity of 7135.29 eggs. Absolute fecundity increases with increasing length, mass and age. The average weighted relative fecundity is 3384.23.

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III. Biological monitoring of whiting (*Merlangius merlangus*) landings

III.1 Objectives

The purpose of biological monitoring is to collect data that will be used to analyze whiting catches, as well as to form a database. The collection of biological samples of whiting catches in **I-VI 2024** includes the following tasks:

1. To collect and analyze the dynamics of length, weight and age distribution.
2. To determine the state of the of whiting using the so-called state factor (Ricker, 1975).
3. Characteristics of the reproductive biology of whiting.
4. Collection of data on ports of landing, sampling vessels, number of samples collected, number of specimens tested, geographical catch data.

III. 2 Sampling

The sampling was carried out by the actively operating fishing fleet in Bulgaria.

III. 2.1 Geographic area coverage

Data of present analysis were collected from Bulgarian Black Sea coast. In **I-VI 2024, 2 samples with 159 specimens** were collected and processed. Information on the size of the catches was also collected.

III. 2.2 Sampling period

In 2023, the biological data for the species were collected from a total of **2 landings at the ports of Nesebar and Sozopol**. Information on the size of the catch was also collected. Ports and ships from which monitoring was carried out to collect biological data from landings are presented in Table III.2.2.1.

Table III.2.2.1 Ports and ships from which monitoring was carried out to collect biological data of whiting landings.

№	Date	Sampling ports	WHG	Fishing vessel	External marking	Fishing gear	Catch/kg	Coordinates
1	20.2.2024	Nesebar	WHG	BUREVESTNIK	BS 032	OTM	22	42.6846847534 27.9002037048
2	28.5.2024	Sozopol	WHG	Sveti Nikola	BS 265	OTM	98	42.3398 27.9[illegible]1

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III. 2.3 Statistical analysis of data

See section statistical analysis of sprat.

III.3 Results

III.3.1 Landings statistics

The highest catches were realized in the month of February 2024 (Figure III.3.1.1).

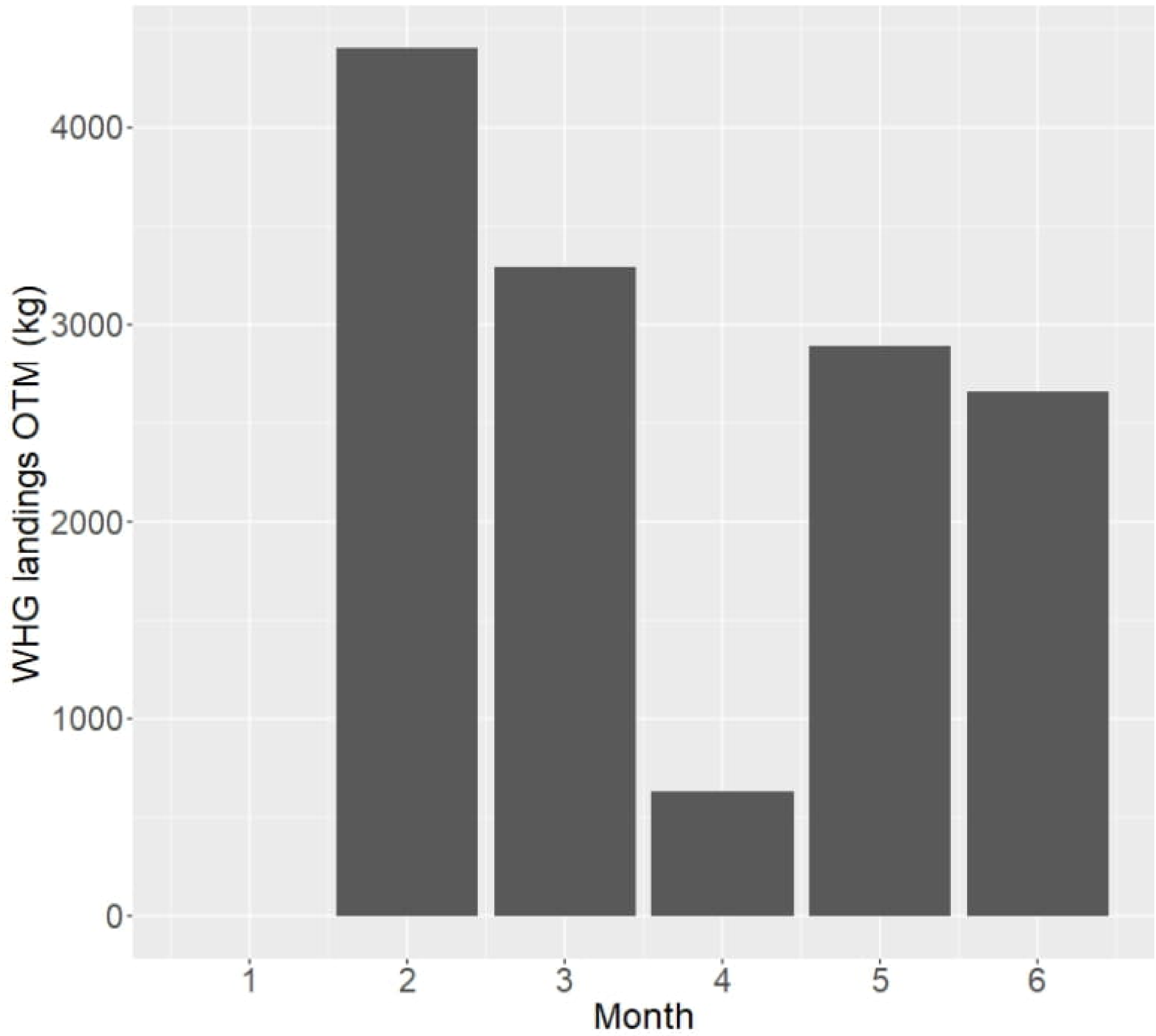

Figure III.3.1.1 Landings statistics of whiting.

III.3.2 Length structure of landings

In the first and second quarters of 2024 the length distribution follows a normal distribution with predominant classes 10.0 and 13.0 cm (I-III) and 12.5 – 14.5 cm (IV-VI).
For the first quarter, the classes in the range of 14.5 to 17 cm have the lowest share in the landings - under 3%. The size groups of 13.5 cm and 10 cm occupy a relatively higher percentage share in the landings - respectively 13.33 % and 12.22 %. The size class 11.5 cm is also represented with a high percentage of 11.11%. For the second quarter, the size classes with

the lowest percentage are 13.5 cm (4.35%), 11.5 cm (5.79%) and 13 cm (7.25%). In the second quarter, the size class 14 cm predominates, followed by the size class 12.5 cm, and 10.5 cm and 15 cm participating with 17.39%, 13.04% and 11.59%. The size classes 12 and 17 cm participate equally in the catches with 10.14%. In the second quarter, the size class 10.5 cm also performed well with 11.59% (Figure III.3.2.1).

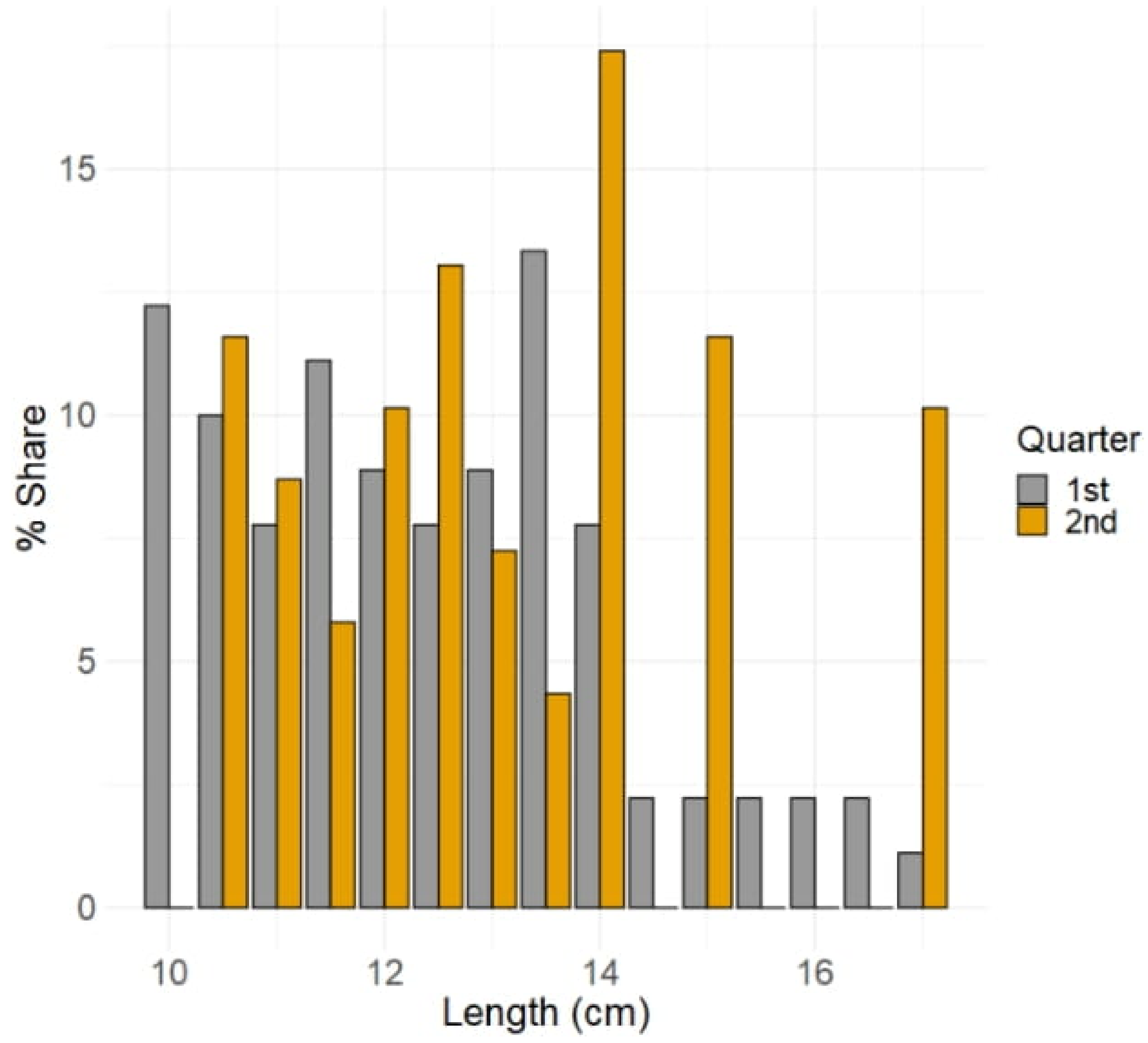

Figure III.3.2.1 Whiting length frequency from landings.

III.3.3 Age structure of landings

The three readers determined the age of whiting otoliths, and reader 1 read all otoliths twice. Specimens **(n = 159)** were used for age determination. The age structure of the whiting is represented by 4 age classes- 1,2,3 and 4 years old (Figures III.3.3.1).

In the first quarter, the highest percentage share – 45.55% is occupied by individuals in the age group 1-1+, followed by 2-2+ (41.11%). With the lowest share – about 3.33% are the age groups 4-4+. The second quarter shows that the age groups 1-1+ and 2-2+ have the highest share – 30.43% and 44.93% and significantly prevail over the others. Below 3% are the age group 4-4+ (2.89%). In the second quarter of 2024, 3-3+ year-old individuals reach 21.74%

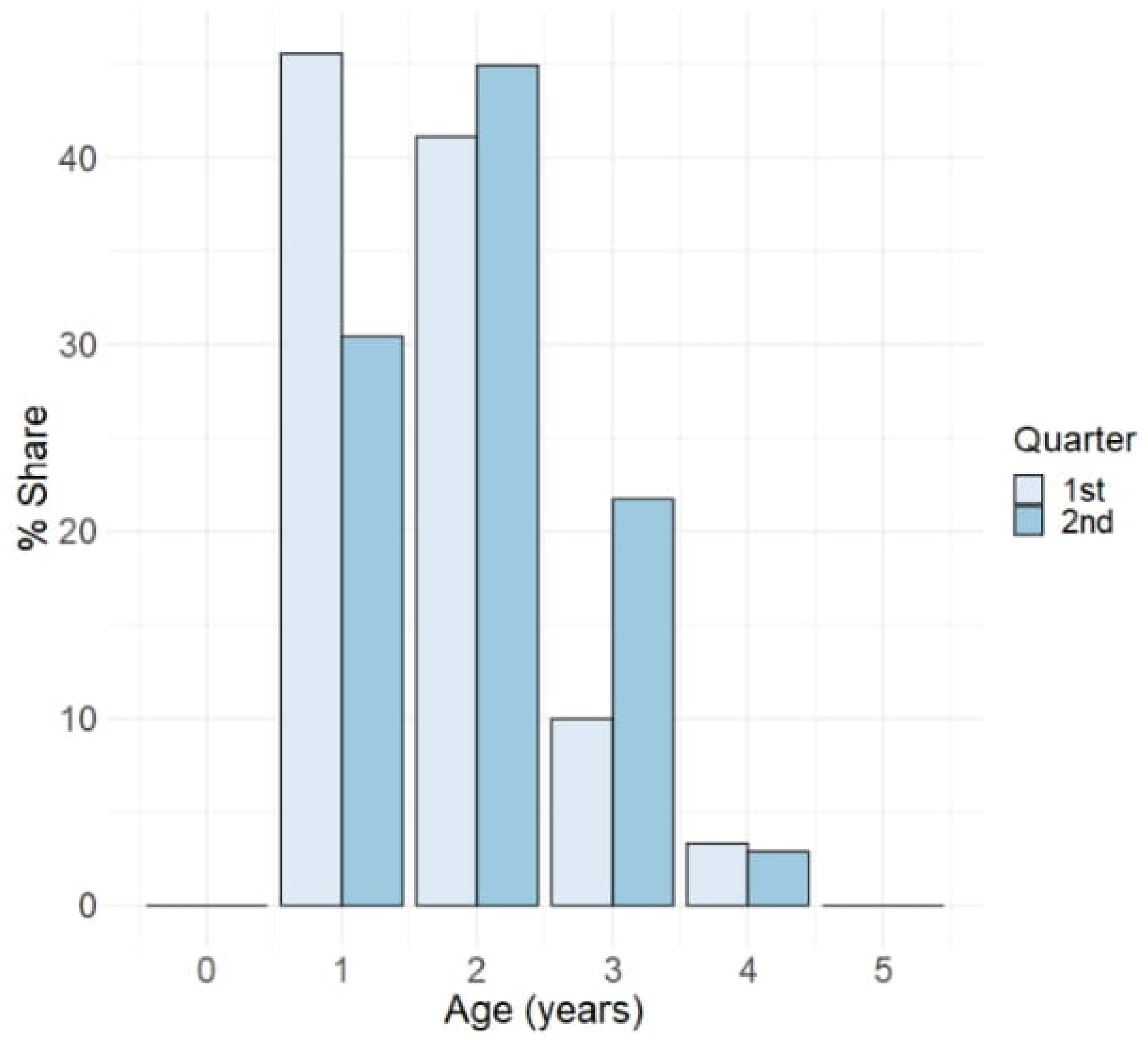

Figure III.3.3.1 Age distribution of whiting.

Results of analytical age slicing methods – similar to the analysis of the age structure of the sprat, the analyzes were made in the R programming environment (Program Language for Statistical Modeling and Analysis) using the ELEFAN functions (ELectronic LEngth Frequency Analysis). and the growth and other biological parameters of the studied species were evaluated based on the measured lengths of individuals of different age groups. The samples are restructured for the purposes of the analysis in order to track the evolution of the cohorts over time (Fig. III.3.3.2 - the first frequency diagram shows the size structure of the collected specimens in the corresponding months, and the second - the restructured data with a moving average MA = 7).

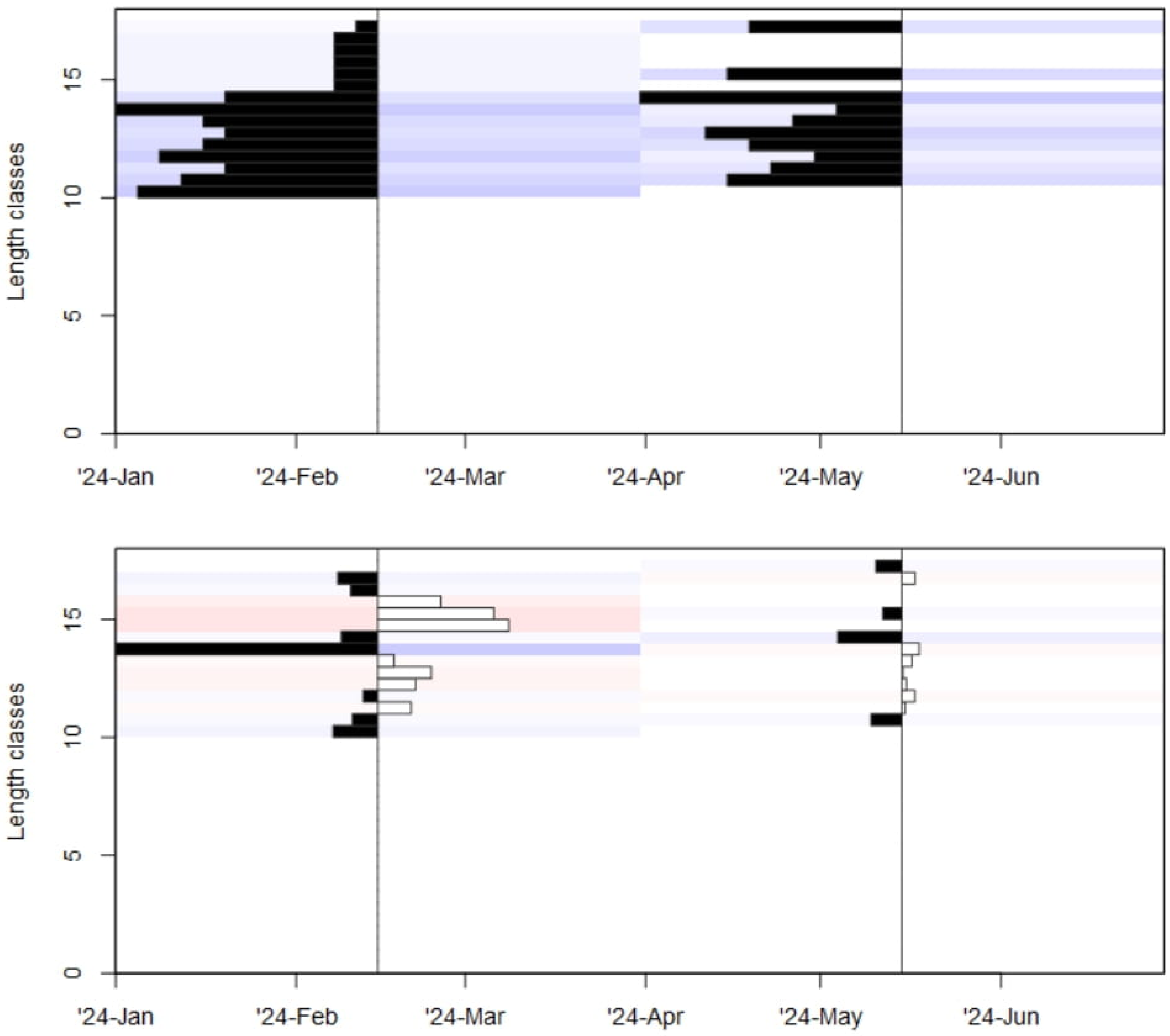

Fig. III.3.3.2 Size-frequency samples visualized as (diagram above) number of individuals in a size class and (diagram below) restructured data with a moving average MA = 7 for the purposes of frequency analysis and determination of growth parameters.

III.1.3.3.1 Frequency analysis RSA – response surface analysis

Growth parameters determined by RSA with initial conditions: conditional interval of for the asymptotic length L_∞ in the sample [17;22 cm] (based on a literature review of the characteristics and biological potentials of the species for the Black Sea) and the parameter that determines the growth rate to L_∞ - $K = \exp\ (seq(from\ log(0.1), to\ \log(1)$with an upper limit for L_∞ = 22 cm. The parameters in the von Bertalanffy model are calculated as follows: L_∞=19.68 cm; K = 0.23 and t_0=-0.11. The ages with their corresponding lengths were calculated from the latter, as the calculated values do not correspond to the characteristics of the species and the experimentally determined ages, probably due to an incorrectly identified coefficient of the growth rate to the asymptotic length, and for this reason they were not taken into account in the analysis of the growth parameters.

III.1.3.3.2 Frequency analysis ELEFAN with simulated annealing (ELEFAN SA)

The growth parameters determined under initial conditions: a conditional interval for the asymptotic length 〖L_∞〗 $_{guess}$=18.68 cm and a search for the value of L_∞ in the interval [$L_{(\infty guess)}$*0.8 ÷$L_{(\infty guess)}$*1.2], and for K in the interval [0÷1]. The parameters in the von Bertalanffy model were calculated as follows: L_∞=22.36 cm; K = 0.4 and t_0=-0.25. The heights with their corresponding lengths were calculated using the latter (Table III.1.3.3.3.1, Figure III.1.3.3.3.1).

III.1.3.3.3 Frequency analysis ELEFAN – Genetic algorithm (ELEFAN GA)

The growth parameters determined under initial conditions: a conditional interval of for the asymptotic length 〖L_∞〗 $_{guess}$=18.68 cm and a search for the value of L_∞ in the interval [$L_{(\infty guess)}$*0.8 ÷$L_{(\infty guess)}$*1.2], and for K in the interval [0÷1]. The parameters in the von Bertalanffy model were calculated as follows: L_∞=18.65 cm; K = 0.5 and t_0=-0.515. The ages with their corresponding lengths were calculated using the latter (Table III.1.3.3.3.1, Figure III.1.3.3.3.2).

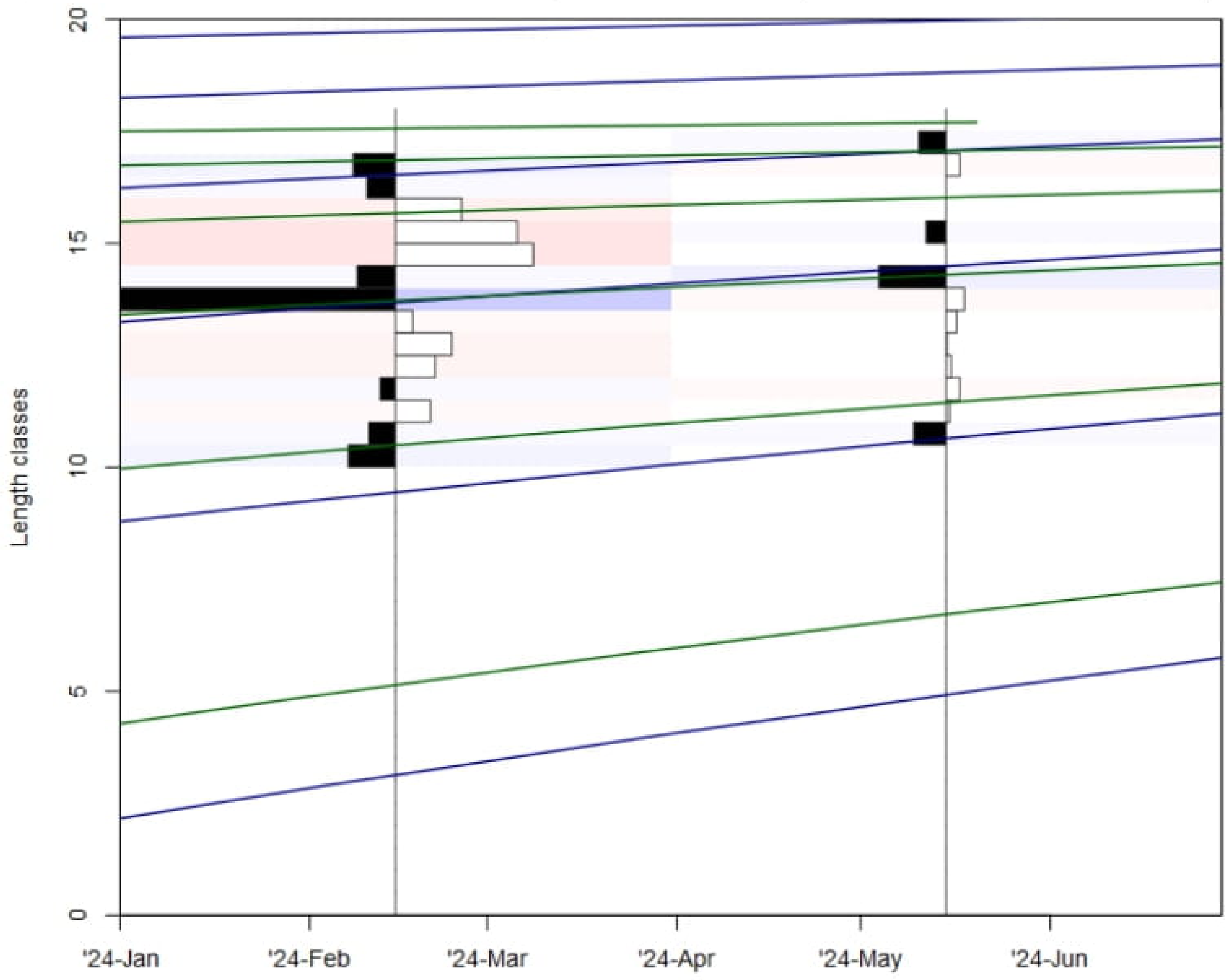

Figure III.1.3.3.3.1 Growth curves (in blue according to ELEFAN SA, and in green according to ELEFAN GA), visualized on the restructured data for the purpose of visualizing the tracking of cohorts over time.

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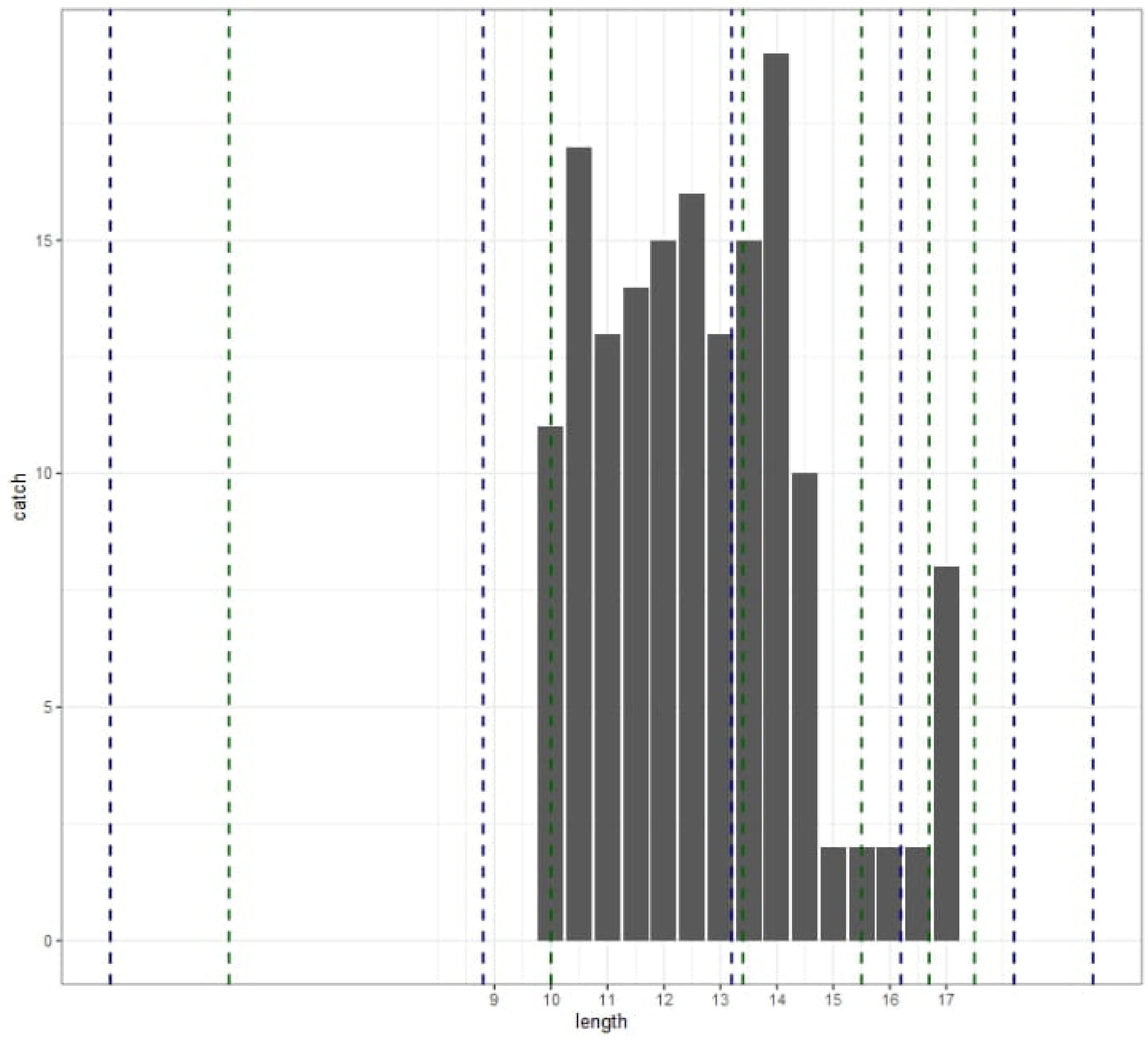

Figure III.1.3.3.3.2 The ages represented with dashed lines, calculated based on the growth parameters determined by ELEFAN SA (in blue) and ELEFAN GA (in green), are overlaid on the cumulative sample of the composition of commercial catches of whiting, aiming to visualize the range of size classes represented in a specific age group (0-5 years) for the first half of 2024.

Table III.1.3.3.3.1 Ages with their corresponding lengths for the studied species calculated using von Bertalanffy parameters, obtained with RSA, ELEFAN with simulated annealing, ELEFAN with genetic algorithm and compared with experimentally determined ages from otoliths.

	ELEFAN SA	ELEFAN GA	Experimentally determined
age	La	La	L_{mean}
0	2.2	4.3	n/a
0.5	5.8	7.5	
1	8.8	10.0	11
1.5	11.2	11.9	
2	13.2	13.4	13.25
2.5	14.9	14.6	
3	16.2	15.5	15.5
3.5	17.3	16.2	
4	18.2	16.7	16.5
4.5	19.0	17.2	

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5	19.6	17.5	n/a

The result from ELEFAN GA is closest to the experimentally determined ages and, accordingly, the biological potentials of horse mackerel for the first half of 2024 are best described by the following analytically determined parameters: L_∞=18.65 cm; K = 0.5 and t_0=-0.515.

III.3.4 Condition factor

Fulton's condition factor showed the highest values for the 10.5 cm size class and for 4-year-old specimens (Fig.III.3.4.1-2).

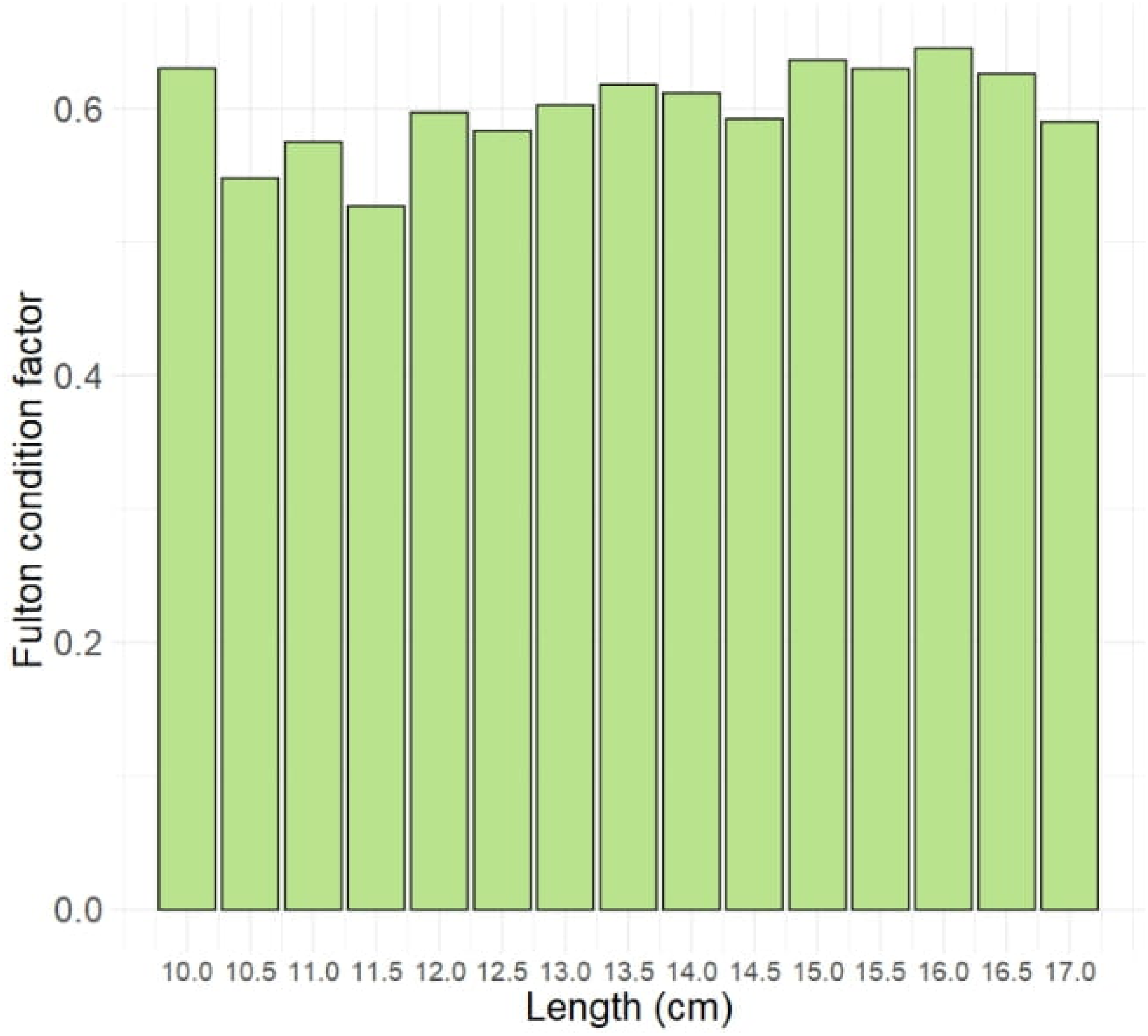

Figure III.3.4.1 Condition factor by size classes of whiting for the first half of 2024.

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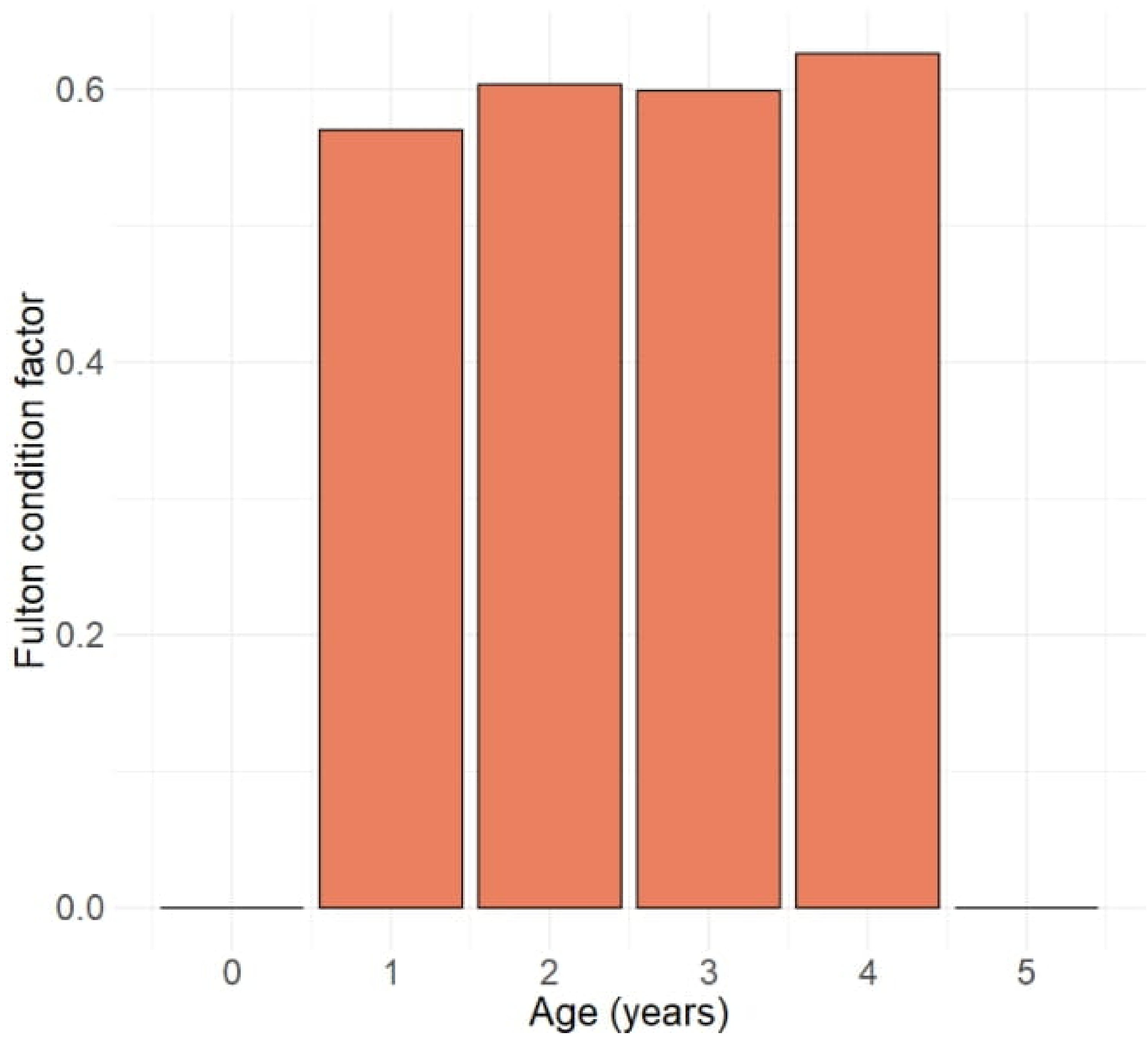

Figure III.3.4.2 Condition factor by age group of whiting for the first half of 2024.

III.3.5 Weight structure of whiting

The weight was measured on 159 specimens. In terms of weight, the largest age groups 3-3 + and 4-4 + year-old individuals have the largest contribution (highest average weights on average) to the catch (Fig. III.3.5.1). The graph shows the distribution of the average weight of whiting by age groups for two quarters. A gradual increase in the average weight is observed in relation to the age groups for both quarters. For age group 1-1+ the lowest average weight is -7.59g (63 number of whiting), and for group 4-4+ the highest average weight is observed - 28.13g (4 number of whiting). For the remaining age groups the weights are as follows: 2-2+- 14.04g (71 number); 3-3+-22.31g (21 number).

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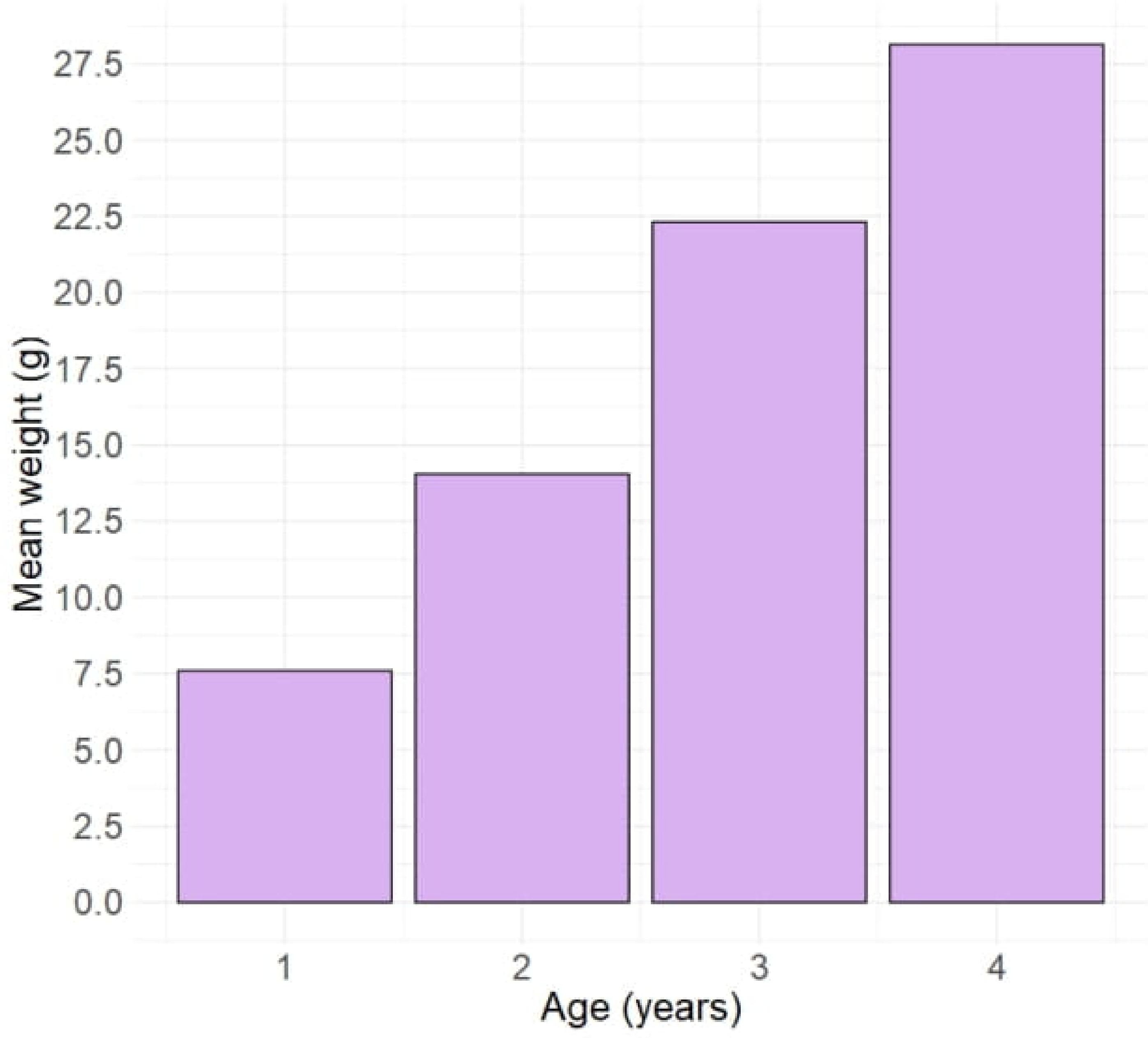

Fig. III. 3.5.1 Mean weight by age group.

The most common size group -14cm represents 11.95% and has a measured average weight of 16.78g. The largest average weight is measured at 17cm-28.99g (8 number).

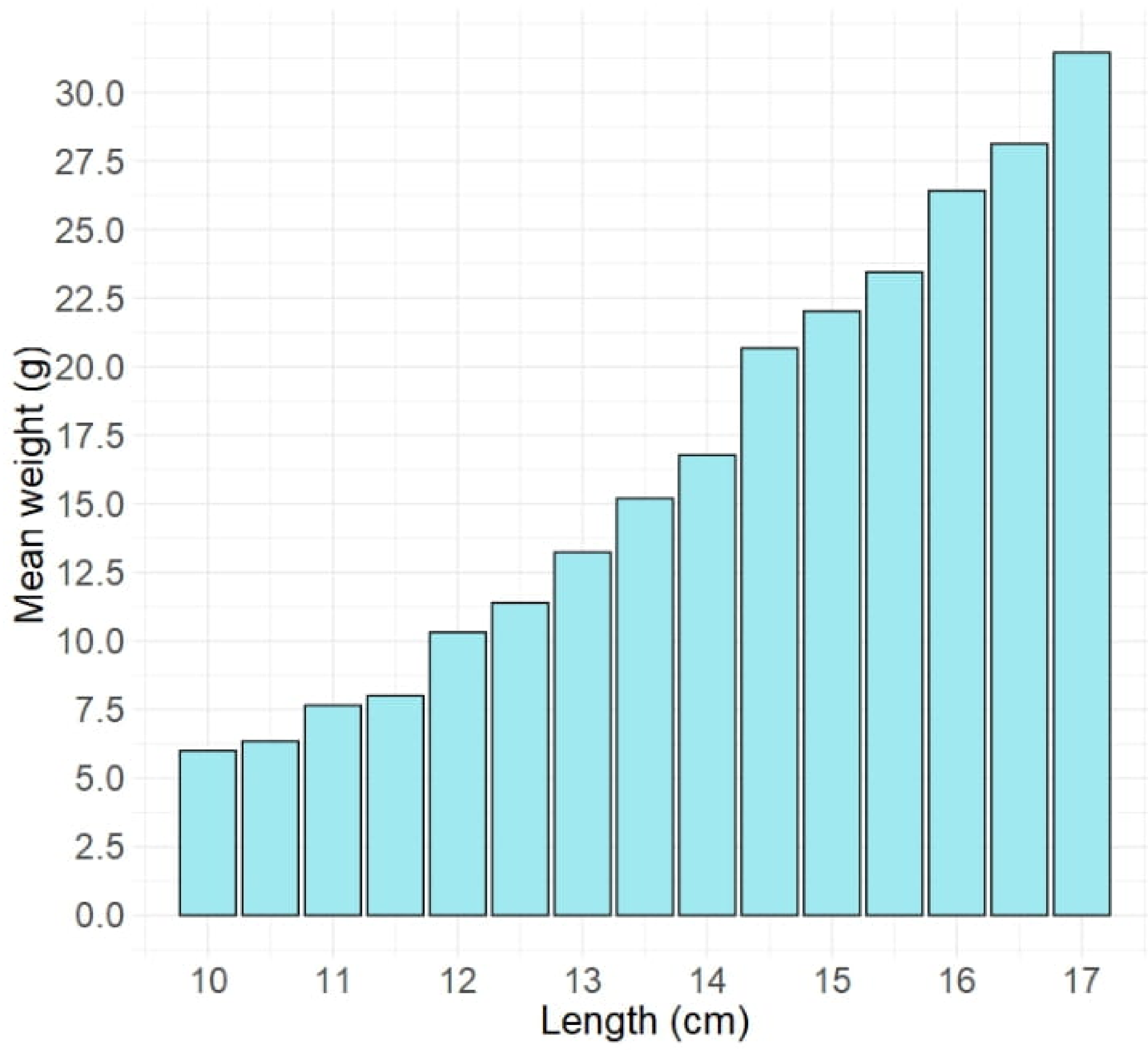

Fig. III. 3.5.2 Mean weight by size classes.

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III.3.6 Size structure of whiting by age group

На **159 екземпляра** е измерена дължината на рибите. The length of the fish was measured on 159 specimens. No large fluctuations were registered in the considered period of 2024 in relation to the average lengths by age for I-VI 2024 (Table IV.3.6.1).

Table III.3.6.1 Mean size of the whiting by age groups.

L_{mean}/cm	Age
10,95	1-1+
13,22	2-2+
15,33	3-3+
16,63	4-4+

III.3.7 Length- weight relationship

The resulting model (Table III.3.7.1.) is statistically significant, the value of the scaling coefficient a in the length-weight relationship model $W_i = aL_i^b$ e: $a = 0.003$, and of the allometry coefficient b=3.17, which indicates a positive allometric growth of the species in the first half of the year, or the increase in weight is proportional to or greater than the increase in length.

Table III.3.7.1 Results of modeling the length L-weight W relationship.

```
Call:
Lm (formula = logW ~ logL, data = weight_l)

Residuals:
     Min        1Q    Median        3Q       Max
-0.104004 -0.022177  0.008837  0.024567  0.099281

Coefficients:
            Estimate Std. Error t value          Pr(>|t|)
(Intercept) -5.57007    0.20517  -27.15 0.0000000000007807 ***
logL         3.17527    0.07907   40.16 0.0000000000000051 ***

Signif. Codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.04991 on 13 degrees of freedom
Multiple R-squared:  0.992,      Adjusted R-squared:  0.9914
F-statistic:  1613 on 1 and 13 DF,  p-value: 0.000000000000005097
```


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III.3.8 Sex ratio

On **50 specimens**, the sex ratio was determined in I-VI 2024. Females ♀ predominate over males ♂. The average lengths of females♀ by age group are higher (Figure III3.8.2).

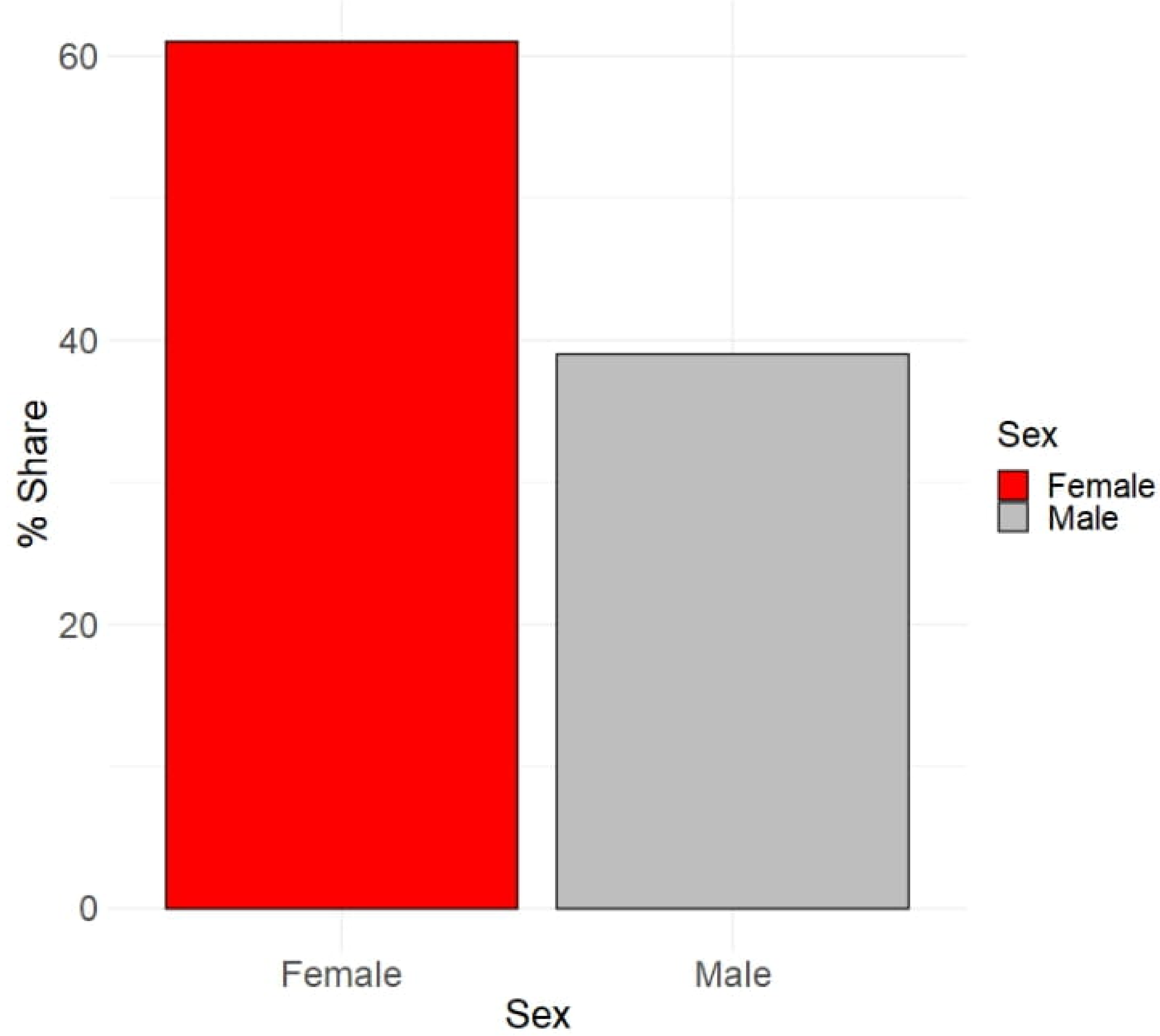

Figure III.3.8.1 Percentage ratio between the sexes of whiting.
The average lengths of females♀ by age group are higher (Figure 3.8.2).

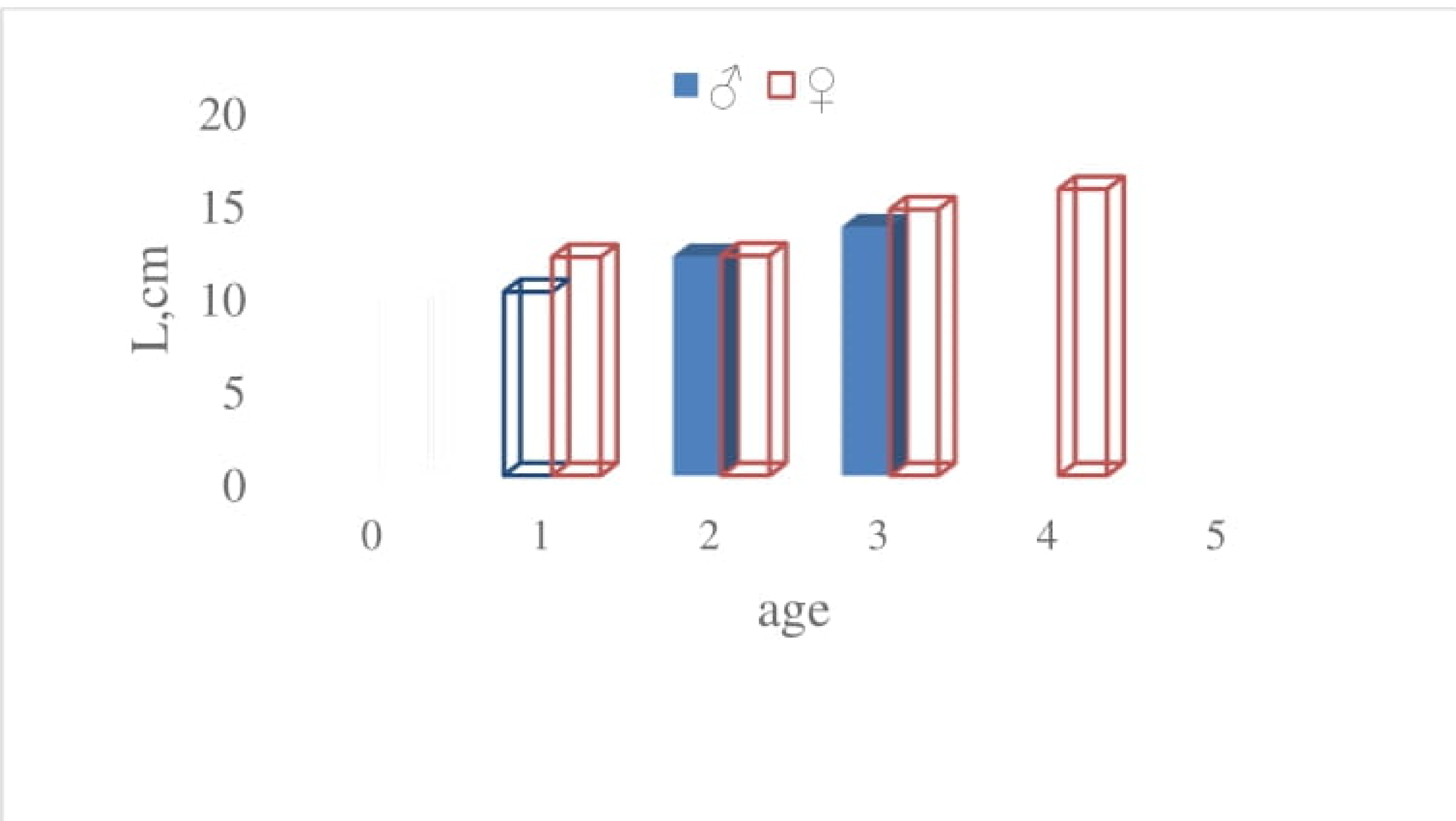

Figure III. 3.8.2 Sex ratio (♂♀) by size and age of whiting.

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III.3.9 Fertility

Fertility was determined on **50 specimens**. Gonado somatic index is highly dependent on the on the sexual glands weights (R^2=0.8145), which is correlated with the high maturation of females in the late spring and summer spawning processes of whiting (Figure III.3.9.1).

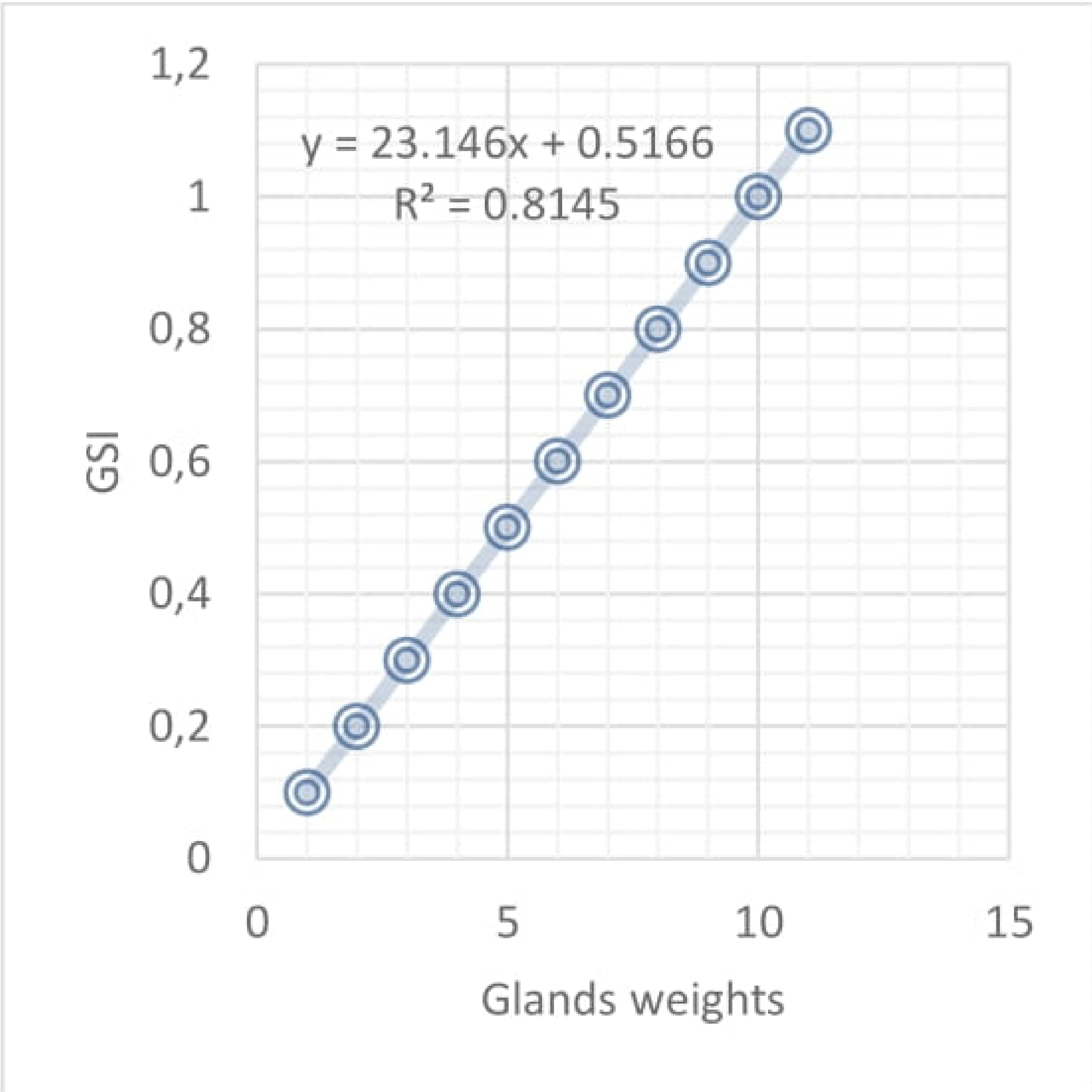

Figure III.3.9.1 Glandule weight (g) vs.GSI for whiting.

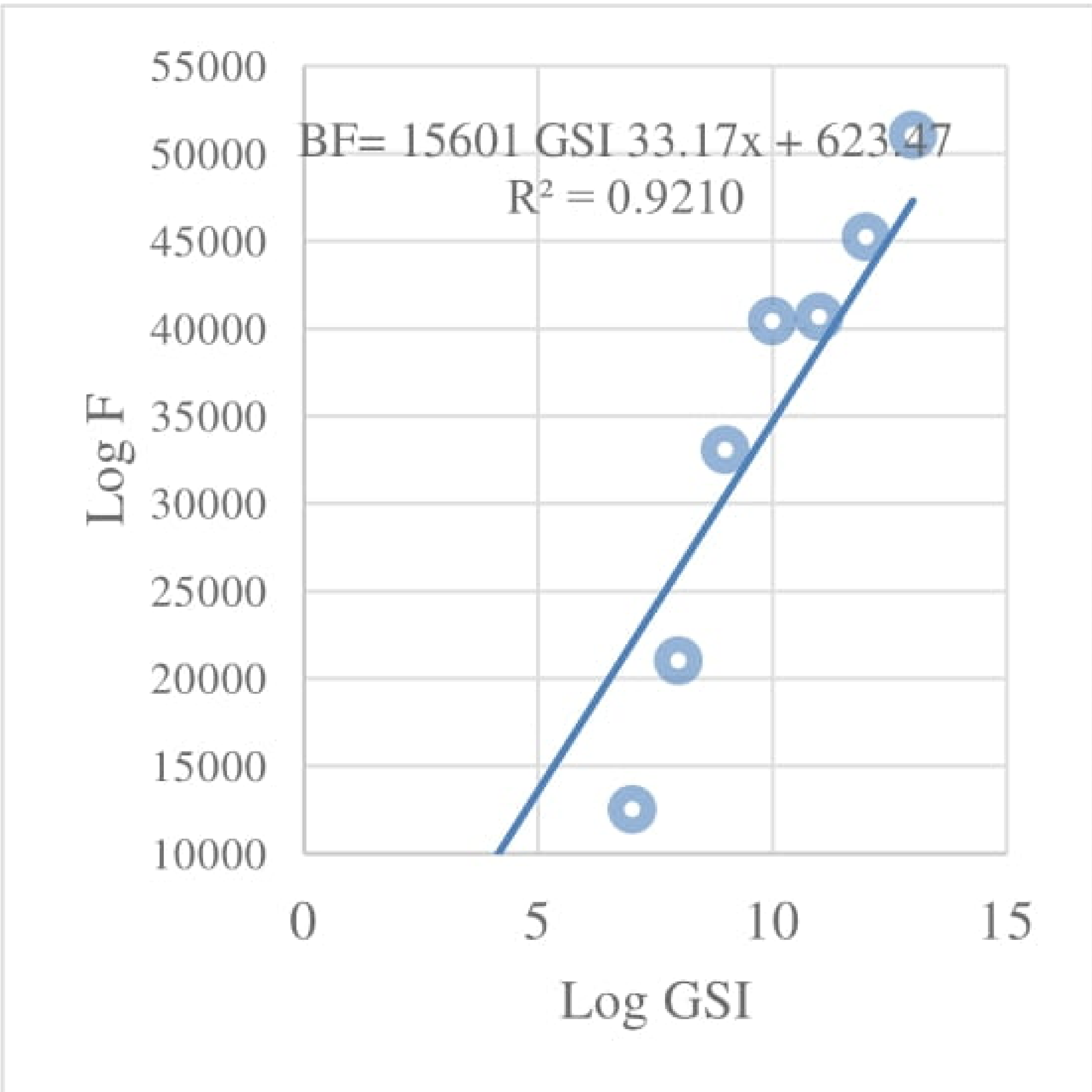

Figure III.3.9.2 LOG relationship between portion fecundity and GSI of whiting.

Absolute fertility increases with increasing length, weight and age. The weighted average relative fertility is 1680,206 (Table III3.9.1).

Table III.3.9.1 Absolute and relative fertility.

Size class	Average body weight (W, g)	Absolute fertility F, caviar grains)	Relative fertility	Number (n) ♀
10	6, 47	8400	1298,3	5
10,5	7, 12	8512	1195,506	5
11	8,057	9835	1220,678	4
11,5	10,18	11706	1149,902	5
12	11, 38	14800	1300,527	5
12,5	13, 23	28120	2125,472	6
13	15,27	36035	2359,856	5
13,5	16, 36	44200	2701,711	5
14	18,12	56834	3136,534	5
14,5	21,44	6723	313,5728	5
		22516,5	1680,206	50

III.3.10 Sexual maturity

Sexual maturity was determined on **50 specimens**. During this period it is actively used in sexual products, with 100% of the degree of maturity being IV-VI.

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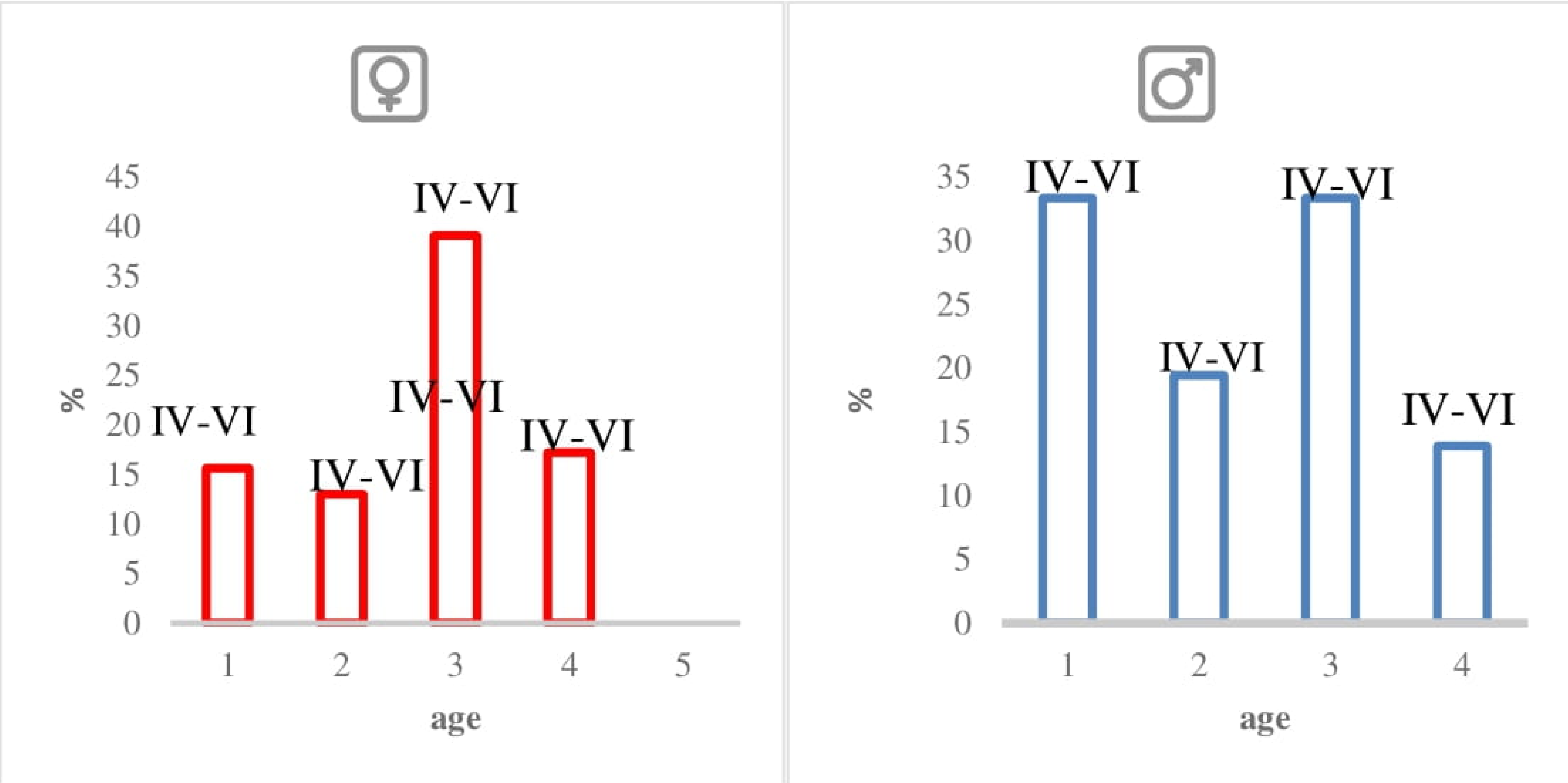

Figure III.3.10.1 Sexual maturity by age of whiting - female♀ and male ♂.

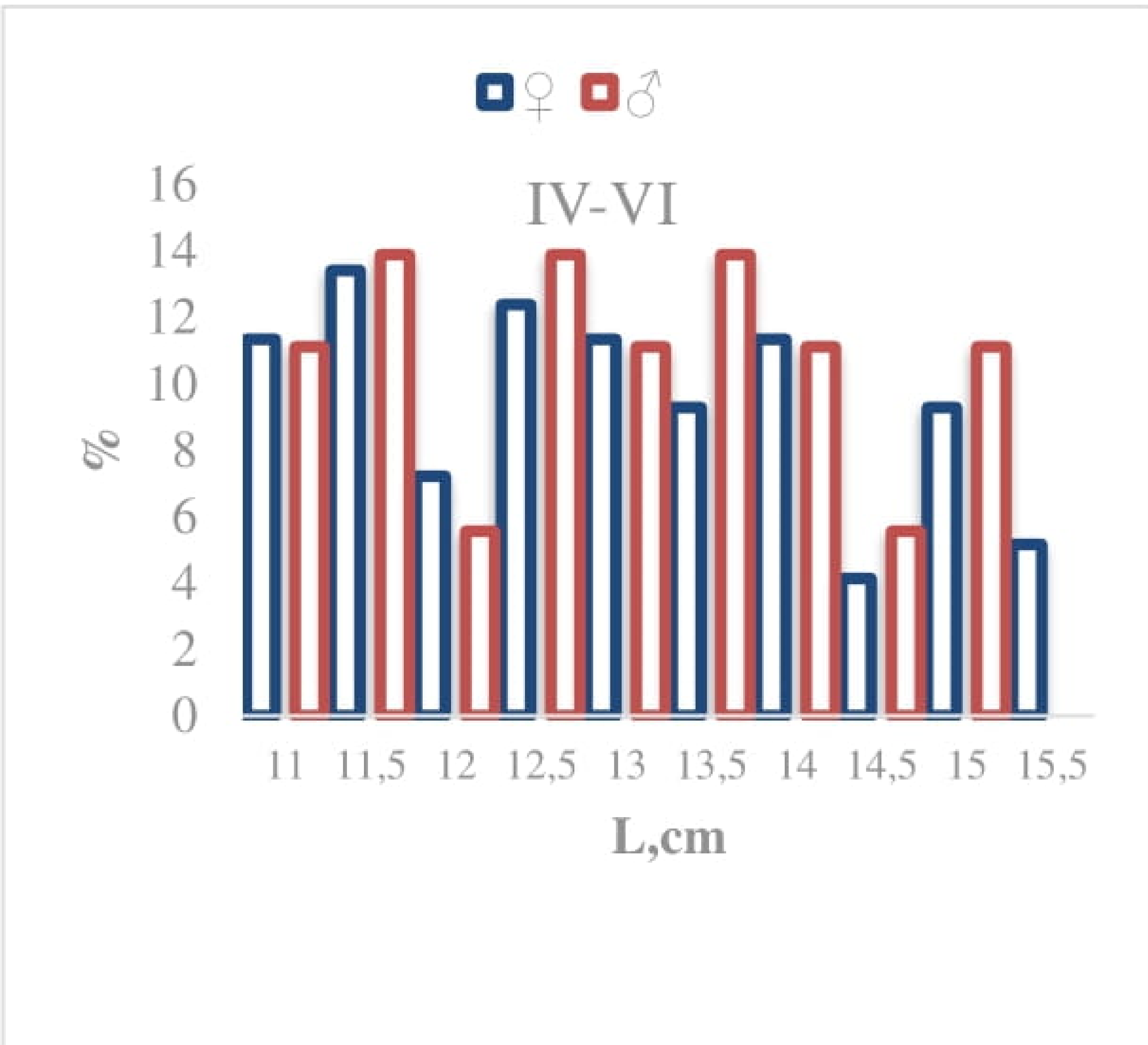

Figure III.3.10.2 Sexual maturity along the length (cm) of whiting - female ♀ and male ♂.

III.3.11 Catch numbers and biomass by age and length

The monthly catch with OTM (in tons) together with the average weights of whiting were used to derive the monthly catch numbers. The proportion (%) by age group and catch numbers are used to create a ***catch-at-age matrix*** (Table III.3.11.1).

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Table III.3.11.1 Catch at age (10^{-6}) matrix and biomass (kg) of whiting with OTM.

*Catch-at-Age *10⁻³ (in thousands)*		
Age groups	*I^th^ quarter*	*II^nd^ quarter*
1-1+	0,23582	0,189281
2-2+	0,265766	0,213317
3-3+	0,078607	0,063094
4-4+	0,014973	0,012018
∑	**0,595165**	**0,477709**
	Biomass	
Age groups	*I^th^ quarter*	*II^nd^ quarter*
1-1+	1790,222	1436,922
2-2+	3731,89	2995,403
3-3+	1753,697	1407,605
4-4+	421,1916	338,0696
∑	**7697**	**6178**

The monthly catch with OTM (in tons) together with the average weights of the of whiting were used to obtain the monthly catch numbers. Proportion (%) by age group and catch abundance were used to create a ***catch-by-length matrix*** (Table III.3.11.2).

Table III.3.11.2 Catch at length (10^{-6}) matrix and biomass (kg) of whiting with OTM.

*Catch-at-Age *10⁻³ (in thousands)*		
Length group (cm)	*I^th^ quarter*	*II^nd^ quarter*
10	0,041175	0,033049
10,5	0,063634	0,051076
11	0,048661	0,039058
11,5	0,052404	0,042062
12	0,056148	0,045067
12,5	0,059891	0,048071
13	0,048661	0,039058
13,5	0,056148	0,045067
14	0,07112	0,057085
14,5	0,037432	0,030045
15	0,007486	0,006009
15,5	0,007486	0,006009
16	0,007486	0,006009

16,5	0,007486	0,006009
17	0,029945	0,024036
∑	**0,595165**	**0,477709**
	Biomass	
Length group (cm)	*I^th^ quarter*	*II^nd^ quarter*
10	259,4021	208,2092
10,5	403,608	323,9561
11	372,4395	298,9387
11,5	419,9095	337,0405
12	579,3314	465,0006
12,5	682,4185	547,7435
13	644,2381	517,0979
13,5	853,4442	685,0173
14	1193,638	958,0741
14,5	675,8305	542,4556
15	160,7245	129,0056
15,5	175,555	140,9093
16	197,7894	158,7558
16,5	210,5162	168,9709
17	868,1549	696,8249
∑	**7697**	**6178**

III.3.12 Conclusions

The analysis of the biological parameters of the whiting makes it possible to draw the following **conclusions**:

1) In the first and second quarters of 2024 the length distribution follows a normal distribution with predominant classes 10.0 and 13.0 cm (I-III) and 12.5 – 14.5 cm (IV-VI). The remaining length classes are represented by lower percentages.

2) The age structure of the whiting is represented by 4 age classes- 1,2,3 and 4 years old.
3) The result from ELEFAN GA is closest to the experimentally determined ages and, accordingly, the biological potentials of horse mackerel for the first half of 2024 are best described by the following analytically determined parameters: L_∞=18.65 cm; K = 0.5 and t_0=-0.515.
4) Fulton's condition factor showed the highest values for the 10.5 cm size class and for 4-year-old specimens.

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5) In terms of weight, the largest age groups 3-3 + and 4-4 + year-old individuals have the largest contribution (highest average weights on average) to the catch.

6) No large fluctuations were registered in the considered period of 2024 in relation to the average lengths by age for I-VI 2024.

7) The resulting model is statistically significant, the value of the scaling coefficient a in the length-weight relationship model $W_i = aL_i^b$ e: $a = 0.003$, and of the allometry coefficient *b*=3.17, which indicates a positive allometric growth of the species in the first half of the year, or the increase in weight is proportional to or greater than the increase in length.

8) Gonado somatic index is highly dependent on the on the sexual glands weights (R^2=0.8145), which is correlated with the high maturation of females in the late spring and summer spawning processes of whiting.

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IV. Biological monitoring of red mullet (*Mullus barbatus*) landings

IV.1 Objectives

The purpose of biological monitoring is to collect data that will be used to analyze red mullet catches, as well as to form a database. The collection of biological samples of red mullet catches in **I-VI, 2024** includes the following tasks:

1. *To collect and analyze the dynamics of length, weight and age distribution.*
2. *To determine the state of the of red mullet using the so-called state factor (Ricker, 1975).*
3. *Characteristics of the reproductive biology of red mullet.*
4. *Collection of data on ports of landing, sampling vessels, number of samples collected, number of specimens tested, geographical catch data.*

IV.2 Sampling

The sampling was carried out by the actively operating fishing fleet in Bulgaria.

IV.2.1 Geographic area coverage

Data of present analysis were collected from landing ports of Bulgarian Black Sea coast. **In I-VI 2024**, **4 samples of red mullet containing 266 specimens** were collected. Information on the size of the catches was also collected.

IV.2.2 Sampling period

In 2024, the biological data on species were collected from a total of **4 landings** at the ports of Tsarevo Varna and Balchik. Information on the size of the catches was also collected. Ports and ships from which monitoring was carried out to collect biological data from landings are presented in Table IV.2.1.1.

Table IV.2.1.1 Ports and vessels from which monitoring was carried out to collect biological data from red mullet landings.

№	Date	Harbor	Species code MUT	Fishing vessel	xternal marking	Fishing gear	Catch	Coordinates
1	19.2.2024	Balchik	MUT	ElIS	BCH5322	OTM	5	43.2879 28.401
2	26.3.2024	Varna	MUT	TESI	VN 7750	OTM	70	43.0079 27.9123
3	23.5.2024	Tsarevo	MUT	CYCLAMA	AH215	OTM	20	42.2513198853 27.9131641388
4	24.6.2024	Varna	MUT	IVA - 1	VN 8194	OTM	72	43.0216 28.0127

IV.2.3 Statistical analysis of data

See section statistical analysis of sprat.

IV.3 Results

IV.3.1 Landings statistics

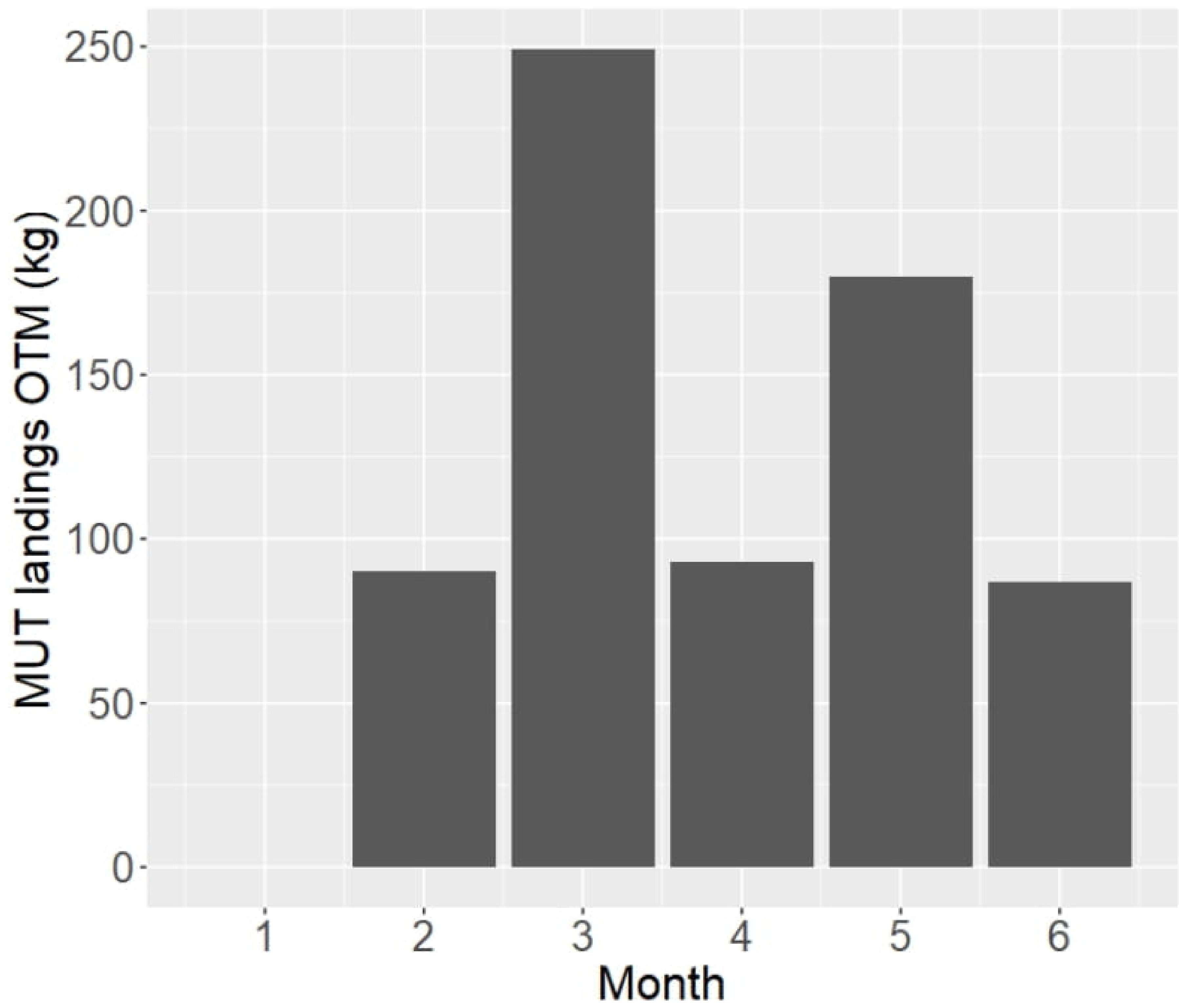

Fig. IV.3.1.1 Official statistics records for red mullet landings by month in the first half of 2024.

IV.3.2 Length structure of landings

The size structure showed a modal distribution with a peak in size group 9.0 representing 15.44%. An increase in the percentage of individuals from the size groups 10 cm and 11 - 11.5 cm representing 13.24% 11.03% and 12.50% is also noted. A sharp decline in the presence is noted by the group 12.5 and 13 cm participating with 2.21 and 2.94%. For the first quarter, the classes with a length of 13.5 cm and 14 cm have a low share in the landings - 4.41% and 5.15%. For the second quarter, the lowest percentage is the size class 12.5 cm and 13.5 cm participating with the same percentage 2.31%. In the second quarter, the size class 10.5 cm predominated, followed by size class 9, participating with 18.46 and 15.38%. The classes in the range 8 - 8.5 cm occupied a relatively higher percentage share in the landings - 6.92 % and 5.38%, respectively.

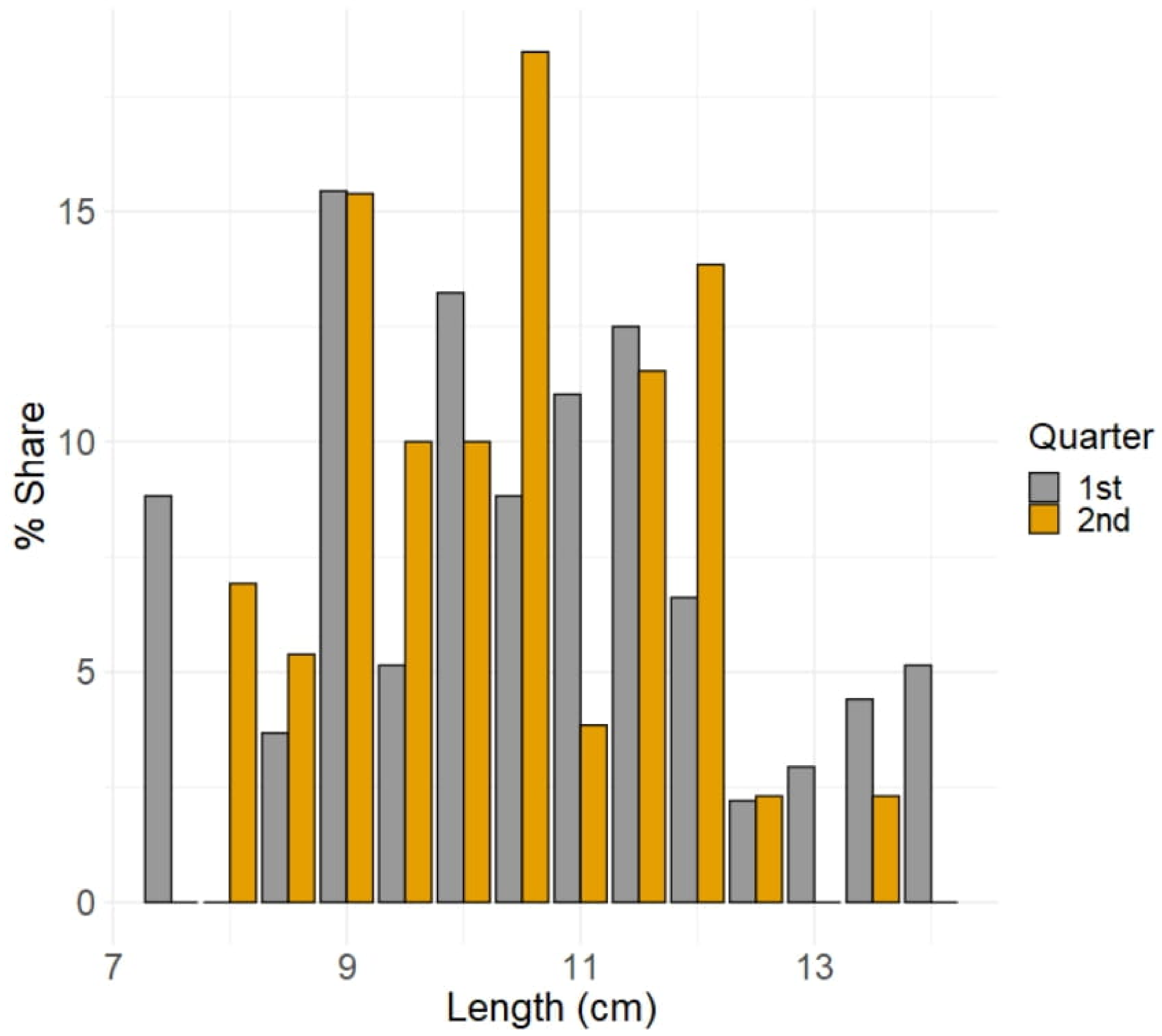

Fig.IV.3.2.1 Size structure and percentage share of length classes in the catch composition.

IV.3.3 Age structure of landings

The three age readers determine the age of the red mullet otoliths, and determinant 1 reads all otoliths twice. Samples (**n = 266**) were used to determine age. During the first six months, the highest percentage share was occupied by individuals in the age group 3-3+, followed by 2-2+. In the first quarter, the highest percentage share – 38.97% was occupied by individuals in the age group 3-3+, followed by 2-2+ with 23.53%. With the lowest share – 10.29% and 11.76% were the age groups: 0-0+ and 4-4+. The second quarter shows that the age groups 3-3+ have the largest share – 40% and significantly prevail over the others. In the second quarter of 2024, a good participation of individuals aged 2-2+ was also registered, with 2-2+ individuals reaching 23.08% (Figure IV.3.3.1).

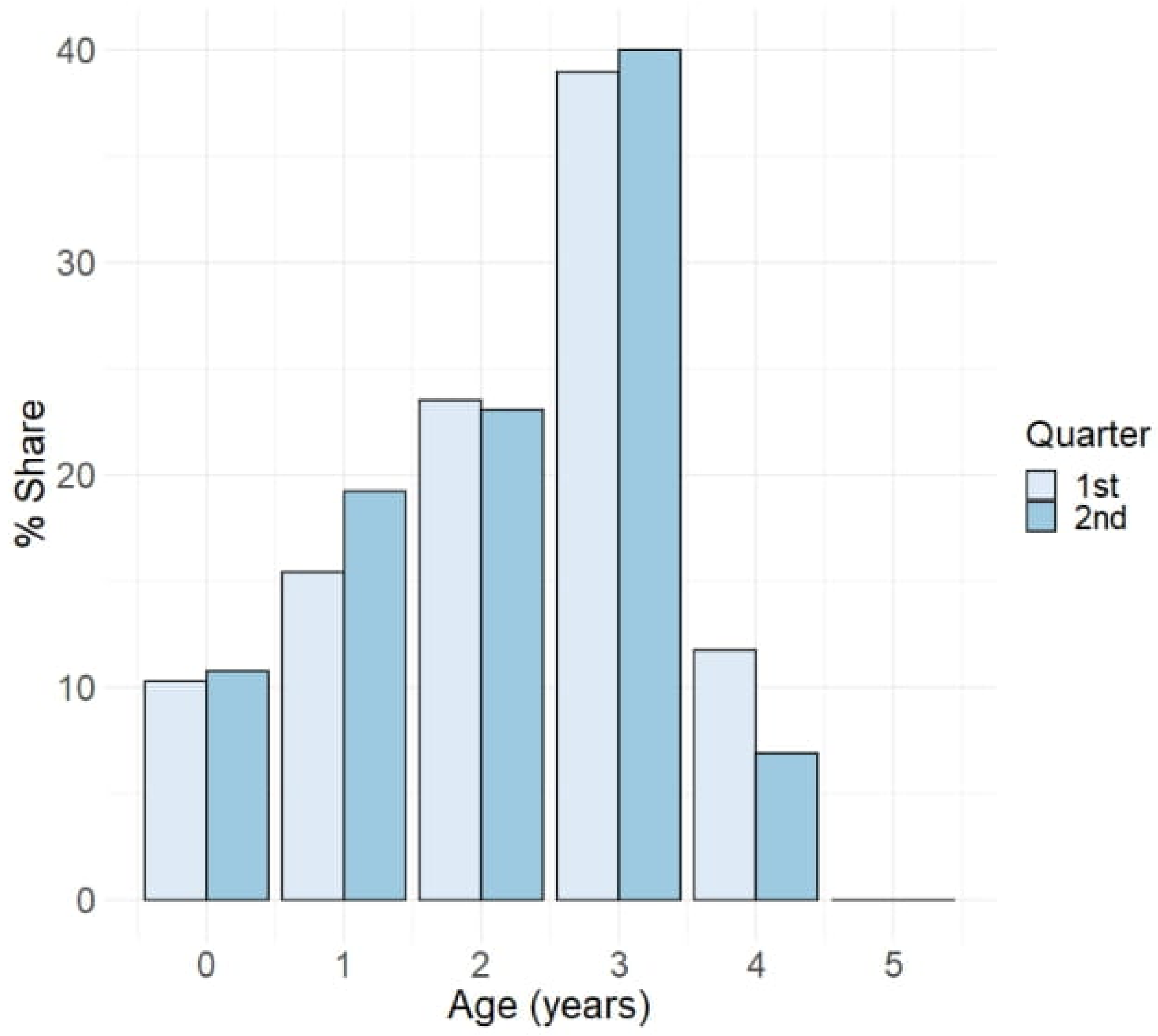

Figure IV.3.3.1. Age structure and percentage share of age groups in the catch composition in the period January-June 2024.

In the analysis (age slicing) of size-frequency samples for determining the growth parameters of the red mullet, only ELEFAN RSA gave relatively good results, the results of the other two methods showed average lengths for 5-year-old specimens of the order of 10 cm, which is not typical for the species and the probable reason is an insufficient number of measurements and poor representation of the size structure in the samples. The latter is expected when determining growth parameters based on samples that do not represent the development of the species on an annual basis.

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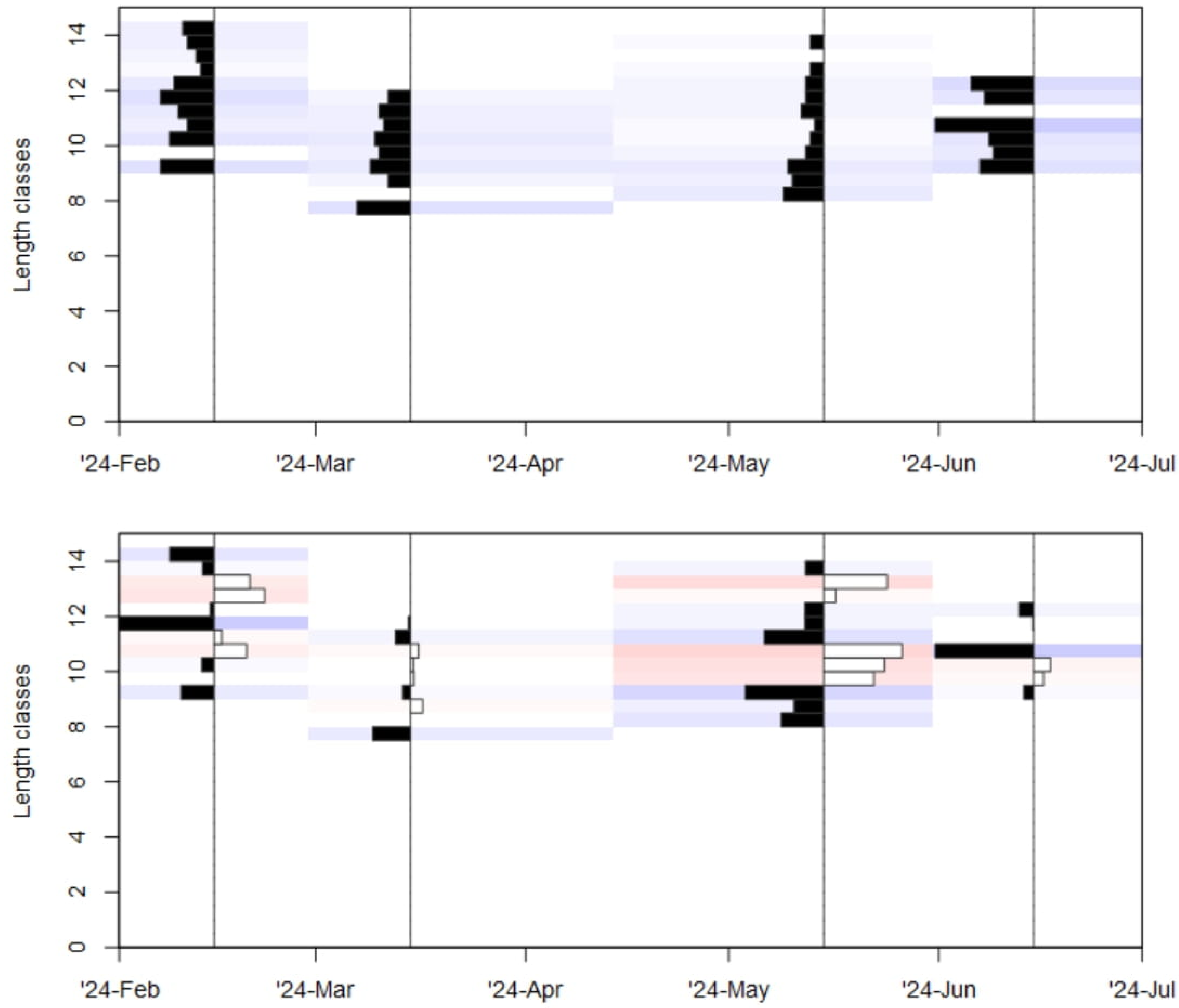

Figure IV.3.3.2 Size-frequency samples, visualized as (top diagram) number of individuals in size class and (bottom diagram) the restructured data with moving average MA = 5 for the purposes of frequency analysis and determination of growth parameters.

IV. 1.3.3.1 Frequency analysis RSA – response surface analysis

The growth parameters determined by RSA with initial conditions: a conditional interval of for the asymptotic length L_∞ in the sample [14;17.5 cm] (based on a literature review of the characteristics and biological potentials of the species for the Black Sea) and the parameter that determines the growth rate to L_∞ - $K = \exp\,(seq(from\ log(0.1), to\ \log(1)$ with an upper limit for L_∞ = 17.5 cm (**Fig. IV. 1.3.3.2**).

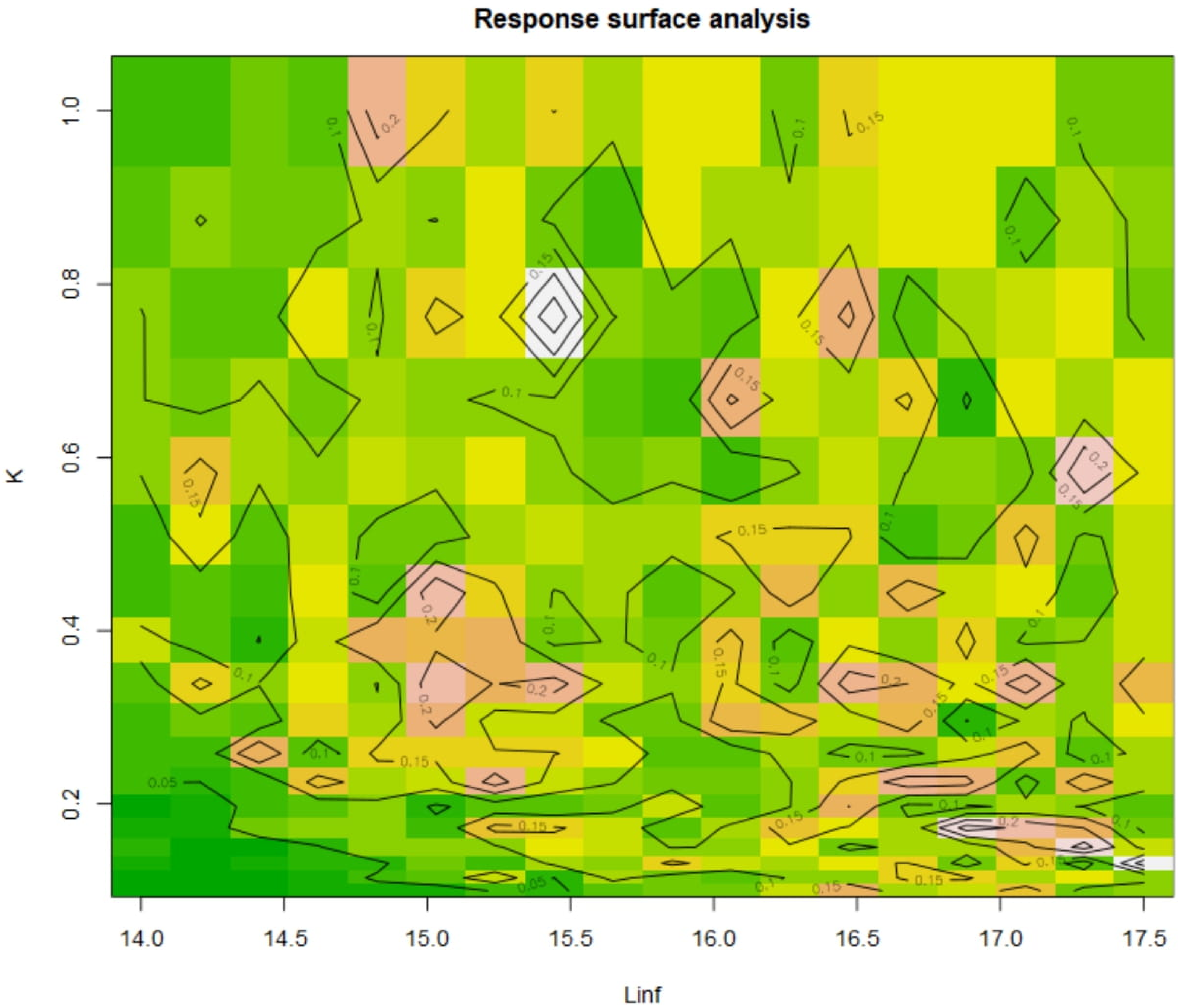

Figure IV. 1.3.3.2 Contour plot visualizing the identified asymptotic length L_∞ and the corresponding value of the parameter K in the defined intervals.

The parameters in the von Bertalanffy model were calculated as follows: L_∞=15.44 cm; K = 0.76 and t_0=-0.67. The ages with their corresponding lengths were calculated using the latter (Table IV. 1.3.3.2, Figure IV. 1.3.3.3.1) and compared with the experimentally determined ones.

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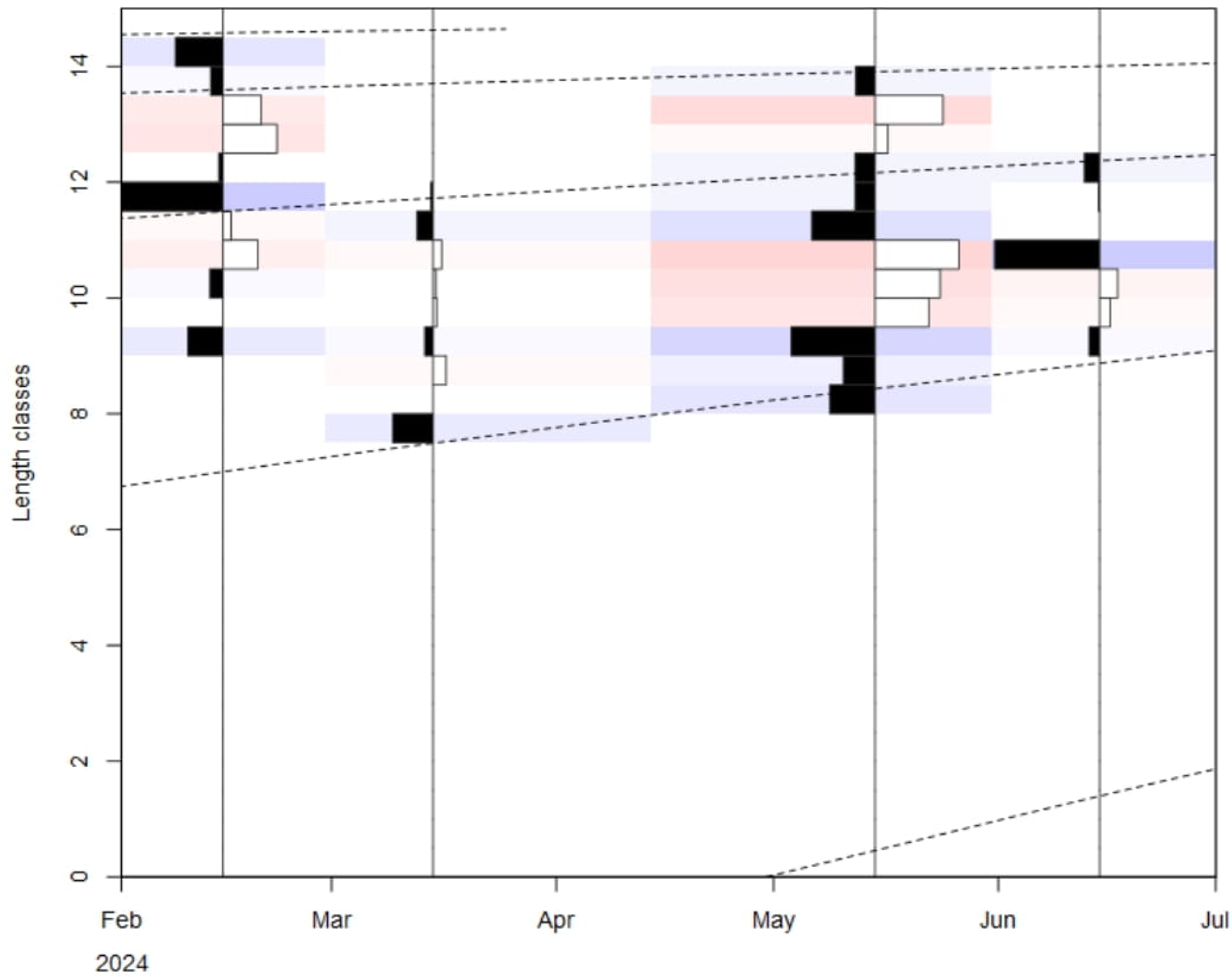

Figure IV. 1.3.3.3.1 Growth curves determined with ELEFAN RSA, visualized on the restructured data for the purpose of visualizing the tracking of cohorts over time.

Table IV. 1.3.3.2 Ages with their corresponding lengths for the studied species calculated using von Bertalanffy parameters obtained with RSA and compared with experimentally determined ages from otoliths.

	ELEFAN RSA	Experimentally determined
age	La	L_{mean}
0	6.2	
0.5	9.1	8.0
1	11.1	9.25
1.5	12.5	
2	13.4	10.5
2.5	14.1	

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3	14.5	11.5
3.5	14.8	
4	15.0	13
4.5	15.1	
5	15.2	-

Given the results obtained, the analysis of growth parameters should be repeated again at the end of the year, when richer biological information about the species will be available.

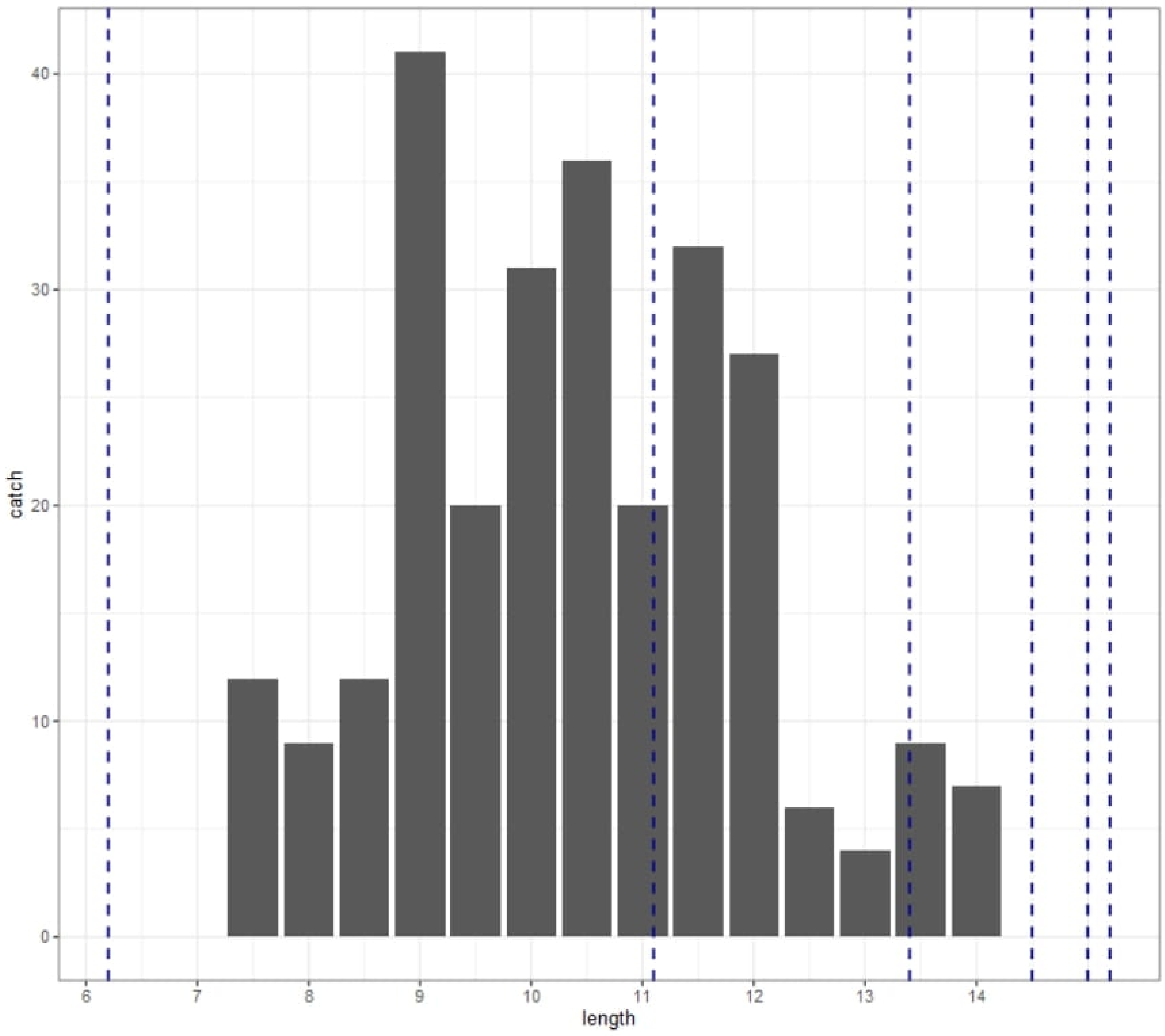

Figure IV. 1.3.3.3.2 The ages represented with dashed lines, calculated based on the growth parameters determined by ELEFAN RSA, are overlaid on the cumulative sample of the composition of commercial catches of red mullet, aiming to visualize the range of size classes represented in a specific age group (0-5 years) for the first half of 2024.

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IV.3.4 Condition factor

The graph (Fig.I.3.4.1) shows that during the first six months K is highest for size classes 13.0-13.5-14.0 cm. The remaining size groups also have good condition, which indicates food security and good physiological condition.

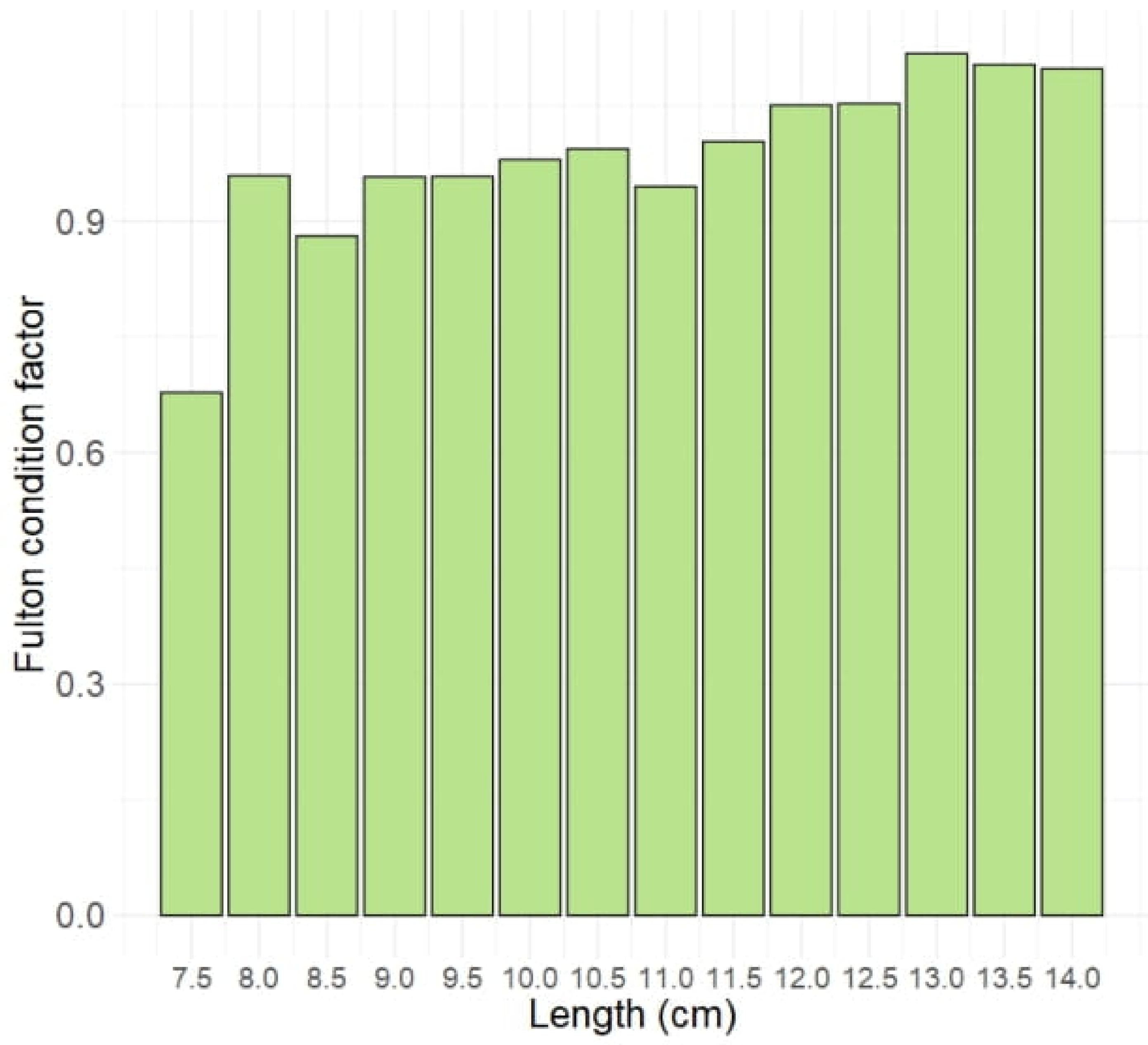

Figure IV.3.4.1 Fulton coefficient of red mullet by length classes.
The condition of the red mullet by age shows a predominance of 4-4+ year olds (Fig. IV.3.4.3).

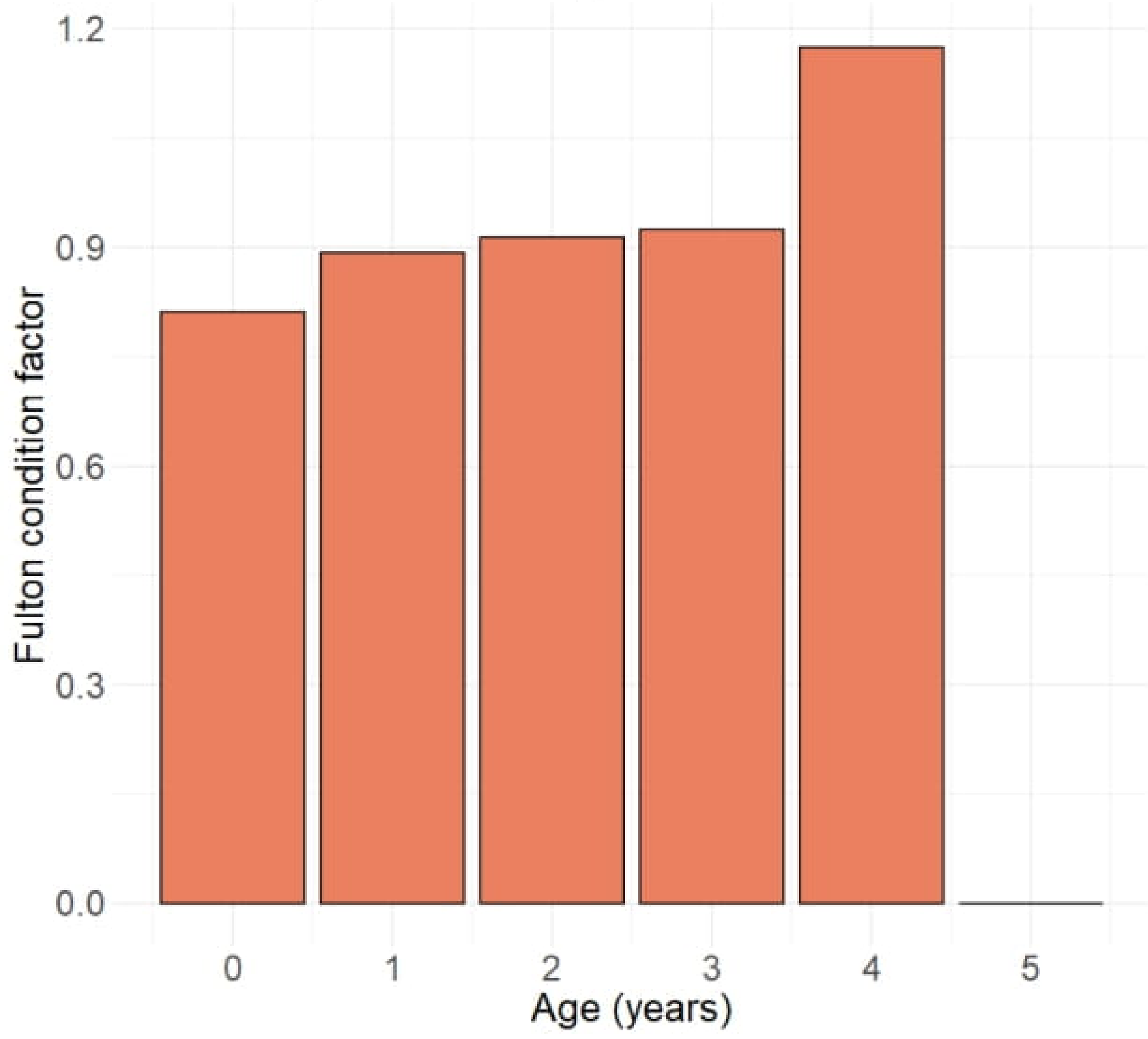

Figure IV.3.4.3 Condition factor of red mullet by age group.

IV.3.5 Weight structure of red mullet

Weight was measured from **266 specimens**. A gradual increase in average weight is observed across age groups. For the age group 0-0+ the lowest average weight is 4.15g (28 number of red mullet), and for the group 4-4+ the highest average weight is observed of 25.79g (25 number). For the other age groups the weights are as follows: 1-1+-7.07g (46 number); 2-2+-10.58g (62 number); 3-3+-14.06g (105 number of red mullet).

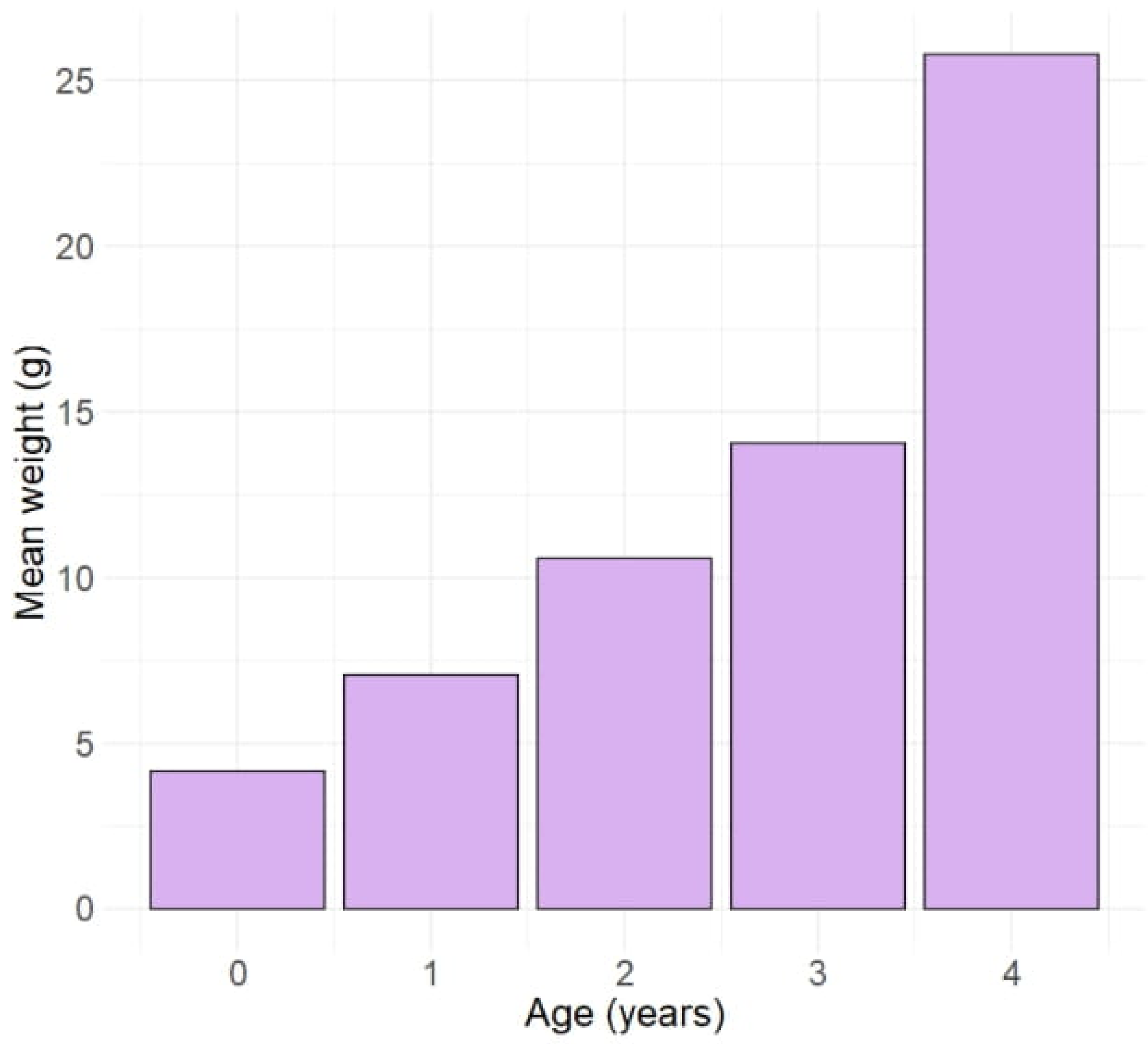

Figure IV.3.5.1 Average weights by age groups.

The most numerous size classes 10-10.5cm participated in the catch with 11.65% and 13.53% and weighed 9.80g and 11.51g.

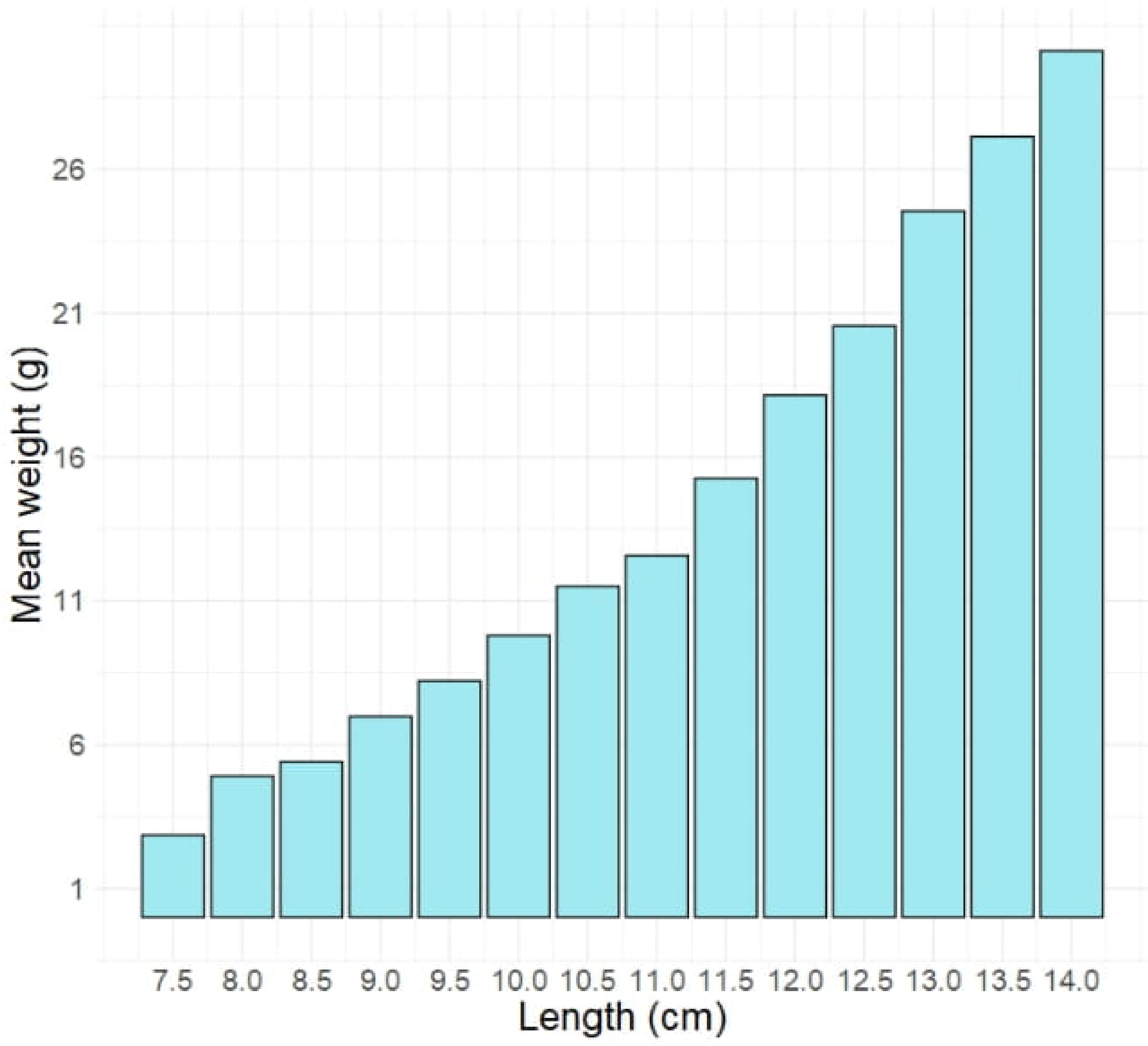

Figure IV.3.5.2 Average weights of red mullet by size classes.

IV.3.6 Weight structure of red mullet by age groups

The length of the fish was measured from **266 specimens**.

Table IV.3.6.1 Size structure by age groups.

L_{mean}/cm	Age
7,91	0-0+
9,04	1-1+
10,18	2-2+
11,13	3-3+
13,28	4-4+

IV.3.7 Length-weight relationship

The resulting model (Table IV.3.7.1) is statistically significant, the value of the scaling coefficient a in the length-weight relationship model $W_i = aL_i^b$ e: $a = 0.002$ and the allometry coefficient b=3.52, which indicates positive allometric growth of the species in the first half of the year, or the increase in weight is proportional to or greater than the increase in length.

Table IV.3.7.1 Results from modeling the length-weight relationship.

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```
Call:
lm(formula = logW ~ logL, data = weight_l)

Residuals:
      Min        1Q    Median        3Q       Max
-0.186878 -0.017504  0.007273  0.029429  0.126176

Coefficients:
            Estimate Std. Error t value          Pr(>|t|)
(Intercept)  -5.8617     0.2377  -24.66 0.000000000011968 ***
logL          3.5234     0.1005   35.05 0.000000000000186 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.07235 on 12 degrees of freedom
Multiple R-squared:  0.9903,	Adjusted R-squared:  0.9895
F-statistic:  1228 on 1 and 12 DF,  p-value: 0.000000000000186
```

IV.3.8 Sex ratio

The sex ratio is set at **150 individuals.** Males ♂ predominate over females♀.

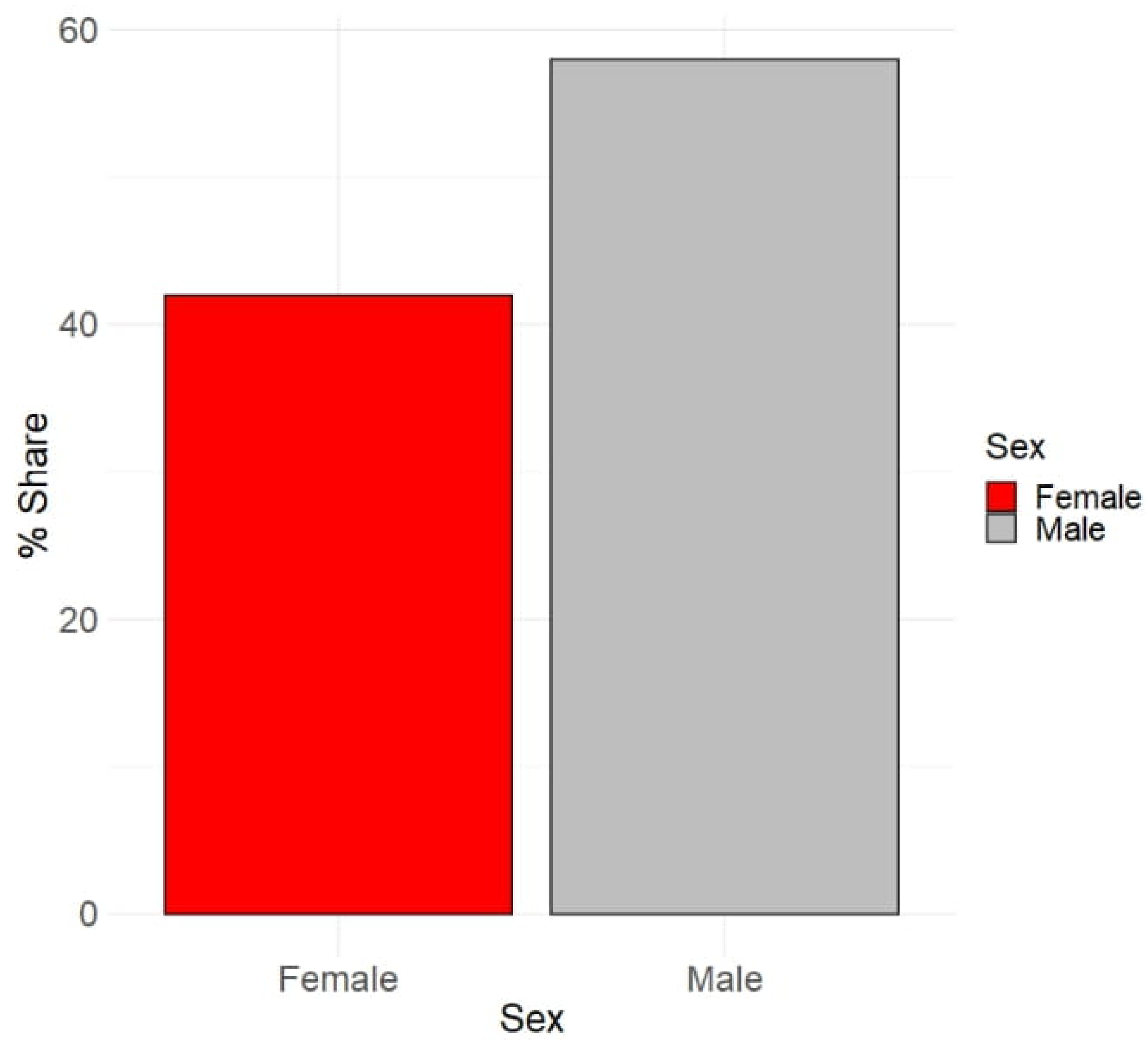

Figure IV.3.8.1 Percentage ratio between genders.

IV. 3.9 Fecundity

50 red mullet fecundity specimens were studied. The production rate during the study period shows a very weak level of determinism ($R^2 = 0.10$), which is directly dependent on the resting period of the reproductive process (Fig. IV.3.9.1).

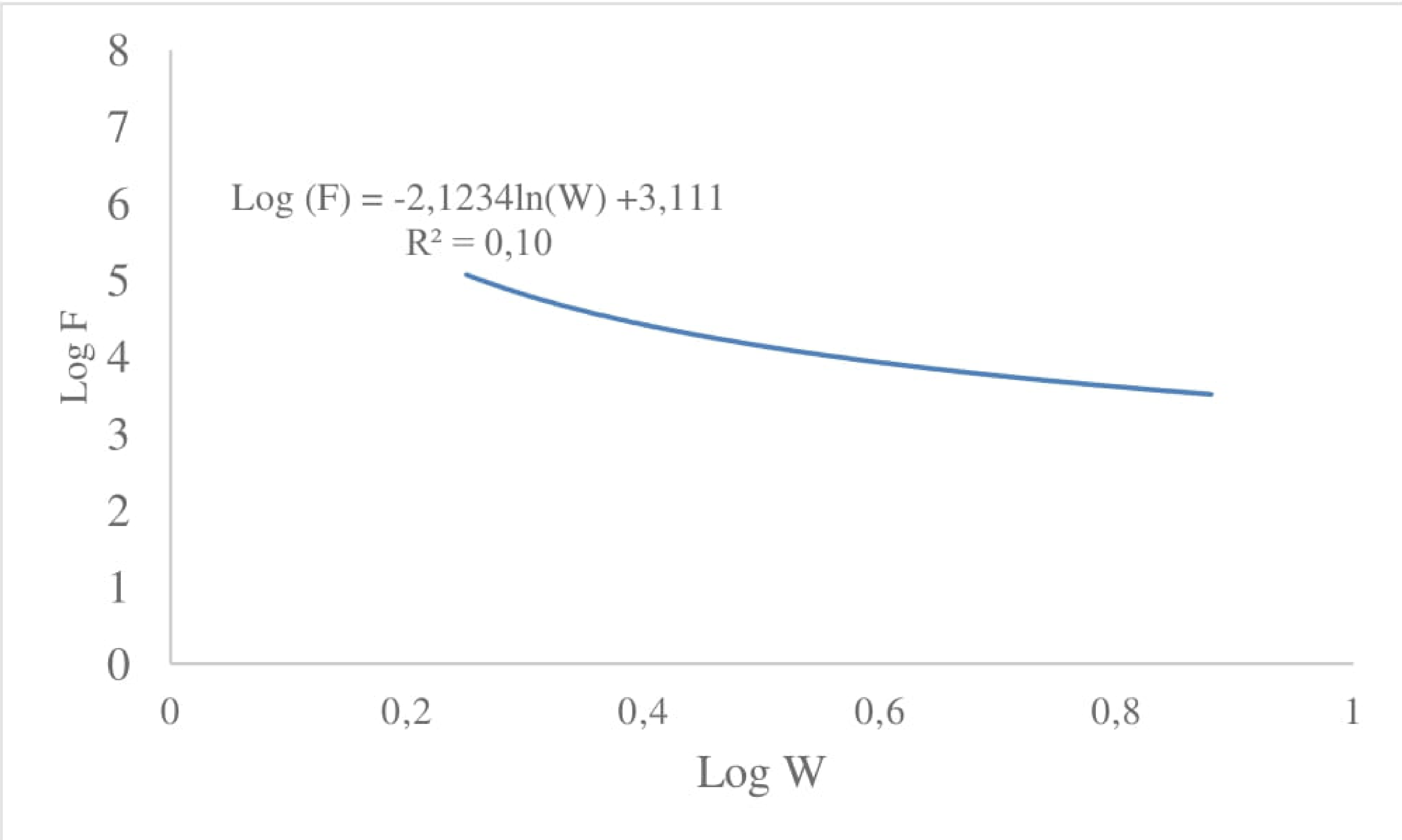

Figure IV. 3.9.1 Dependence of portion fertility on red mullet weight.

The relationship between the weight and the fertility of the turbot showed a weak relationship (R^2=0.128), of the Gonado-somatic index and the weight of the gland (ovaries) (Figure IV. 3.9.2). The coefficient of reproduction during the studied period shows a very weak level of determinism, which is directly related to the active reproductive processes during the considered period (Fig. IV.3.9.2).

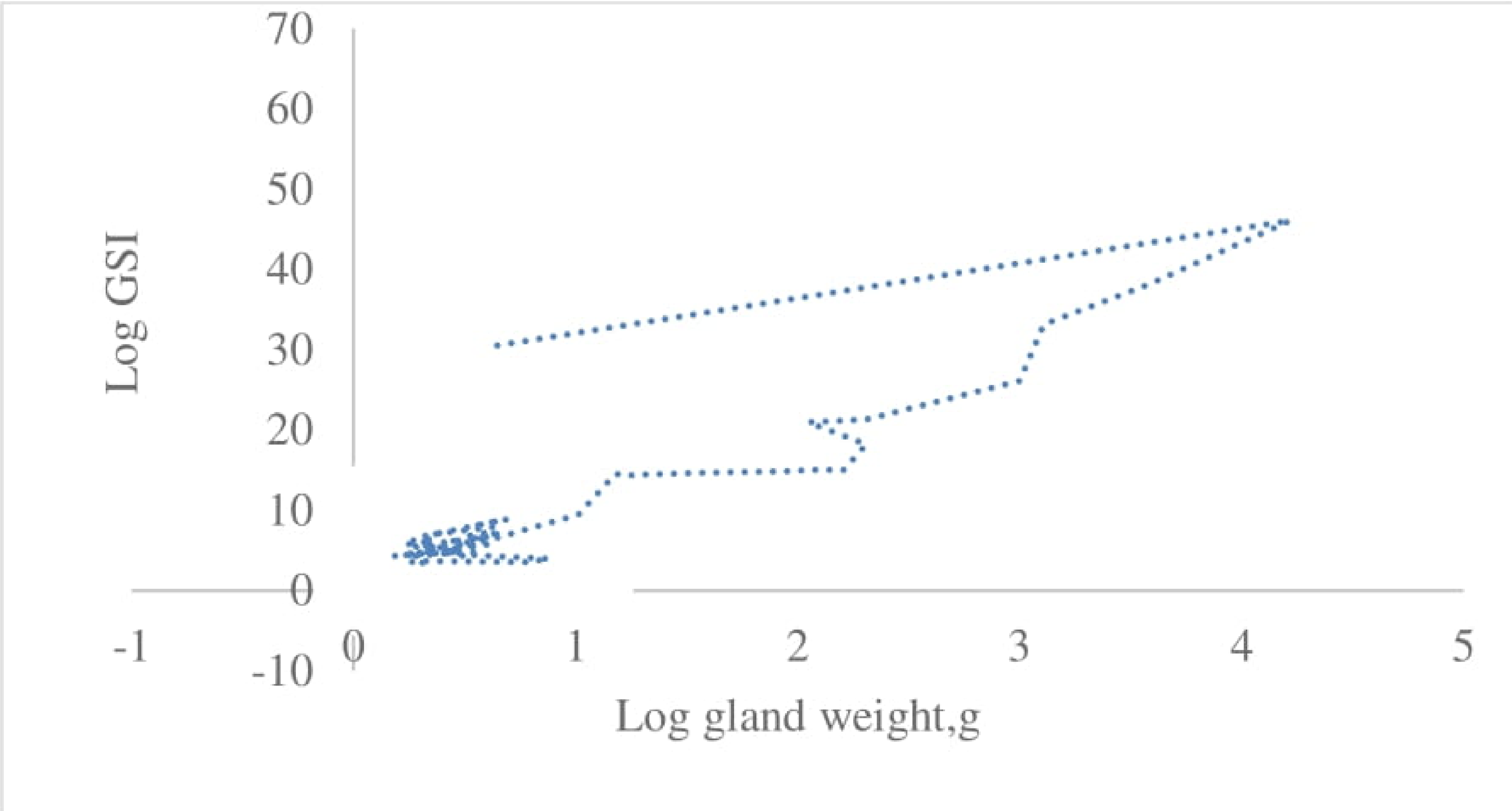

Figure IV.3.9.2 Dependence of log gland weight on log gonadosomatic index (GSI).

Absolute and relative fecundity with average lengths and weights are described in Table IV.3.9.1. The average value of absolute fecundity was estimated at 7844.4 spawn grains. The average value of relative fertility is 4944.25.

Table IV.3.9.1 Absolute and relative fertility of red mullet from the first six months of 2024.

Size class	Average body weight (W, g)	Absolute fertility F, caviar grains)	Relative fertility	♀N
9,5	9,234	6220	6735,98	12
10	9,902	6922	6990,51	6
10,5	11,53	7144	6196,01	11
11	13,325	7901	5929,46	1
11,5	15,55	7099	4565,27	3
12	19,12	7923	4143,83	3
12,5	20,26	8456	4173,74	4
13	22,92	8096	3532,29	3
13,5	25,23	9453	3746,73	2
14	26,92	9230	3428,68	5
		7844,4	4944,25	50

IV.3.10 Sexual maturity

150 specimens were used to determine maturity. The red mullet is a summer-breeding species. Males (Figure IV.3.10.1-2) have fewer age groups and a mature gland at age 2-2 + y^{-1}, and an average length of 10.00 cm. Females have a greater range of ages, as replenishment was observed only for them, and correspondingly the greatest range of mean lengths.

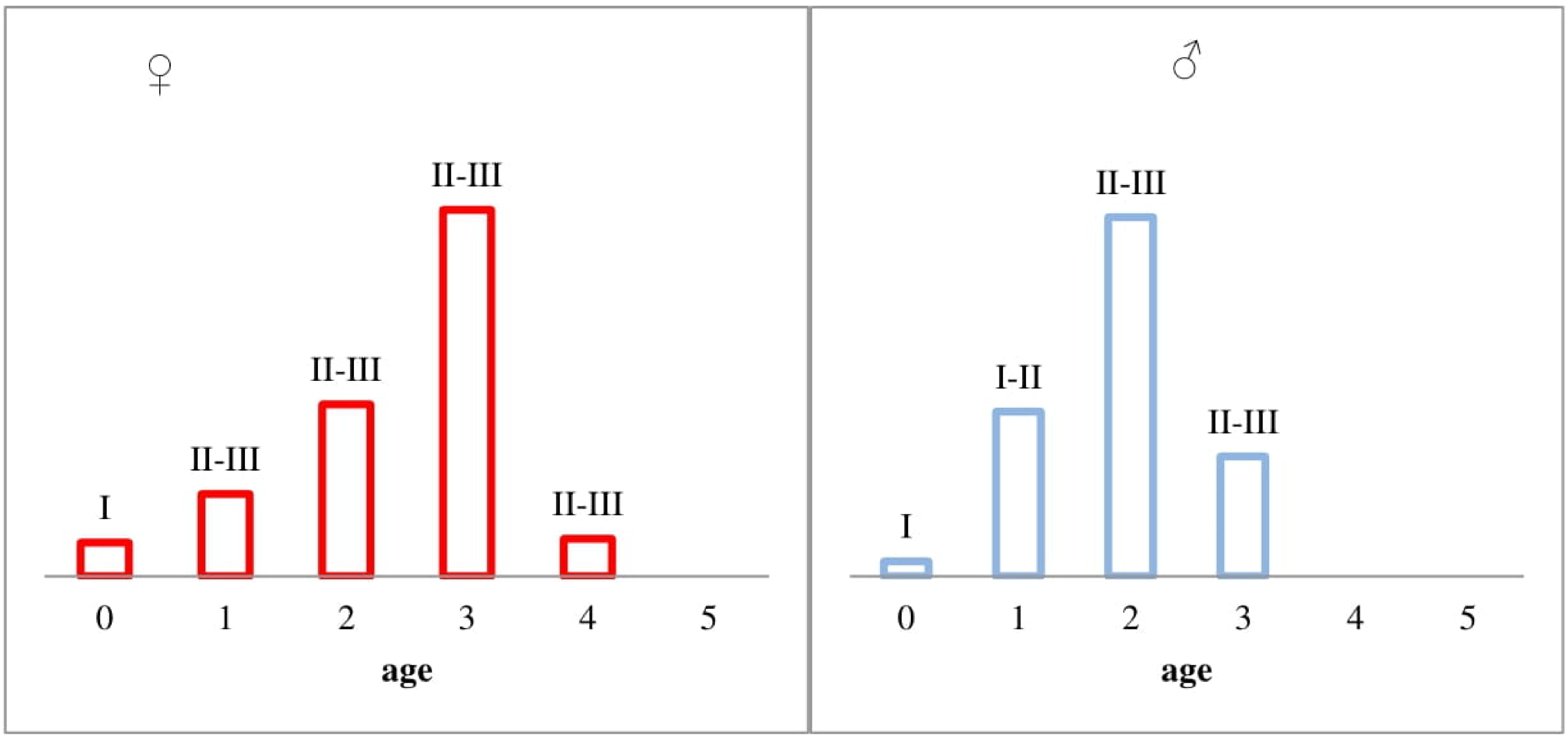

Figure IV.3.10.1 Sexual maturity by age for red mullet - female (♀) and male (♂).

The red mullet is a summer breeding species. Males (Figure IV.3.10.2) have fewer age groups and a mature gland at ages 1-1+ and 2-2 + y^{-1} and an average length of 10.00 cm. Females have a larger age range, as they are the only ones to see fill and the correspondingly largest range in average length.

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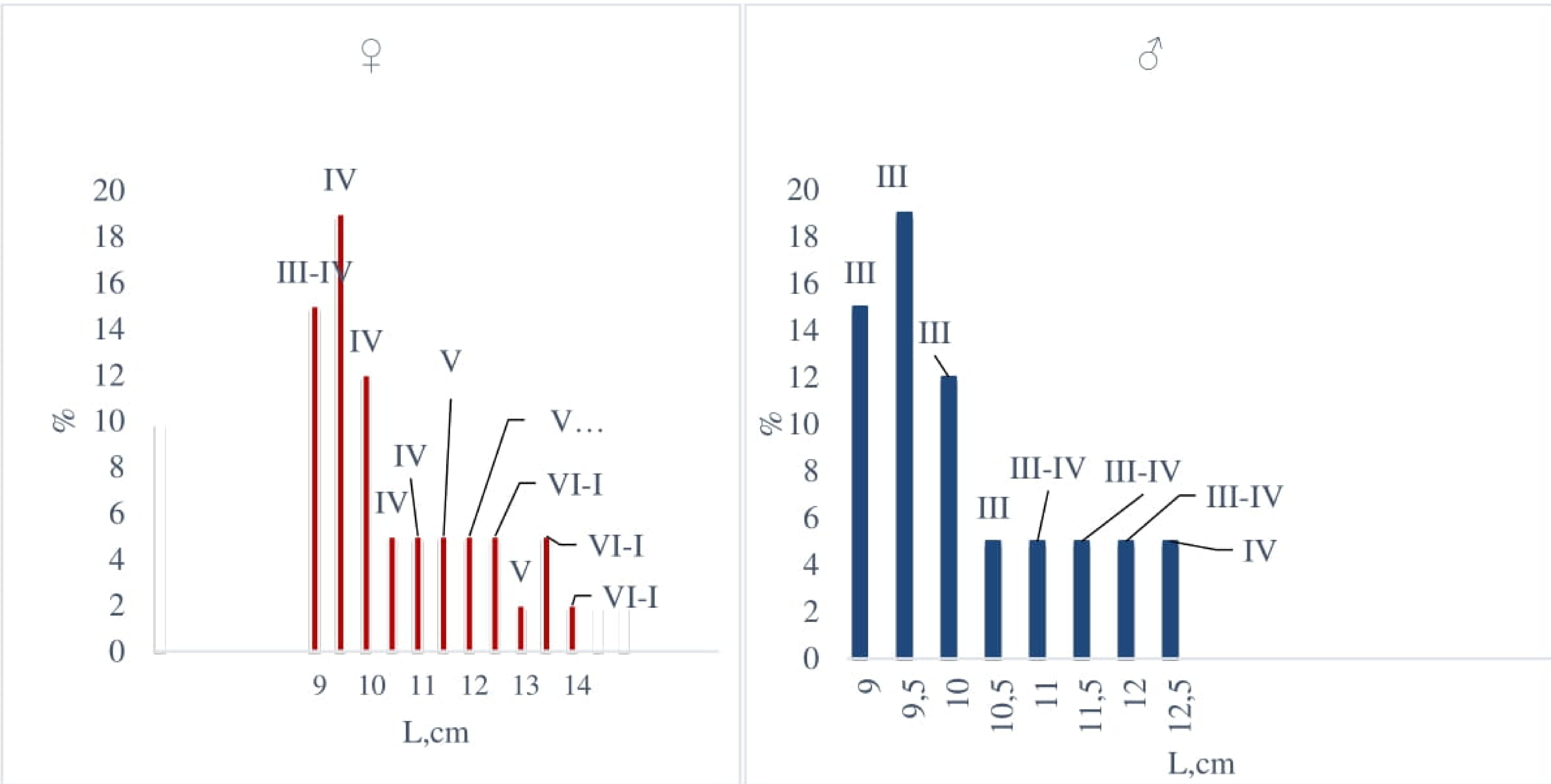

Figure IV.3.10.2 Sexual maturity by length (cm) of red mullet - female (♀) and male (♂).

IV.3.11 Catch numbers and biomass by age and length

Monthly catches with OTM (in tonnes) together with mean weights of red mullet were used to derive the monthly catch numbers. The share (%) by age groups and catch numbers were used to create ***catch-at-age matrix*** for selected months by age groups (Table IV.3.11.1).

Table IV.3.11.1 Matrix of catch with OTM by age (10-6) and biomass (kg) of red mullet.

Catch in numbers *10^{-3}		
Age groups (yr	*Ist quarter*	*IInd quarter*
0-0+	2,94926408	3,13196186
1-1+	4,84521956	5,14536591
2-2+	6,53051332	6,9350584
3-3+	11,0597403	11,744857
4-4+	2,6332715	2,79639452
∑	**28,0180088**	**29,7536376**
	Biomass (kg)	
Age groups (yr	*Ist quarter*	*IInd quarter*
0-0+	12,25841	13,01778

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1-1+	34,23883	36,35982
2-2+	69,08669	73,3664
3-3+	155,49	165,1221
4-4+	67,92612	72,13393
∑	**339**	**360**

The monthly OTM catch (in tonnes) together with the average red mullet weights were used to obtain the monthly catch numbers. Proportion (%) by age group and catch abundance were used to create a ***catch-by-length matrix*** (Table IV.3.11.2).

Table IV.3.11.2 Catch at length (10^{-6}) matrix and biomass (kg) of red mullet whiting with OTM.

Catch in numbers *10^-3		
Length classes (cm)	*I-st quarter*	*II-nd quarter*
7,5	1,26397	1,342269
8	0,947978	1,006702
8,5	1,26397	1,342269
9	4,318565	4,586087
9,5	2,106617	2,237116
10	3,265257	3,467529
10,5	3,791911	4,026808
11	2,106617	2,237116
11,5	3,370588	3,579385
12	2,843933	3,020106
12,5	0,631985	0,671135
13	0,421323	0,447423
13,5	0,947978	1,006702
14	0,737316	0,78299
∑	**28,01801**	**29,75364**
Biomass (kg)		
Length classes (cm)	*I-st quarter*	*II-nd quarter*
7,5	3,614955	3,83889
8	4,654571	4,942907
8,5	6,838079	7,261677
9	30,14675	32,01424
9,5	17,3027	18,37455
10	31,9953	33,97731
10,5	43,62804	46,33066
11	26,49387	28,13508
11,5	51,43201	54,61806

12	51,62055	54,81828
12,5	12,99361	13,79853
13	10,34349	10,98424
13,5	25,72812	27,32189
14	22,20796	23,58367
∑	**339**	**360**

IV. 3.12 Conclusions

The analysis of the biological parameters allows us to draw the following conclusions:

1) The size structure showed a bimodal distribution with a peak in the size group 9.0 and 10.5 cm. A slight increase in the percentage of individuals from the size group 10.0 and 11.5 cm is also noted. A sharp decrease in the presence is noted in the group 12.5 and 13 cm.
2) During the first six months, the highest percentage share was occupied by individuals in the age group 3-3+, followed by 2-2+.
3) In the analysis (age slicing) of size-frequency samples for determining the growth parameters of the red mullet, only ELEFAN RSA gave relatively good results, the results of the other two methods showed average lengths for 5-year-old specimens of the order of 10 cm, which is not typical for the species and the probable reason is an insufficient number of measurements and poor representation of the size structure in the samples. The latter is expected when determining growth parameters based on samples that do not represent the development of the species on an annual basis.
4) The graph shows that during the first six months K is highest for size classes 13.0-13.5-14.0 cm.
5) A gradual increase in average weight is observed across age groups. The 0-0+ age group has the lowest average weight, while the 4-4+ age group has the highest average weight.
6) The resulting model is statistically significant, the value of the scaling coefficient a in the length-weight relationship model $W_i = aL_i^b$ e: $a = 0.002$ and the allometry coefficient b=3.52, which indicates positive allometric growth of the species in the first half of the year, or the increase in weight is proportional to or greater than the increase in length.
7) In the first half of 2024, there was no portion reproduction of the species, as it is a summer breeder.

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V. Biological monitoring of anchovy (*Engraulis encrasicolus*)

V.1 Objectives

The purpose of biological monitoring is to collect data that will be used to analyze anchovy catches, as well as to form a database. The collection of biological samples of anchovy catches in **I-VI 2024** includes the following tasks:

1. To collect and analyze the dynamics of length, weight and age distribution.
2. To determine the state of the of anchovy using the so-called state factor (Ricker, 1975).
3. Characteristics of the reproductive biology of anchovy.
4. Collection of data on ports of landing, sampling vessels, number of samples collected, number of specimens tested, geographical catch data.

V.2 Sampling

The sampling was carried out by the actively operating fishing fleet in Bulgaria.

V.2.1 Geographic area coverage

Data of present analysis were collected from Bulgarian Black Sea coast. In **I-VI 2024, 2 samples with 255 specimens** were collected and processed. Information on the size of the catches was also collected.

V.2.2 Sampling period

In **I-VI 2024**, the biological data for the species were collected from a total of **2 landings at the ports of Nesebar and Burgas**. Ports and ships from which monitoring was carried out to collect biological data from landings are presented in **Table V.2.2.1.**

Table V.2.2.1 Ports and ships from which monitoring was carried out to collect biological data of anchovy landings.

№	Date	Sampling ports	ANE	Fishing vessel	External marking	Fishing gear	Catch/ kg	Coordinates
1	4.1.2024	Nesebar	ANE	R/K 40	BS258	OTM	470	42.6438 27.7598
2	3.6.2024	Burgas	ANE	VALNOBOR	BS390	OTM	200	42.3667 27.8[illegible]5

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V.2.3 Statistical analysis of data

See section statistical analysis of sprat.

V.3 Results

V.3.1 Landings statistics

In January 2024, anchovy landings were the highest. In February, March, April and May, no catches were observed with OTM (Fig. I.3.1.2).

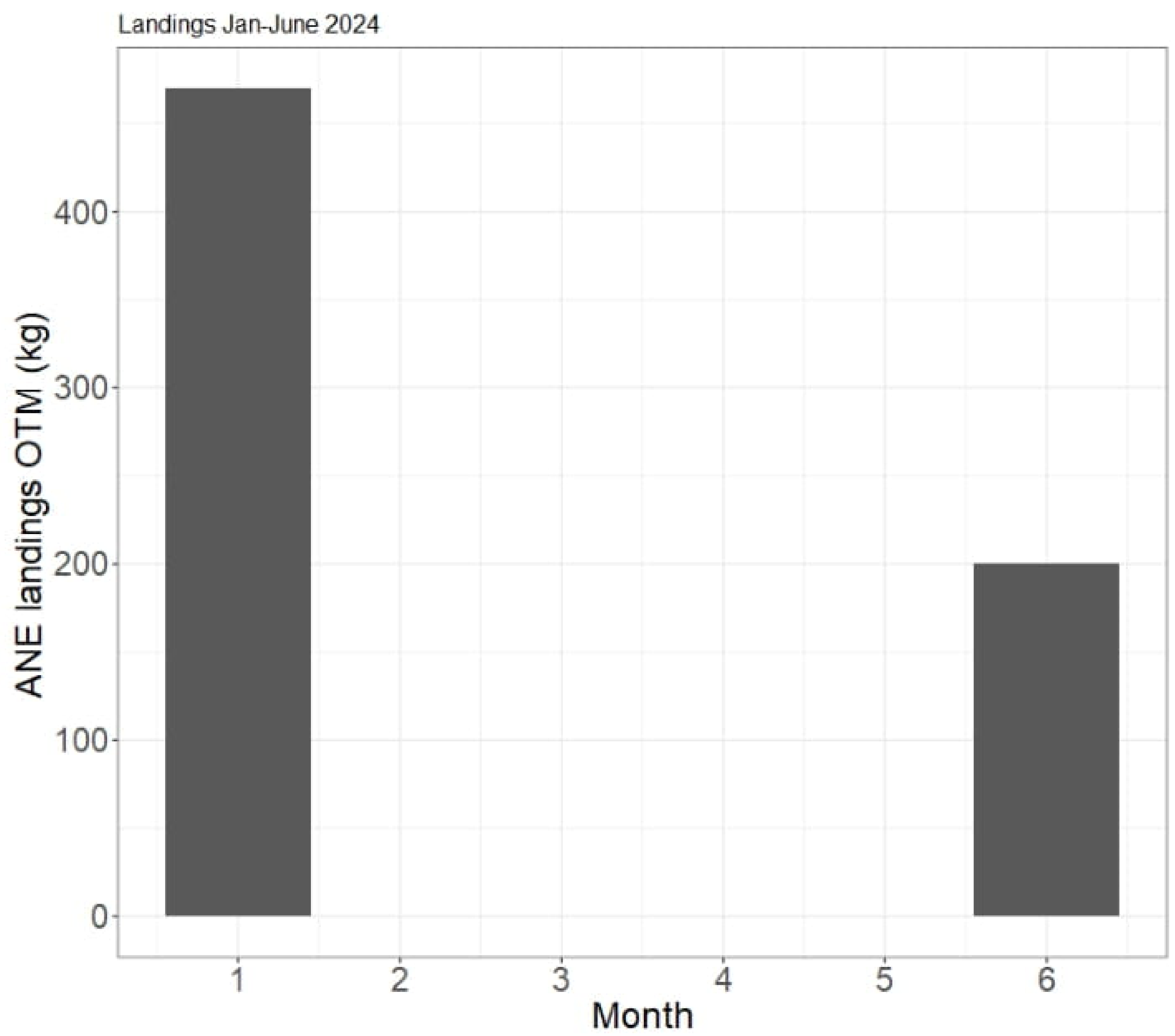

Figure V.3.1.1 Anchovy landing statistics.

V.3.2 Length structure of landings

The size structure during the period I-III month of 2024. would show a modal distribution with a peak at size group 9.5 and 10.0 cm. An increase in the percentage of individuals from the size group 11.00 cm is also noticed. During the months IV-VI, an increase in the percentage of size groups 9.5 and 12.0 cm was found in the catches (Figure V.3.2.1).
From the distribution of individuals by size groups in the first quarter of 2024, it is found that the groups 9.5 cm and 10 are represented most massively with 21.64% and 19.40%. Fish with a body length in the range of 11 cm represent 17.91% of the catch, followed by 10.5 cm with

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13.43%. Anchovy with a body length in the range of 12 cm are 9 pieces, which represents 6.72%. The graph shows that in both quarters the percentage distribution of catches by size classes is uneven. In the second half of the year, the individuals with the highest values are in the size classes of 9.5 cm (15.70%) and 12 cm (16.53%), and the size classes of 10 and 11 cm with the lowest percentage value are, respectively, with 9.91% and 9.09%.

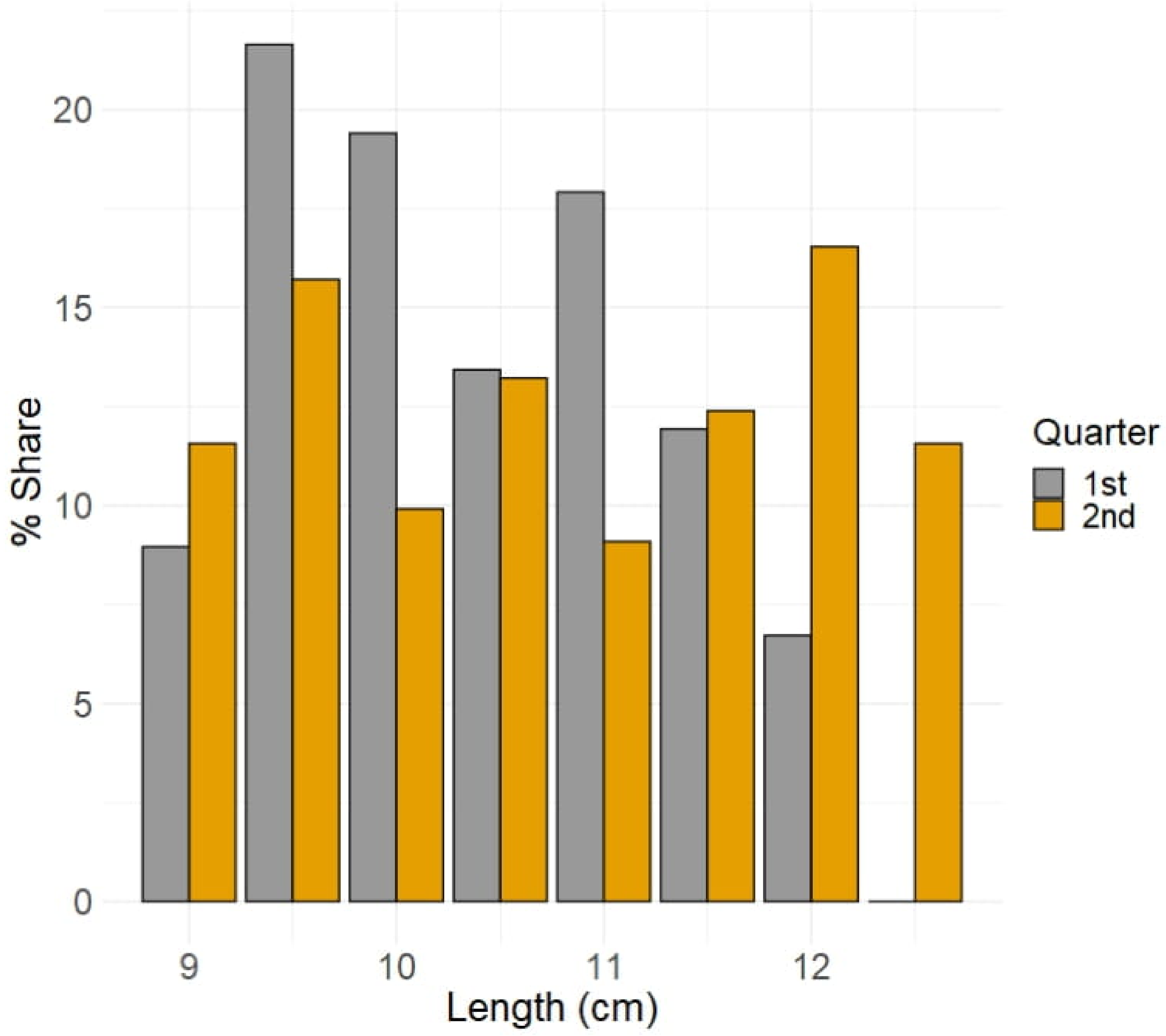

Figure V.3.2.1 Percentage representation of anchovy size classes in the composition of catches in the first and second quarters of 2024.

V.3.3 Age structure of landings

255 fish were used for age determination. In the first quarter, the highest percentage is occupied by individuals in the age group 2-2+, followed by 3-3+. The age group 4-4+ has the lowest share. The second quarter shows that the age groups of 3-3+ and 4-4+ significantly prevail over the others (Figure V.3.3.1).
The graph shows a gradual increase in the catch values for anchovy (%) for the first quarter, with the age group 1-1+ having the lowest possible catch values of 22.39%, and 3-3+ having the highest percentage performance in catches - 37.31%. In the second half of the year, catch results are best represented in the groups 3-3+, and 4-4+ with 29.75% and 30.57% respectively.

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In the second quarter, the distribution by age showed the presence of age groups 1-1+ with 16.53% and 2-2+ with 23.14%.

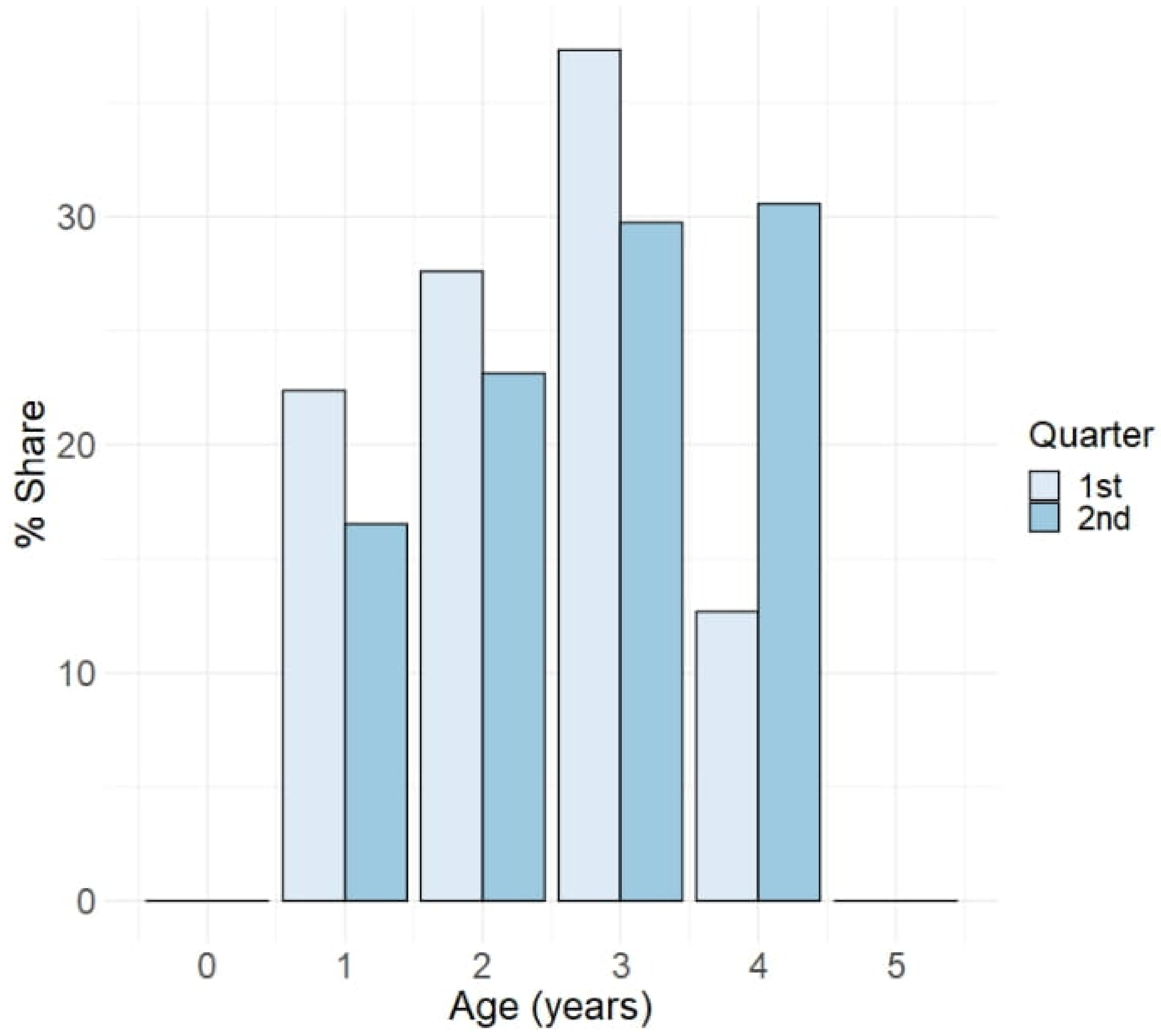

Figure V.3.3.1 Percentage representation of anchovy age groups in the catch composition in the first and second quarters of 2024.

In the analysis (age slicing), the size-frequency samples for determining the growth parameters of the anchovy gave relatively good results only ELEFAN RSA, the results of the other two methods showed an average length for 5-year-old specimens of the order of 9 cm, which is not characteristic of species and the probable cause is insufficient measurement of the number and poor representation of the size structure in the samples. The latter is expected when determining growth parameters based on samples that do not represent the development of the species on an annual basis.

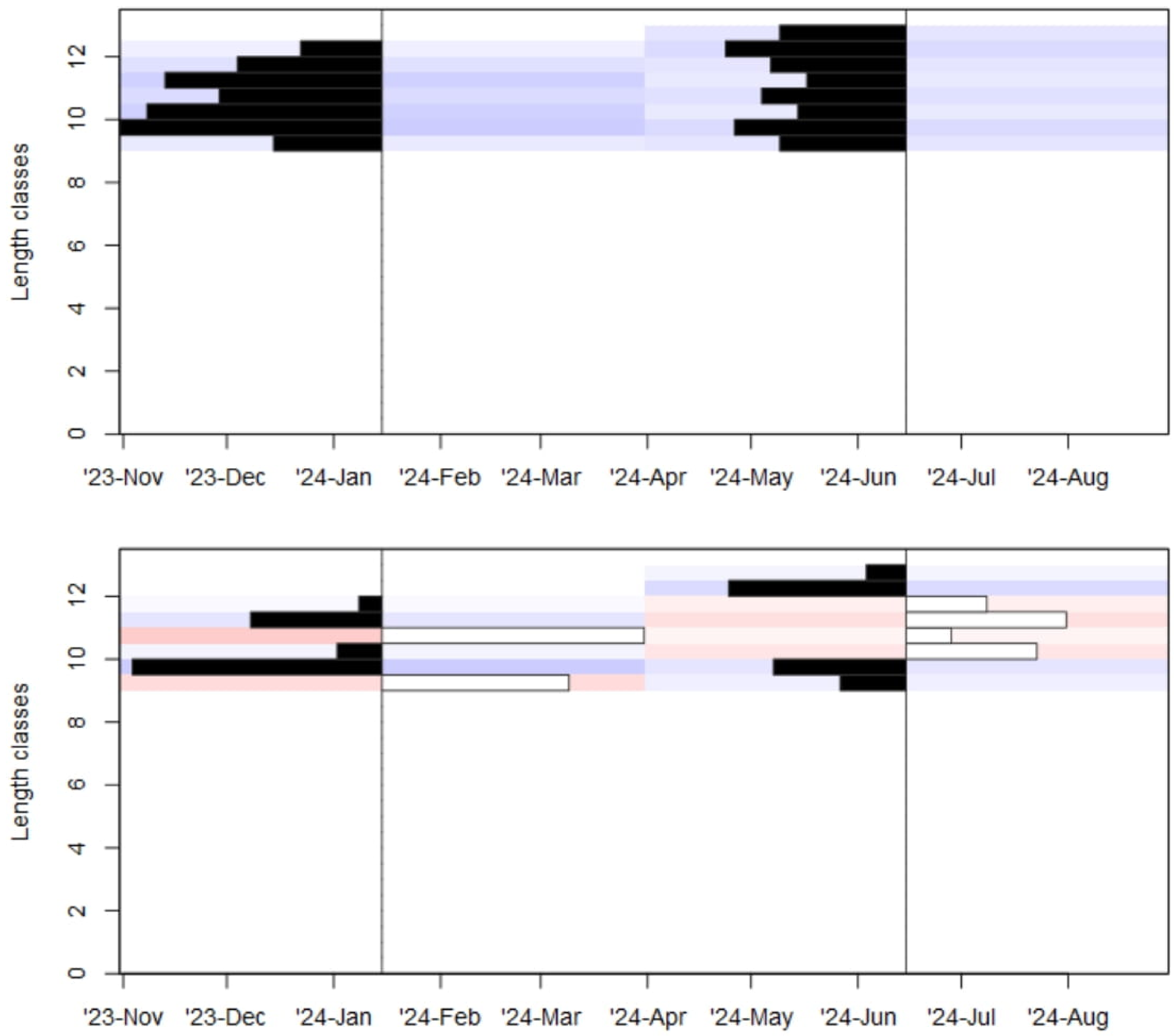

Figure V.3.3.2 Size-frequency samples, visualized as (top diagram) number of individuals in size class and (bottom diagram) the restructured data with moving average MA = 5 for the purposes of frequency analysis and determination of growth parameters.

V.1.3.3.1 Frequency analysis RSA – response surface analysis

Growth parameters determined by RSA with initial conditions: conditional interval of for the asymptotic length L_∞ in the sample [12;15 cm] (based on a literature review on the characteristics and biological potentials of the species for the Black Sea) and the parameter that determines the growth rate to L_∞ - $K = \exp\,(seq(from\ log(0.1), to\ \log(1))$ with an upper limit for L_∞ = 15 cm (Figure V.1.3.3.1.1).

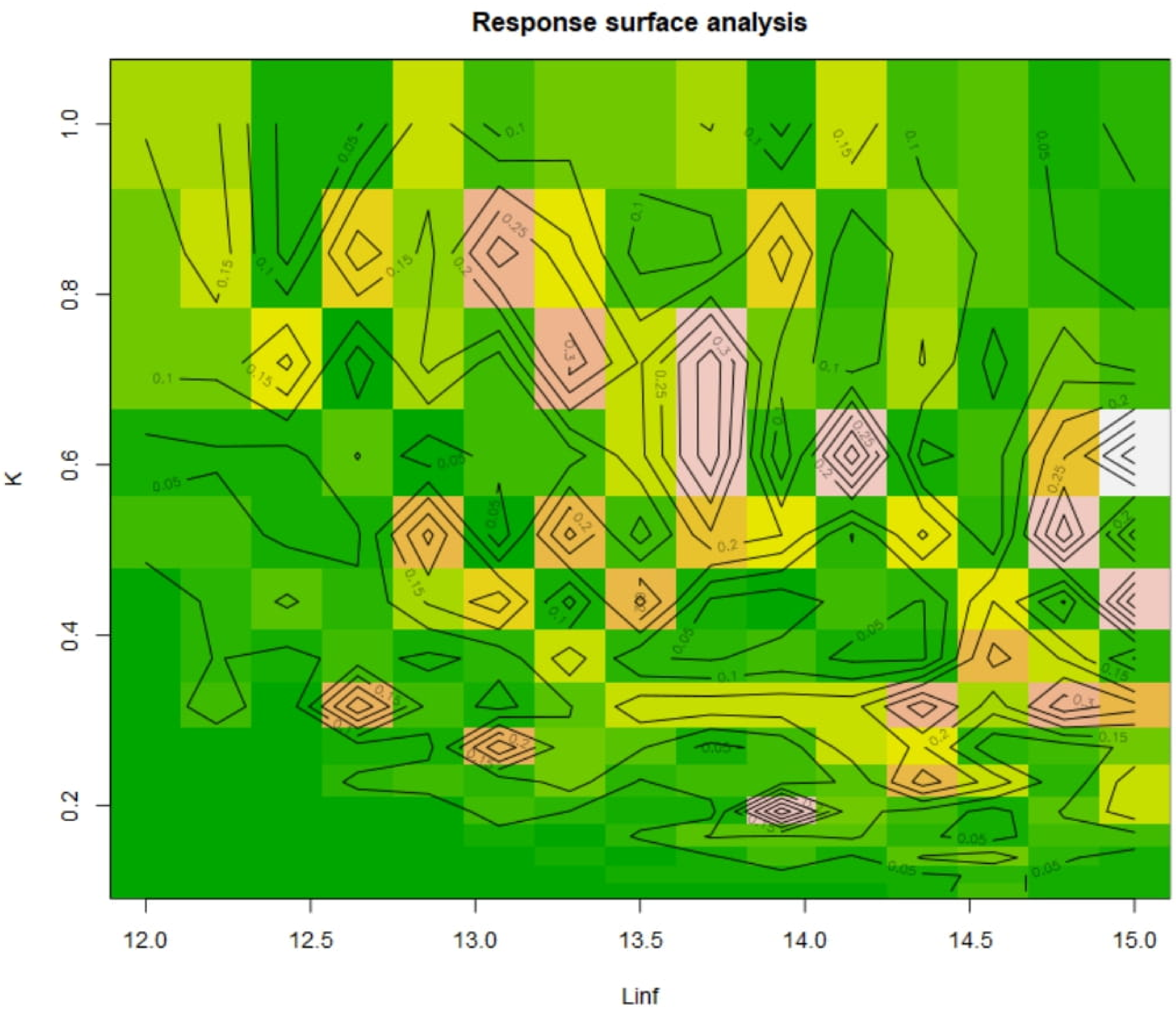

Figure V.1.3.3.1.1 Contour plot visualizing the identified asymptotic length L∞ and the corresponding value of the parameter K in the defined intervals. The parameters in the von Bertalanffy model were calculated as follows: L_{∞}=15.00 cm; K = 0.61 and t_0=-0.33. The ages with their corresponding lengths (Table V.1.3.3.1.1) were calculated using the latter and compared with the experimentally determined ones.

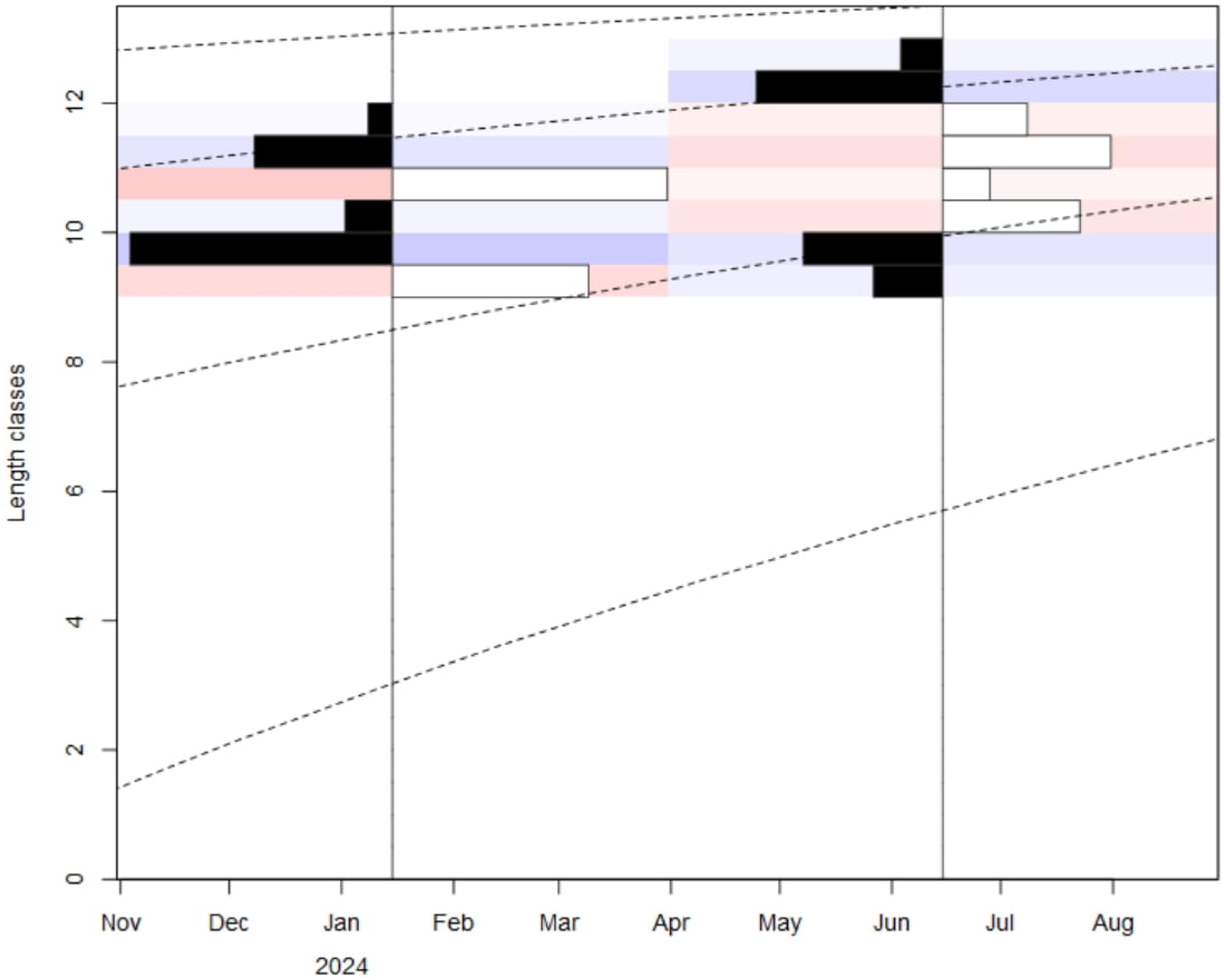

Figure V.1.3.3.1.2 Growth curves determined with ELEFAN RSA, visualized on the restructured data for the purpose of visualizing the tracking of cohorts over time.

Table V.1.3.3.1.1 Ages with their corresponding lengths for the studied species calculated using von Bertalanffy parameters, obtained with RSA and compared with experimentally determined ages from otoliths.

	ELEFAN RSA	Experimentally determined
age	La	L_{mean}
0	2.7	
0.5	6.0	-
1	8.3	
1.5	10.1	9.25

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2	11.4	
2.5	12.3	9.75
3	13.0	
3.5	13.5	11
4	13.9	
4.5	14.2	12
5	14.4	-

Given the results obtained, the analysis of growth parameters should be repeated again at the end of the year, when richer biological information about the species will be available.

V. 3.4 Condition factor

The graph (Fig.V.3.4.1) shows that K is highest for size classes 11-12.5 cm. The remaining size groups also have good condition, which indicates food security and good physiological condition.

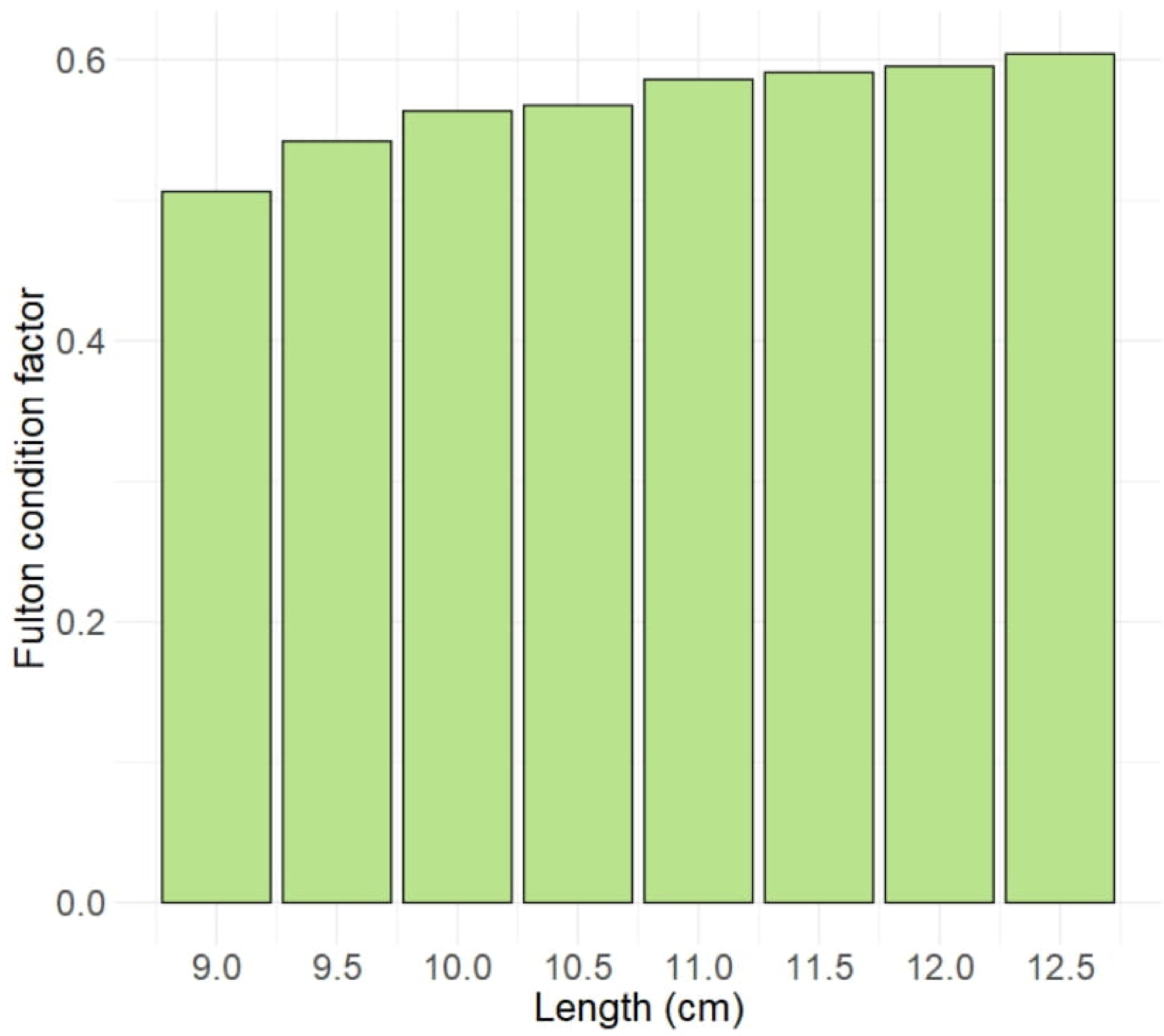
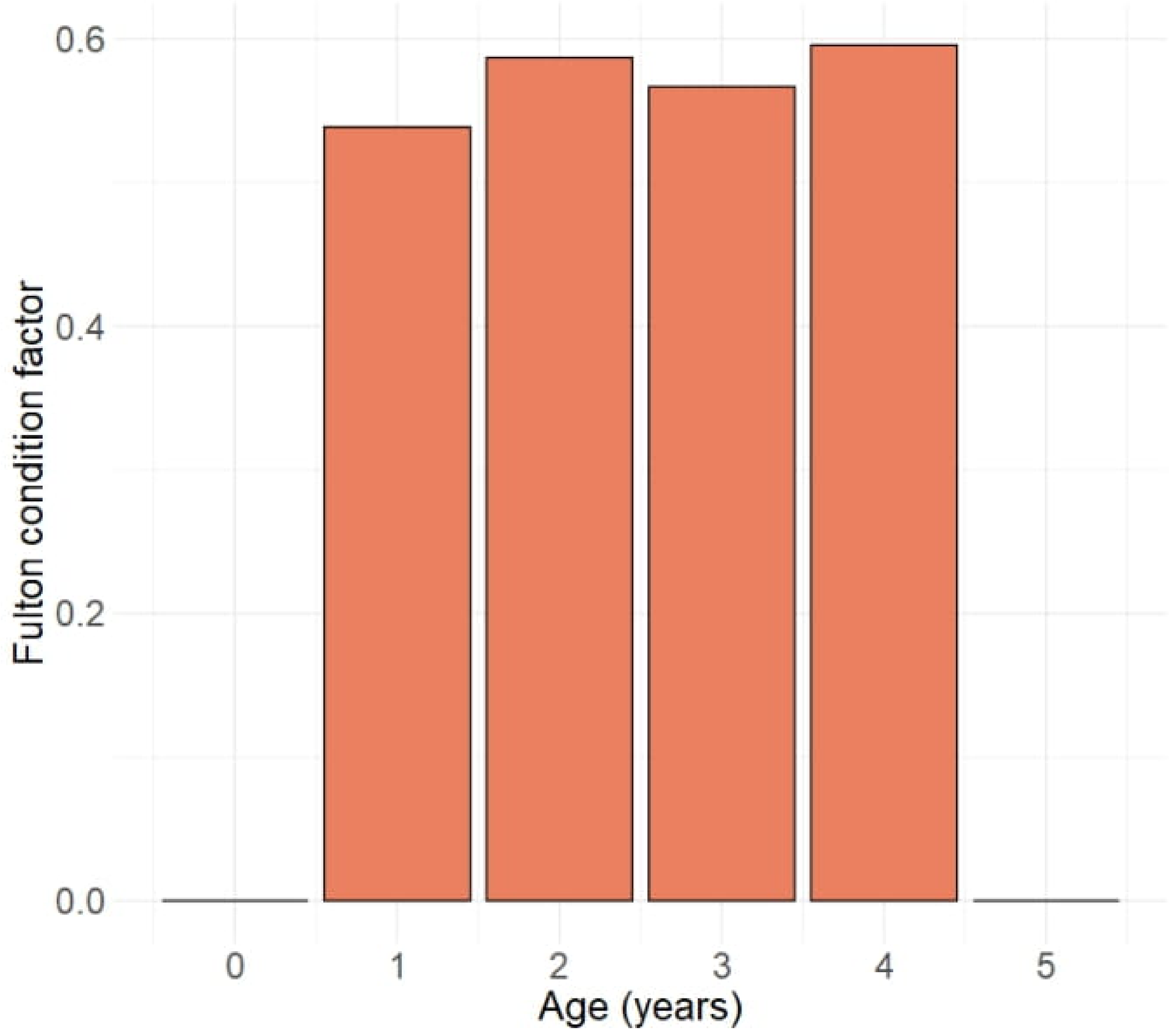


Figure V.3.4.1 Fulton condition factor by anchovy size classes for the first half of 2024.

Fig. V.3.4.2 shows that the second and fourth age groups have the highest values.



V3.5 Weight structure of anchovy

The weight was measured on **255 specimens.** There was a smooth increase in average weight across age groups for both the first and second trimesters. For age group 1-1+, the lowest average weight is 4.26g (50 number of anchovy), and for group 4-4+, the highest average weight is observed -10.29g (54 number). For the other age groups, the weights are as follows: 2-2+-5.44g (65 number); 3-3+-7.55g (86 number)(Fig. V. 3.5.1).

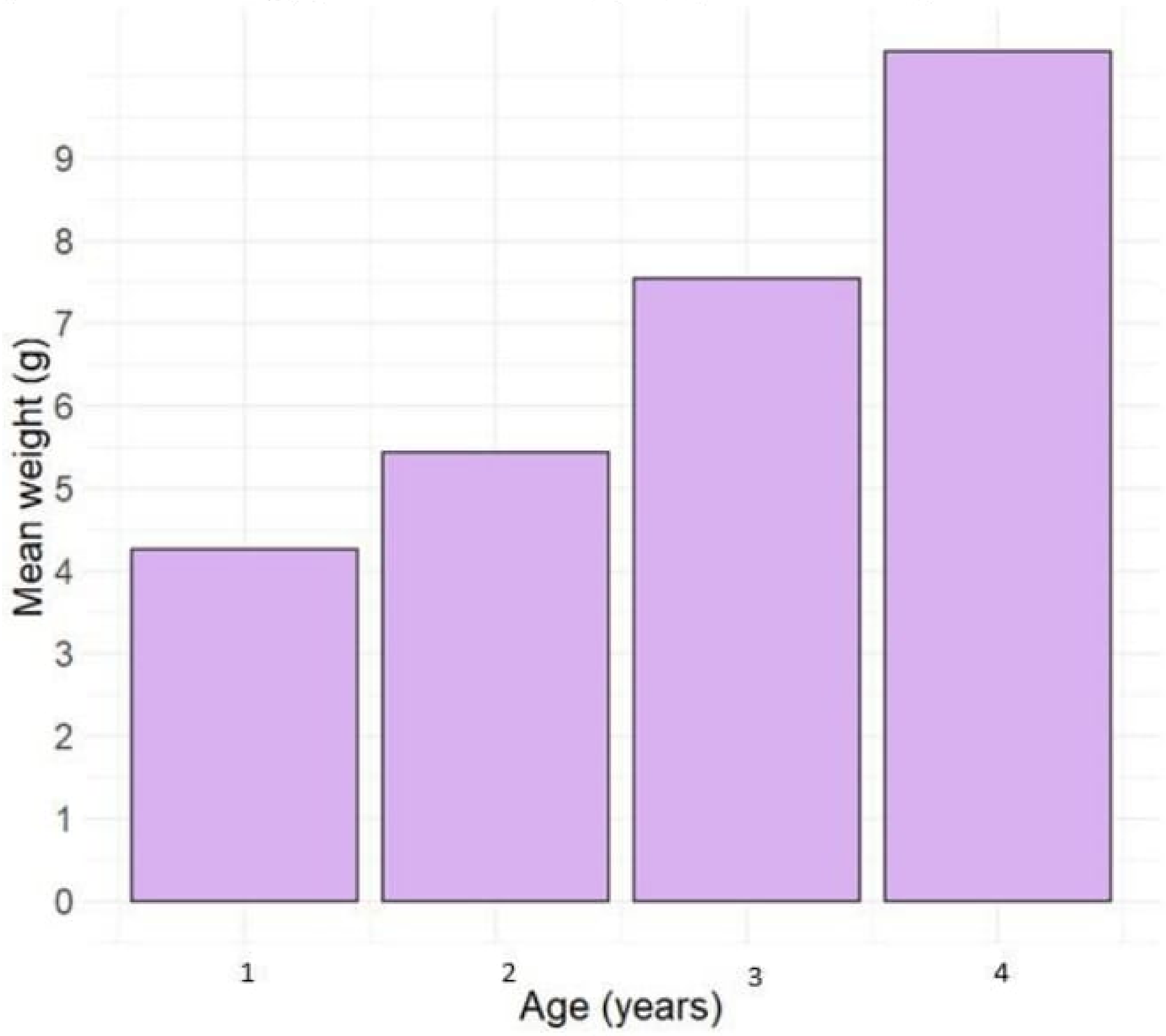


Figure V.3.5.1 Distribution of average anchovy weight by age group, first half of 2024.

The 9.5cm size class, which is the most numerous and is represented by 48 number of anchovy (18.82%), has a measured weight of 4.65g, with the highest weight recorded at 12.5 cm (participating with 5.49%) - 11.8 g.

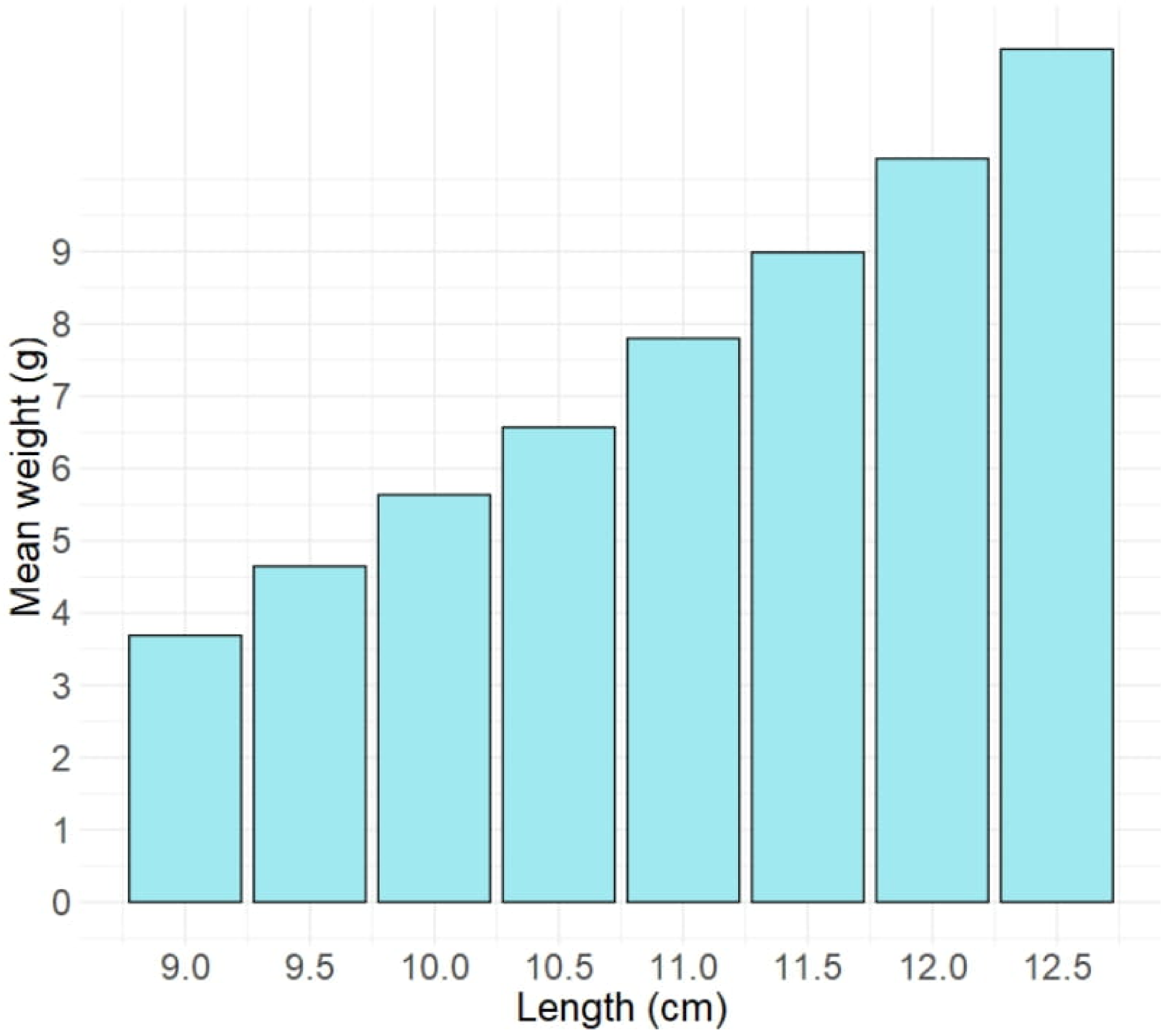


Fig. V.3.5.2 Distribution of average anchovy weight by size groups for the first half of 2024.

V.3.6 Size structure of whiting by age group

The length of the fish was measured **on 255 specimens**. A gradual increase in the average length values is observed across age groups. The lowest average length value is in the age group 1-1+, and the highest is in the group 4-4+ years. (Table III.3.6.1).

Table III.3.6.1 Size structure of anchovy by age groups.

L_{mean}/cm	Age
9,30	1-1+
9,91	2-2+
10,88	3-3+
11,98	4-4+

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V.3.7 Length- weight relationship

The resulting model (Table V.3.7.1) is statistically significant, the value of the scaling coefficient a in the length-weight relationship model $W_i = aL_i^b$ e: $a = 0.002$, and the allometry coefficient b=3.49, which indicates positive allometric growth of the species in the first half of the year, or the increase in weight is proportional and greater than the increase in length.

Table V.3.7.1 Results of modeling the length-weight relationship:

```
Call:
lm(formula = logW ~ logL, data = weight_l)

Residuals:
      Min        1Q    Median        3Q       Max
-0.032928 -0.011526  0.004883  0.010963  0.023397

Coefficients:
            Estimate Std. Error t value       Pr(>|t|)
(Intercept) -6.31886    0.15513  -40.73 0.00000001464 ***
logL         3.48504    0.06541   53.28 0.00000000293 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.01987 on 6 degrees of freedom
Multiple R-squared:  0.9979,	Adjusted R-squared:  0.9975
F-statistic:  2839 on 1 and 6 DF,  p-value: 0.000000002935
```

V.3.8 Sex ratio

The sex ratio was determined on **100 specimens**. Females predominate over males.

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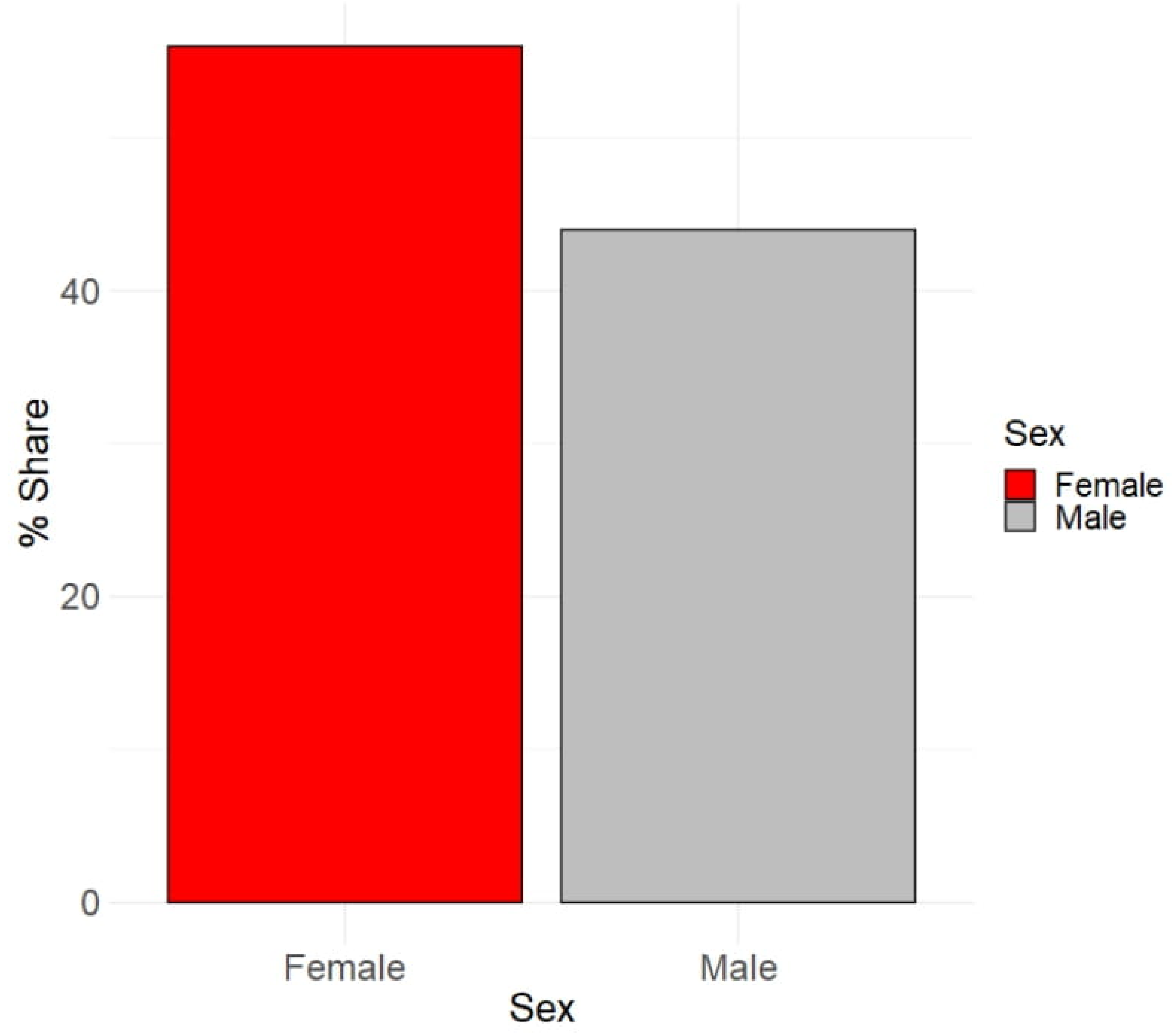

Figure V3.8.1 Anchovy sex structure.

The average lengths of females♀ by age group are higher (Figure V.3.8.2).

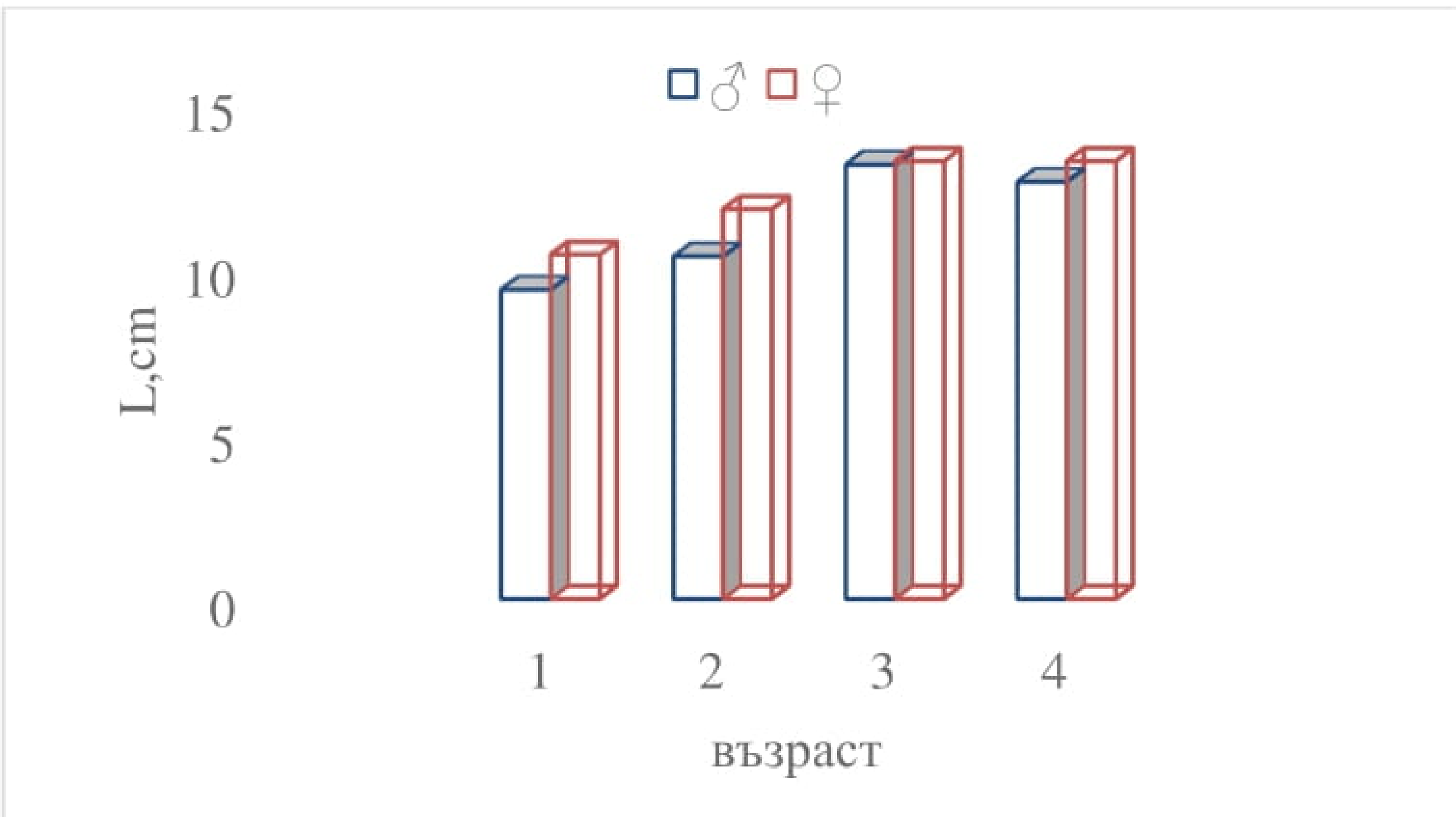

Figure V.3.8.2 Sex ratio(♂♀) by size and age of anchovy.

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V.3.9 Fertility

Fertility was determined on **50 specimens**. The gonadosomatic index is not strongly dependent on the weight of the gonads (R^2=0.6271), which is associated with the not-high rate of maturation of the females (Figure V.3.9.1).

The somatic index for anchovy varies within narrow limits, with a pronounced correlation between the gland weight of the measured specimens (R^2 = 0.8163) (Fig.V.3.9.1).

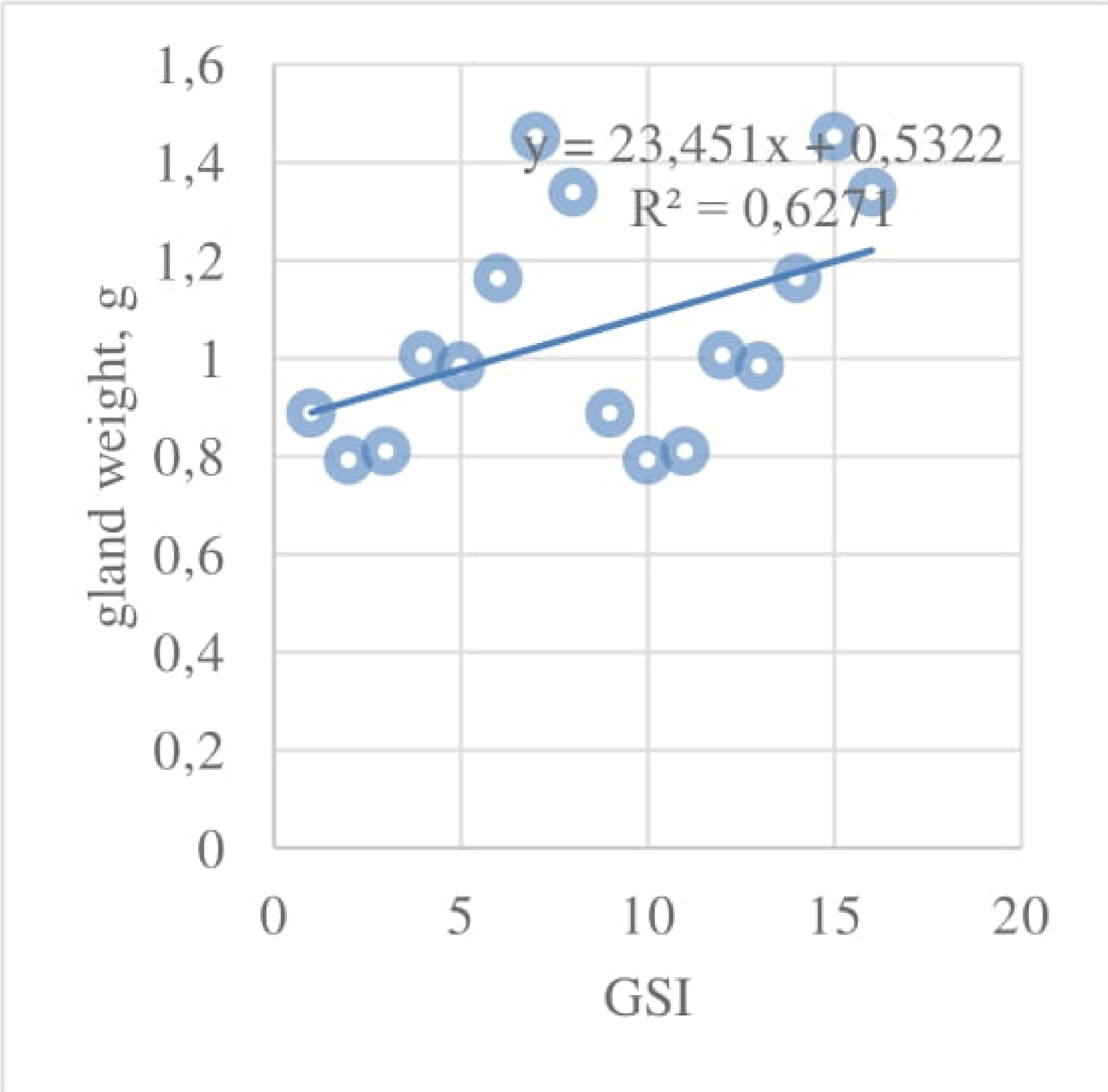

Figure V. 3.9.1 Dependence of gland weight on gonadosomatic index (GSI).

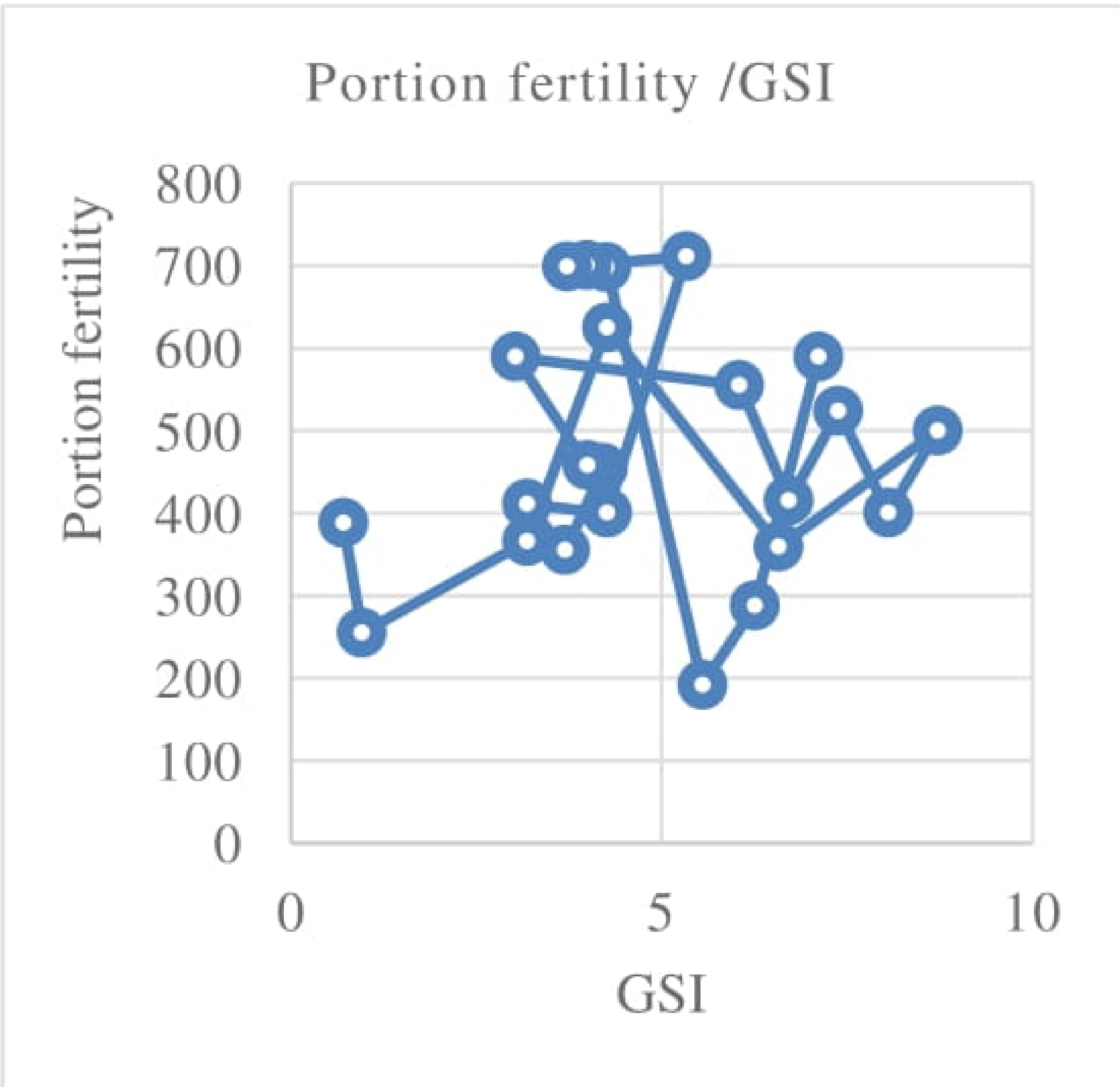

Figure V.3.9.2 Portion fertility to GSI of anchovy.

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The mean absolute fecundity was estimated at 6814,19 spawn. The average value of relative fertility is 890,236 (**Table V.3.9.1**).

Table V.3.9.1 Absolute and relative fertility.

Size class	Average body weight (W, g)	Absolute fertility F, caviar grains)	Relative fertility	Number (n)
9	4,57	3440	752,735	10
9,5	5,75	4331	753,217	10
10	5,16	5103	988,953	7
10,5	6,58	6002	912,158	7
11	7,73	7732	1000,26	7
11,5	8,12	1235,5	152,155	3
12	9,25	13459	1455,03	3
12,5	11,93	13211	1107,38	3
		6814,19	890,236	50

V.3.10 Sexual maturity

Sexual maturity was determined on **50 specimens**. All examined specimens showed degree of gonad discharge (V- III) grade.

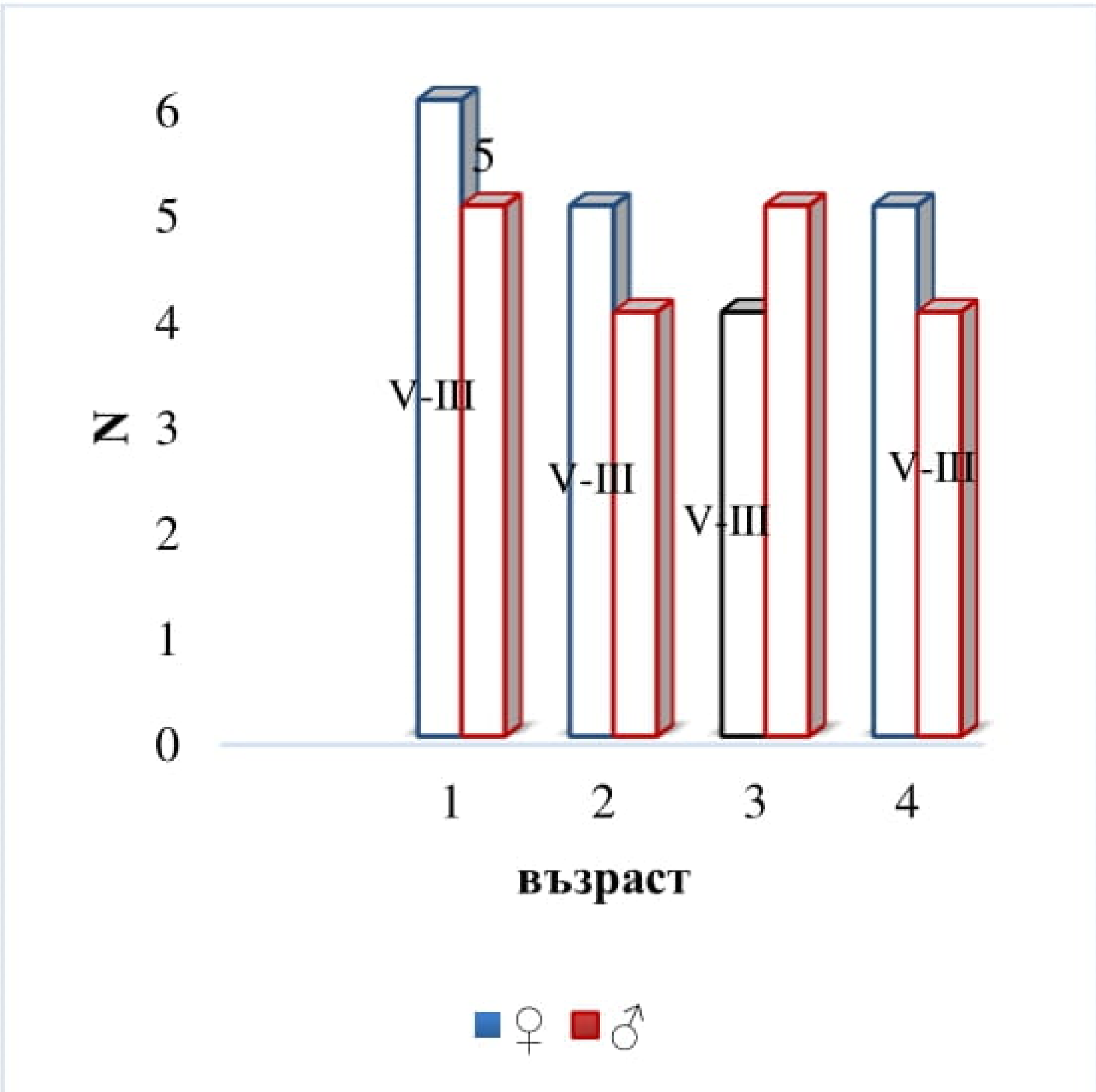

Figure V.3.10.1 Sexual maturity by age of anchovy - female (♀) and male (♂).

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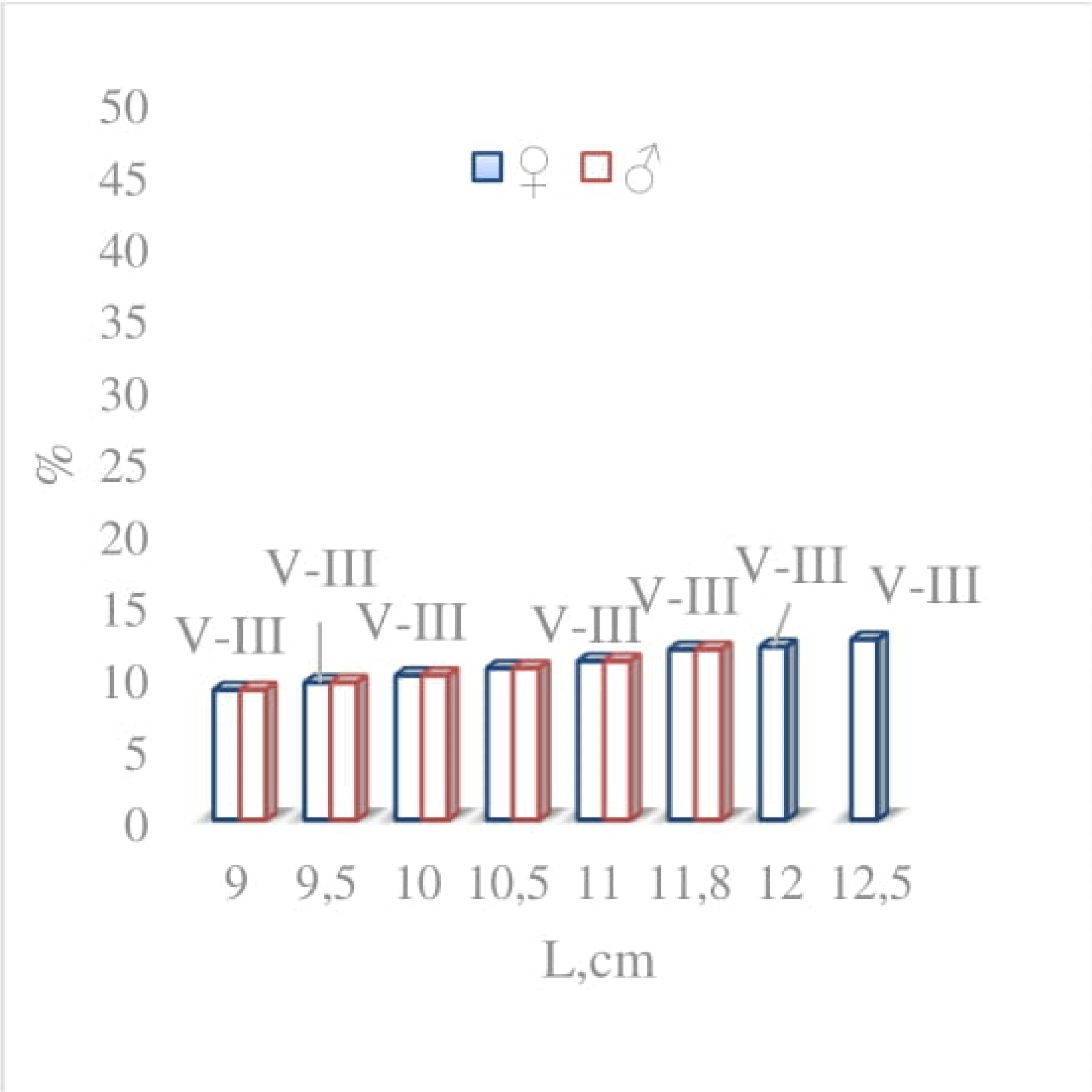

Figure V.3.10.2 Sexual maturity in length (cm) of anchovies - female ♀ and male ♂.

V.3.11 Catch numbers and biomass by age and length

The monthly catch with OTM (in tons) together with the average weights of anchovy were used to derive the monthly catch numbers. The proportion (%) by age group and catch numbers are used to create a ***catch-at-age matrix*** (**Table V.3.11.1**).

Table V. 3.11.1 Catch matrix by age (10^{-6}) and biomass (kg) of anchovy with OTM.

Catch-at-Age *10^{-3} (in thousands)		
Age groups	*III rd quarter*	*IVth quarter*
1-1+	13,265	5,644
2-2+	17,244	7,338
3-3+	22,815	9,709
4-4+	14,326	6,096
∑	**67,649**	**28,787**
Biomass (kg)		
Age groups	*III rd quarter*	*IVth quarter*
1-1+	56,563	24,070
2-2+	93,823	39,925
3-3+	172,139	73,251
4-4+	147,475	62,755
∑	**470,000**	**200,000**

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The monthly catch with OTM (in tons) together with the average weights of the anchovy were used to obtain the monthly catch numbers. Proportion (%) by age group and catch abundance were used to create a ***catch-by-length matrix*** (**Table V.3.11.2**).

Table V.3.11.2. Catch length (10^{-6}) with OTM matrix and biomass (kg) of anchovy.

Catch-at-length *10^{-3} (in thousands)		
Length group (cm)	*III rd quarter*	*IVth quarter*
9.0	6,898	2,935
9.5	12,734	5,419
10.0	10,081	4,290
10.5	9,020	3,838
11.0	9,285	3,951
11.5	8,224	3,500
12.0	7,693	3,274
12.5	3,714	1,580
∑	**67,649**	**28,787**
Biomass (kg)		
Length group (cm)	*III rd quarter*	*IVth quarter*
9.0	25,452	10,831
9,5	59,176	25,181
10	56,815	24,176
10,5	59,255	25,215
11	72,414	30,814
11,5	73,921	31,456
12	79,142	33,677
12,5	43,826	18,649
∑	**470,000**	**200,000**

V.3.12 Conclusions

The analysis of the biological parameters of the anchovy makes it possible to draw the **following conclusions:**

1) The size structure during the period I-III month of 2024 showed a bimodal distribution with a peak in the size group 9.5 and 10.0 cm. An increase in the percentage of individuals from the size group 11.00 cm is also noted. During the months IV-VI, an increase in the percentage of size groups 9.5 and 12.0 cm was found in the catches.

2) In the first quarter, the highest percentage share is occupied by individuals in the age group 2-2+, followed by 3-3+. The second quarter shows that the age groups of 3-3+ and 4-4+ significantly prevail over the others.

3) In the analysis (age slicing) of size-frequency samples for determining the growth parameters of anchovy, only ELEFAN RSA gave relatively good results, the results of the other two methods showed average lengths for 5-year-old specimens of the order of 9 cm, which is not typical for the species and the probable reason is an insufficient number of measurements and poor representation of the size structure in the samples. The latter is expected when determining the growth parameters based on samples that do not represent the development of the species on an annual basis.

4) Condition factor K is highest for size classes 11-12.5 cm. The remaining size groups also have good condition, which indicates food security and good physiological condition.

5) A gradual increase in average weight and size is observed by age group for both the first and second trimesters. The age group 1-1+ has the lowest average weight and size, while the group 4-4+ has the highest average weights and sizes.

6) The resulting model is statistically significant, the value of the scaling coefficient a in the length-weight relationship model $W_i = aL_i^b$ e: $a = 0.002$, and the allometric coefficient *b=3.49*, which indicates positive allometric growth of the species in the first half of the year, or the increase in weight is proportional and greater than the increase in length.

7) Females predominate over males. The average lengths of females♀by age group are higher.

8) Гонадо-соматичният индекс е не е силно зависим от теглото на половите жлези (R^2=0.6271), което е свързано с не-високия процент на съзряване на женските.

VI. Biological monitoring of picked dogfish (*Squalus acanthias*) landings

VI.1 Objectives

The study examines the population structure of specimens caught by Bulgarian fishermen.

VI.2 Sampling

The sampling was carried out by the actively operating fishing fleet in Bulgaria.

VI.2.1 Geographic area coverage

Shark specimens were measured and weighed on board fishing vessels.

VI.2.2 Sampling period

In I-VI 2024, to study the population dynamics of the shark, **50 specimens** were collected and processed, to establish the size, weight and gender composition of the catches. Ports and ships from which monitoring was carried out to collect biological data from picked dogfish landings are presented in Table VI. 2.2.1.

Table VI.2.2.1 Ports and vessels from which monitoring was carried out to collect biological data from spiny dogfish landings .

№	Дата	Пристанище	Вид риба - код DGS	Риболовен кораб	Външна маркировка	Уред	Улов	Кординати
1	**5.2.2024**	Каварна	**DGS**	Р/К 40	ВН4601	**LLS**	52	
2	**11.3.2024**	Балчик	**DGS**	ВЪЛНОБОР	ВН 8112	**LLS**	110	43.2879 28.401

VI.3. Landings statistics of spiny dogfish

Official shark catch statistics for 2024 are presented in Figure VI. 3.1. The highest catches are in March and June.

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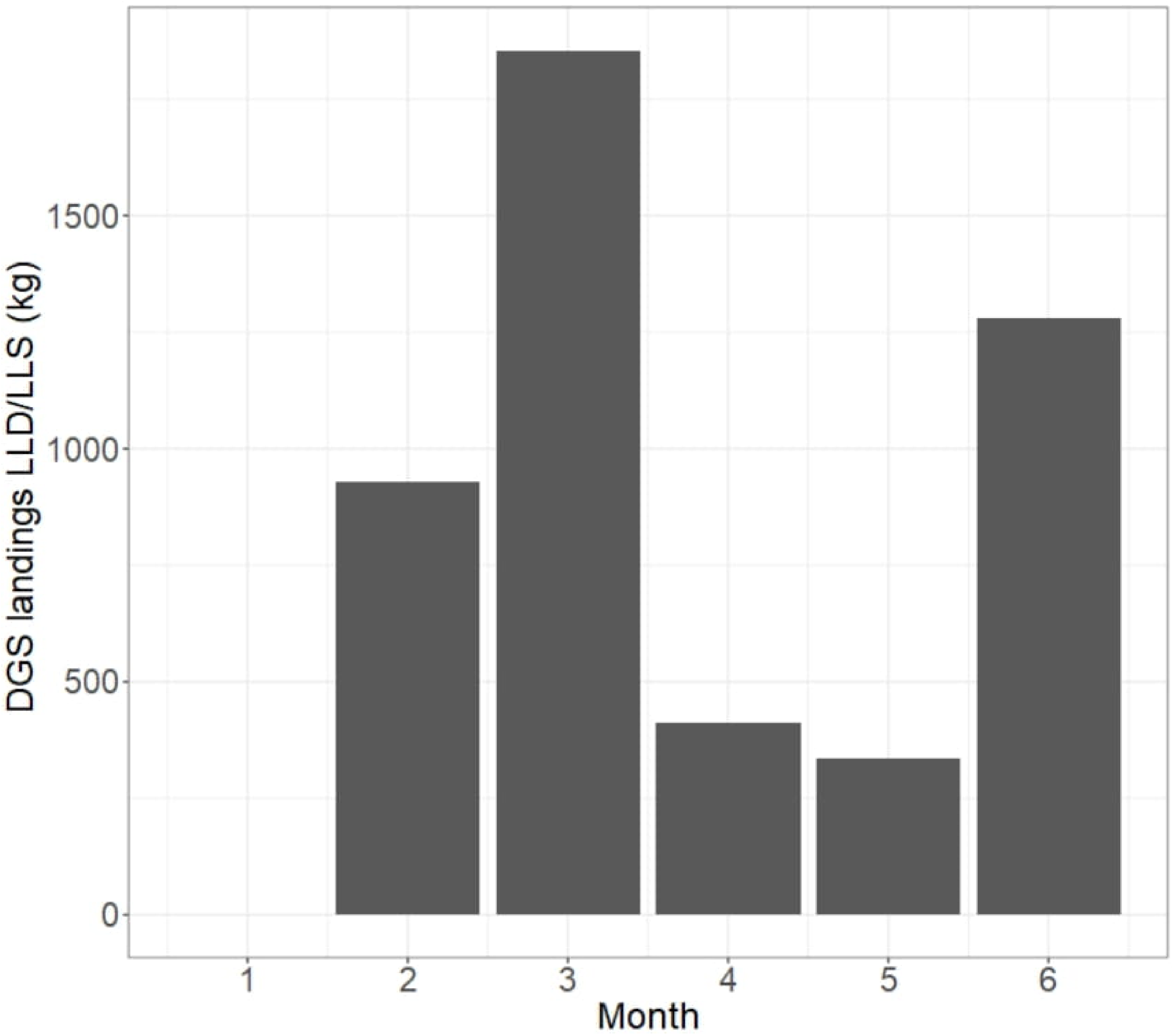

Fig. VI.3.1 Official statistics on shark landings.

VI.4 Results

VI.4.1 Sex, size and weight structure

The average size of a male shark is 117 cm, and the average length of females is 118 cm, (Table VI. 4.1.1-2). Average weights of 6.323 kg have been recorded for females, while the average weights measured for males are 6.389 kg (Table VI. 4.1.1-2).

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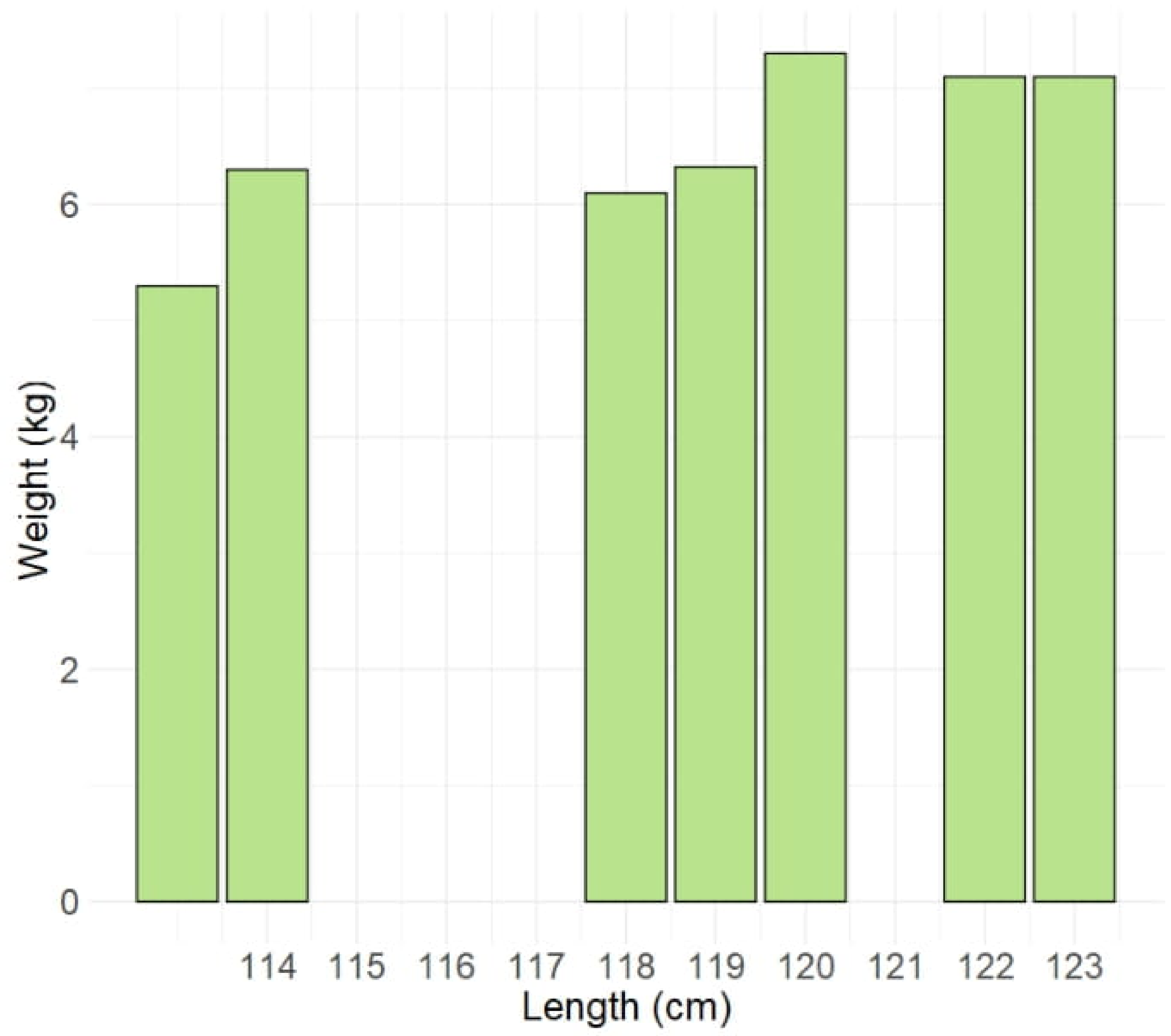

Figure VI.4.1.1 Distribution of weights by length of female♀ specimens in the sample.

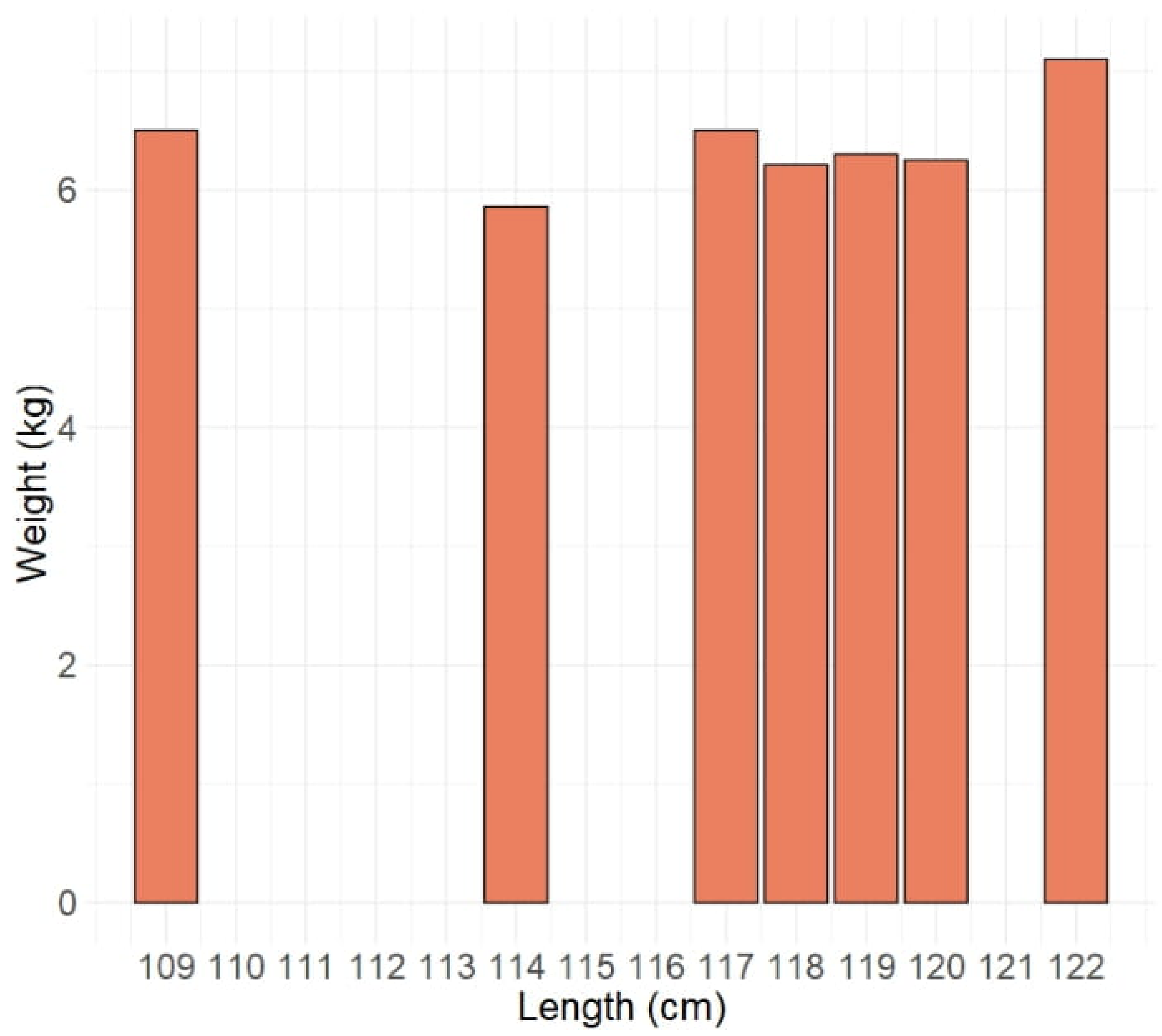

Figure VI.4.1.2 Weight distribution by length of male ♂ specimens in the sample.

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Table VI.4.1.1 Descriptive statistics of the biological parameters (weights and lengths) of the female specimens in the sample (minimum, maximum, mean and median of the total length and weight of the specimens in the samples during the first 6 months of 2024.

Sex Female♀	
length	weight
Min. :113	Min. :5.300
1st Qu.:118	1st Qu.:6.200
Median :118	Median :6.200
Mean :118	Mean :6.323
3rd Qu.:119	3rd Qu.:6.325
Max. :123	Max. :7.300

Table VI.4.1.2 Descriptive statistics of the biological parameters (weights and lengths) of the male specimens in the sample (minimum, maximum, mean and median mean and median of the total length and weight of the specimens in the samples during the first 6 months of 2024.

Sex Male ♂	
length	weight
Min. :109.0	Min. :5.860
1st Qu.:115.5	1st Qu.:6.230
Median :118.0	Median :6.300
Mean :117.0	Mean :6.389
3rd Qu.:119.5	3rd Qu.:6.500
Max. :122.0	Max. :7.100

VI.4.2 Gender structure and size-weight relationship

The sex ratio was determined on **30 specimens** (January-June). Male specimens predominated with 70%, and females were represented with 30% **(Figure VI.4.2.1)**.

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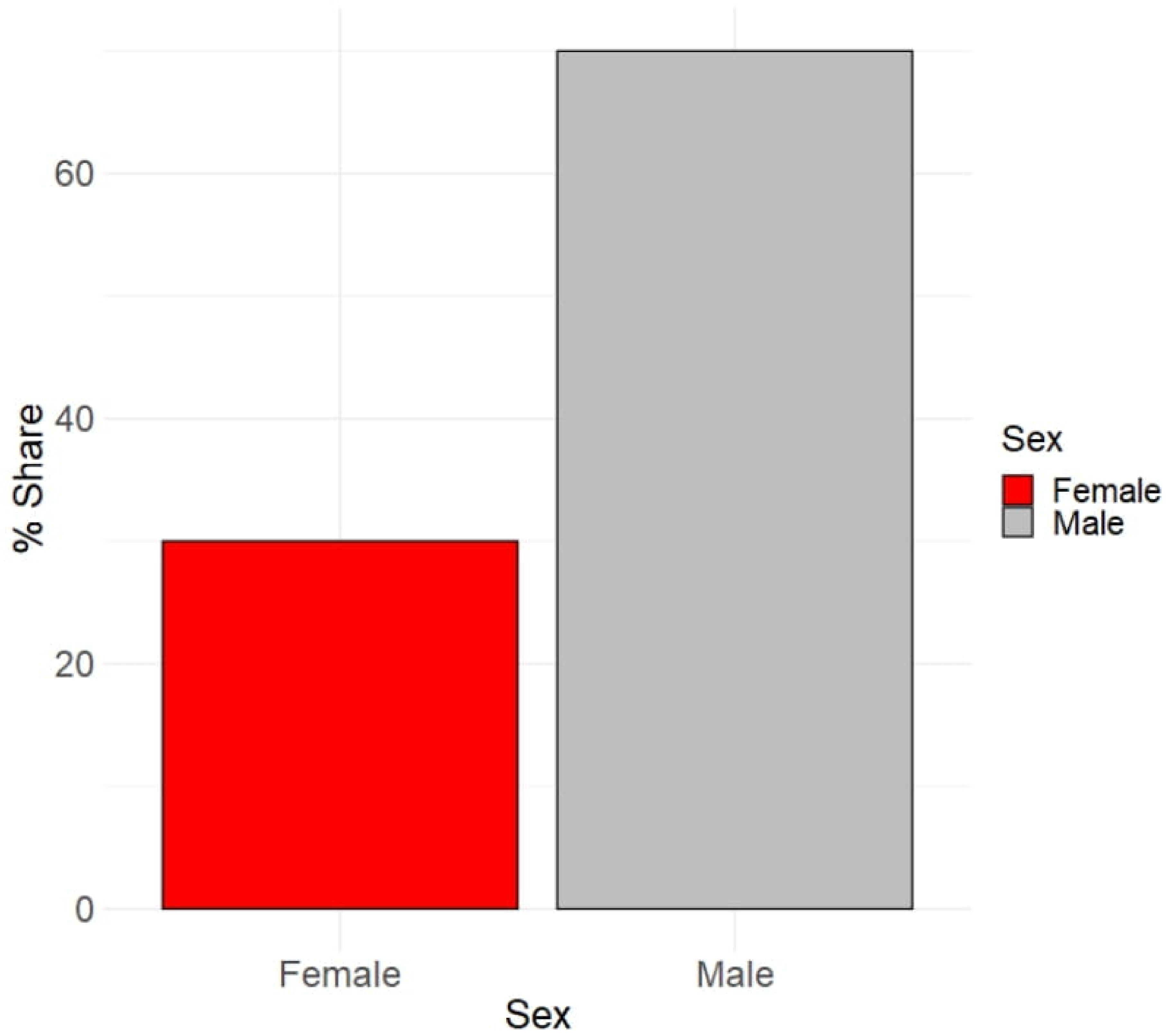

Figure VI.4.2.1 Sex ratio of sharks from the Bulgarian sector of the Black Sea.

An attempt was made to determine the length-weight relationship - the resulting model has a very low determinism $\boldsymbol{R^2_{adj} = 0.23}$, which means that it is not statistically significant. It is possible to describe the relationship with a high-order polynomial, which is not realistic, since the full size structure of the species is not represented in the samples due to the selectivity of the fishing gear.

VI.4.3 Sexual maturity

Sexual maturity was determined in **30 specimens**. In January-June, females are sexually mature with visible embryos.

VI.5 Conclusion

1) The average size of a male shark is 117 cm, and the average length of females is 118 cm. Average weights of 6.323 kg have been recorded for females, while the average weights measured for males are 6.389 kg.
2) Male specimens predominate with 70%, and females are represented with 30%.
3) An attempt was made to determine the length-weight relationship - the resulting model has a very low determinism $R^2_{adj} = 0.23$, which means that it is not statistically significant. It is possible to describe the relationship with a high-order polynomial, which is not realistic, since the full size structure of the species is not represented in the samples due to the selectivity of the fishing gear.
4) In January-June, females are sexually mature with visible embryos.

VII. Anex

Sprattus sprattus	**Number of specimens from the study I-VI,2023**	**Number of specimens from the study VII-XII,2023**	**Number of specimens from the study I-XII,2023**	**Number of specimens Contract 157/13/03/2022, EAFA/IO-BAS**
length	**592**			1250
weight	**592**			1250
age	**592**			1250
sex ratio ♀♂	**250**			500
fecundity	**250**			500
sexual maturity	**250**			500

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Trachurus mediterraneus ponticus

	Number of specimens from the study I-VI,2023	**Number of specimens from the study VII-XII,2023**	**Number of specimens from the study I-XII,2023**	**Number of specimens Contract 157/13/03/2022, EAFA/IO-BAS**
length	**649**			1500
weight	**649**			1500
age	**649**			500
sex ratio ♀♂	**150**			250
fecundity	**100**			100
sexual maturity	**150**			250

Merlangius merlangus euxinus

	Number of specimens from the study I-VI,2023	**Number of specimens from the study VII-XII,2023**	**Number of specimens from the study I-XII,2023**	**Number of specimens Contract 157/13/03/2022, EAFA/IO-BAS**
length	**159**			250
weight	**159**			250
age	**159**			250
sex ratio ♀♂	**50**			100
fecundity	**50**			100
sexual maturity	**50**			100

Mullus barbatus

	Number of specimens from the study I-VI,2023	**Number of specimens from the study VII-XII,2023**	**Number of specimens from the study I-XII,2023**	**Number of specimens Contract 157/13/03/2022, EAFA /IO-BAS**
length	**266**			500
weight	**266**			500

age	**266**			500
sex ratio ♀♂	**150**			250
fecundity	**50**			не по-малко от 100
sexual maturity	**150**			250

Engraulis encrasicolus

	Number of specimens from the study I-VI,2023	Number of specimens from the study VII-XII,2023	Number of specimens from the study I-XII,2023	Number of specimens Contract 157/13/03/2022, EAFA/IO-BAS
length	**255**			500
weight	**255**			500
age	**255**			250
sex ratio	**100**			250
fecundity	**50**			100
sexual maturity	**100**			250

Squalus acanthias

	Number of specimens from the study I-VI,2023	Number of specimens from the study VII-XII,2023	Number of specimens from the study I-XII,2023	Number of specimens Contract 157/13/03/2022, EAFA/IO-BAS
length	**50**			100
weight	**50**			100
sex ratio ♀♂	**30**			30
sexual maturity	**30**			30

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