R E P O R T

BIOLOGICAL MONITORING OF TURBOT (*SCOPHTHALMUS MAXIMUS*) LANDINGS AT THE BULGARIAN BLACK SEA DURING SPRING - SUMMER SEASON 2016

Agricultural Academy, Sofia

Institute of Fisheries and Aquaculture, Plovdiv

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List of authors:

Institute of Fisheries and Aquaculture (IFA, Plovdiv)

- **Prof.Tania Hubenova, PhD;**
- **Prof.Angel Zaikov, PhD;**
- **Assis.Prof. Angelina Ivanova, PhD;**
- **Assis.Prof. Maria Gevezova, PhD;**
- **Assis. Prof.Georgi Rusenov**

1. Purpose

The purpose of the biological monitoring of the turbot catches at the Bulgarian Black Sea was to collect biological data which will be used for analysis of the catches, and for the development of data base for tracking the changes in the catches' structure over the years. The collecting of biological samples, from landing of turbot catches during spring-summer season 2016, consists of the following main tasks:

- Collecting data for landing ports, vessels selected for sampling, number of collected samples, number of studied individuals, geographical data for the catches;
- Determination of size-weight structure of the landings of turbot;
- Characterization of the reproductive biology of turbot.

2. Material and methods

2.1. Collection of biological data from landing operations

The biological data collection is performed in spring-summer period 2016 at the Bulgarian Black Sea coastal zone.

2.1.1. Ports for collection of biological data

From ports where landings of turbot are permitted - Kavarna, Balchik, Nesebar and Varna ports are used for biological data collection.

2.1.2. Vessels for sample collections

Biological data is collected from 17 ships - 9 ships from Kavarna port, 1ships from Balchik port, 6 ships from Varna port and 1 from Nesebar. The information is presented in **Table 1.**

2.1.3. Number of collected samples

Biological data is collected from 22 catches unloaded from the vessels on the 4 ports – Kavarna, Balchik, Varna and Nesebar.

Table 1. Ports and vessels used for monitoring and biological data collection during spring-summer 2016

№	Port Varna
	ships
1.	Jambi/Вн 3428

2.	Mercury/Вн 8000
3.	Traiana/Вн 4320
4.	Hermes 3/Вн 4926
5.	Hermes 4/Вн 8080
6.	Perla/BA 3480
Port of Kavarna	
7.	Viking/Вн8406
8.	Вн 3341
9.	Hishtnik/Вн 6262
10.	Вн 3341
11.	Gondola/Вн 4321
12.	Gulliver/КВ 5562
13.	Delfin/КВ 6275
14.	Hera/Кв 6241
15.	Вн 4601
Port of Balchik	
16.	Sv.George/Вн 3554
Port of Nesebar	
17.	РК 29/Бс 222

2.1.4. Number of measured turbots

The total number of fish used for biological data collection is 154, with required minimum of 100 specimens under contract D-59/05.07.2016 from IAFA.

2.1.5. Geographical data of the fish catches

The coordinates and depth of places of catch for ships that landed at port Kavarna, Balchik, Varna and Nesebar are shown in **Table 2**. For 5 ships the catch places are not presented, but the missing catch sites are close to the ones in the table.

Table 2. Coordinates and depth of the turbot catch places

№	Ship	Coordinates of catch places		Depth of catch places
		latitude	longitude	
1.	Sv. George/Вн 3554	43°16'00"	28°19'00"	60

2.	Trigona/Бс 171	43°16'30"	28°17'40"	62
3.	Perla/BA 3480	43°13'30"	28°17'40"	66
4.	Gulliver/KB 5562	-	-	64-65
5.	Delfin/KB 6275	43°12'59"	28°18'96"	60
6.	Gulliver/KB 5562	-	-	60-62
7.	Hera/KB 6241	43°19'15"	28°26'49"	60-62.5
8.	Hermes 3/Вн 4926	-	-	73
9.	Hermes 4/Вн 8080	-	-	60
10.	Traiana/Вн 4320	-	-	67
11.	Mercury/Вн 8000	43°21'00"	29°15'00"	62.5
12.	Вн 4601	43°11'83"	28°15'85"	63
13.	РК 29/Бс 222	42°38'98"	28°00'68"	65
14.	Вн 3341	43°21'36"	29°02'24"	63
15.	Gondola/Вн 4321	43°18'50"	28°21'20"	60
16.	Hermes 3/Вн 4926	43°03'00"	27°59'35"	66
17.	Hermes 4/Вн 8080	43°10'25"	29°16'17"	63
18.	Jambi /Вн 3428	42°59'65"	28°12'16"	62
19.	Hermes 3/Вн 4926	43°01'48"	27°35'24"	60
20.	Viking/Вн8406	43°25'51"	28°33'40"	62.5
21.	Вн 3341	43°16'86"	28°32'30"	63
22.	Hishtnik/Вн 6262	43°24'50"	28°40'30"	65

2.1.6. Determining the size and weight structure of the turbot landings

The measurements of the fish are made on board of the ships immediately after docking at the port, on fresh ice-cooled subjects. The weight measurement is done with a precision of 0.1 g, while that of total and standard lengths with a precision of 0.1 cm.

The preliminary data of the measurements is presented in the tables in the report.

2.1.7. Characteristics of the reproductive biology of turbot

50 specimens of turbot, caught during the spring and summer season, are bough for gender and age structure analysis. From the collected samples of the reproductive organs gender, sex ratio, gonadosomatic index, maturity stage of the gonads is determined. The correlations between the measured parameters are determined.

• Gender and sex ratio

The gender of 50 specimensof turbot caught during the spring-summer season is determined and the ratio between female and male is expressed.

• Gonadosomatic index (GSI, %)

Gonadosomatic index (GSI,%) is defined during spring - summer season based on 27 female and 23 male fish. The data from the weight measurements of the organs are be used for calculating GSI,%. It is calculated as % of weight for each gender separately, using the data from weight measures of the body and the gonads of female and male fish, according to Wootton formula (1998):

$$\mathbf{GSI\ (\%) = 100 W_G \cdot W^{-1}},$$ where:

$\mathbf{W_G}$ – weight of the gonads, g

W – body weight, g

• **Fecundity of female fish**

The fecundity is determined based on 27 specimens. Females caught in the spring-summer season of 2016. Data on the absolute and relative fertility of individuals are presented.

• **Determination of the maturity stage of the reproductive organs**

The reproductive organs collected from the fish are dissected and fixed in formaldehyde for further processing to determine the degree of maturity of the testis and ovary.

•**Determination of fish age**

The age of the fish is determined by concentric circles (zones) of the otoliths which correspond to periods of growth. Otoliths are taken out through the gills of the fish, without opening the skull, each pair is carefully detached without damaging the otoliths. After the otoliths are cleaned, the age of the individuals is determined by using stereomicroscope.

3.Results

3.1. Number of fish caught by each vessel

The total number of fish, used for biological data collection, is 154. The number of fish caught from each vessel and their percentage distribution is presented in **Fig. 1** and **Fig. 2.** An average of 7 turbot is caught from each ship, with an average weight of 15 kg. The maximum catch is 50 and the minimum – 1 fish.

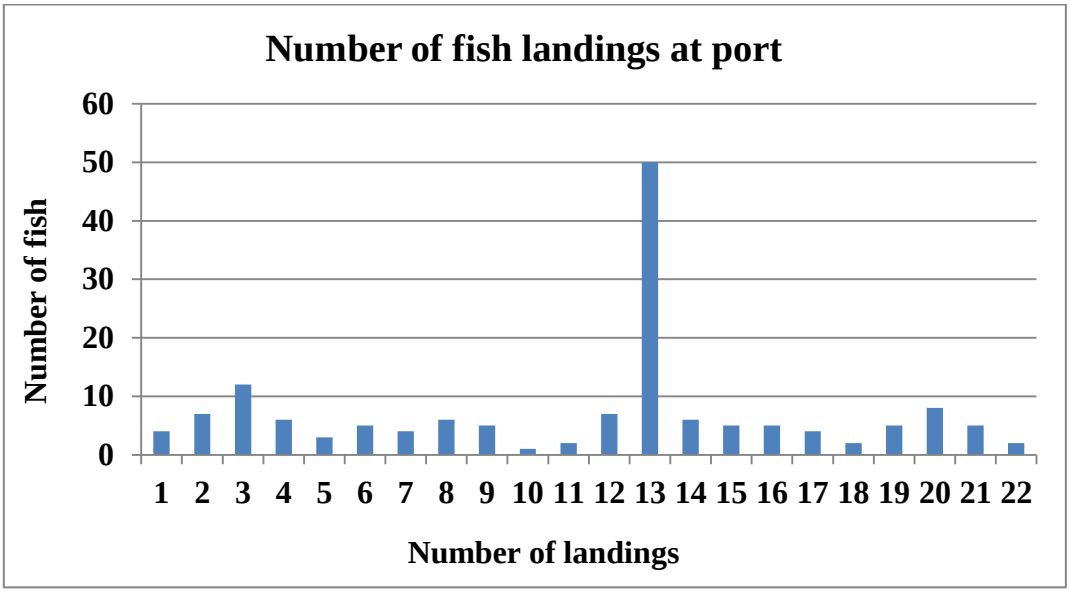


Fig. 1. Number of fish landings

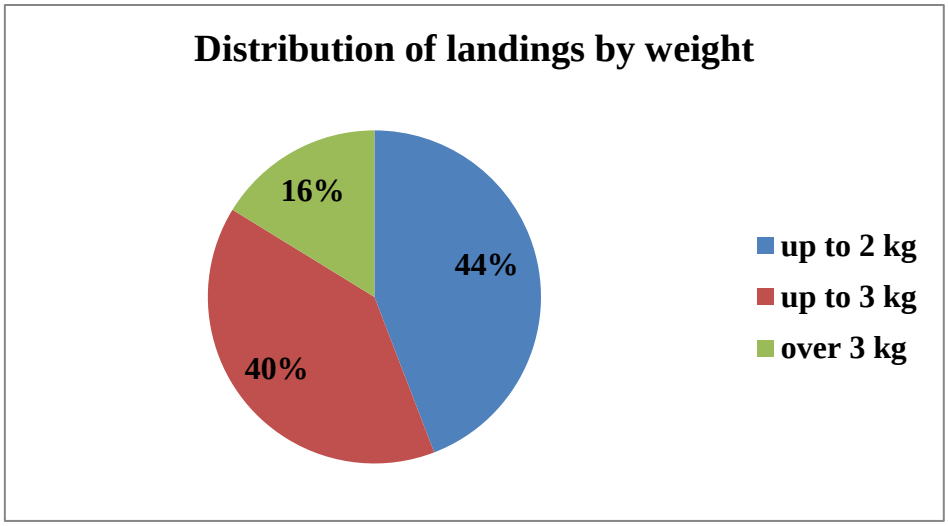


Fig. 2. Percentage distribution of catches from each vessel, %

68 turbot or 44% have weight up to 2 kg and 61 or 40% have weight of 3 kg. Therefore, 84% of all turbot have weight of 3 kg.

3.2. Weight structure of catches

The average weight of the landings of turbot is presented in **Fig. 3.** The average weight of measured turbot is 2.15 kg, and the maximum-minimum range is 4.10-1.25 kg.

Fig. 3 presents the percentage distribution of the different weight groups for all 154 measured turbot. The highest share is that of the weight group from 2.0 to 3.0 kg – 62%, followed by the weight group of 2.00 kg – 44%. With 40% is represented the group of 3.00, with 15% - 4.00 kg, and 1% represents the 4.00 kg to 5.00 kg group.

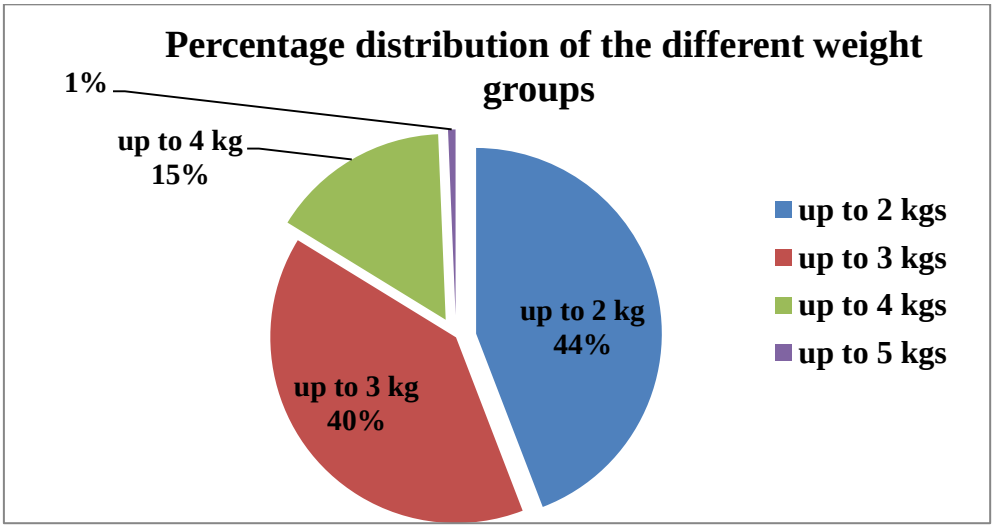


Fig. 3.Percentage distribution of individuals in different groups

3.3. Body size structure (total and standard body length) of turbot catches

The average total body length (TL, cm) of turbot from the landings are presented in **Fig. 4.** The average total length of measured fish is 49.36 cm, the maximum is 62.00 cm, while the minimum – 45.30 cm.

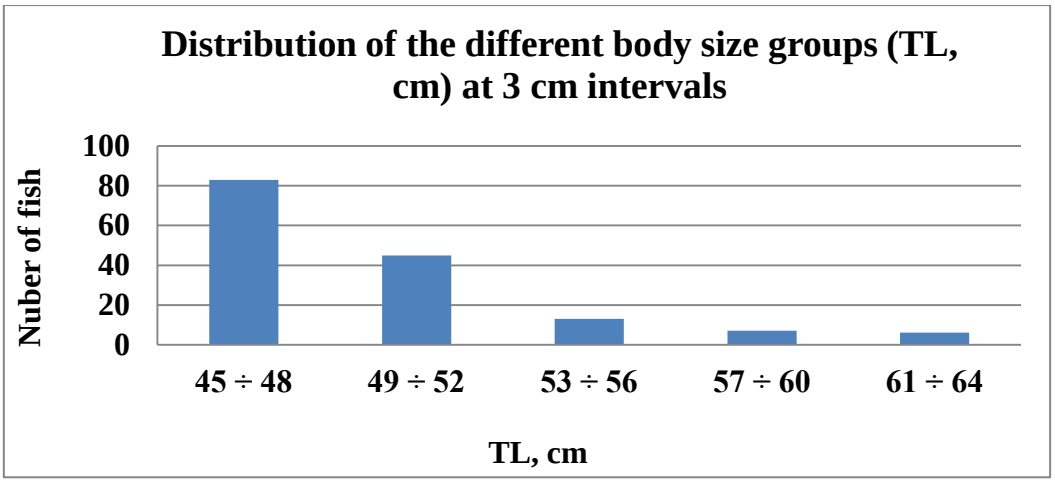


Fig. 4. Distribution of individuals in different size groups, according to the total body length (TL, cm)

The shares of the different size groups (at 3 cm intervals) showed that two size groups are the most frequently encountered: 45-48 cm and 49-52 cm. The most numerous is the group of turbot with total body length (TL, cm) within the range of 45-48 cm (53.90%), followed by 49 – 52 cm group (29.22%). Fish with total body length of 45-52 cm are 83% (128 fish) of all measured turbot. Fish with total length (TL, cm) around 45 cm, which is the minimum allowed length for turbot fishing, are 27 specimens or 17. 50% of all measured fish, but specimens with length in the range of 45-48 cm represent 53.90 % of all measured individuals.

The average standard length body length (SL, cm) of turbots from the landings are presented in **Fig. 5.** The average standard length of measured fish is 39.64 cm, the maximum - 46.50 cm, and the minimum – 35.00 cm.

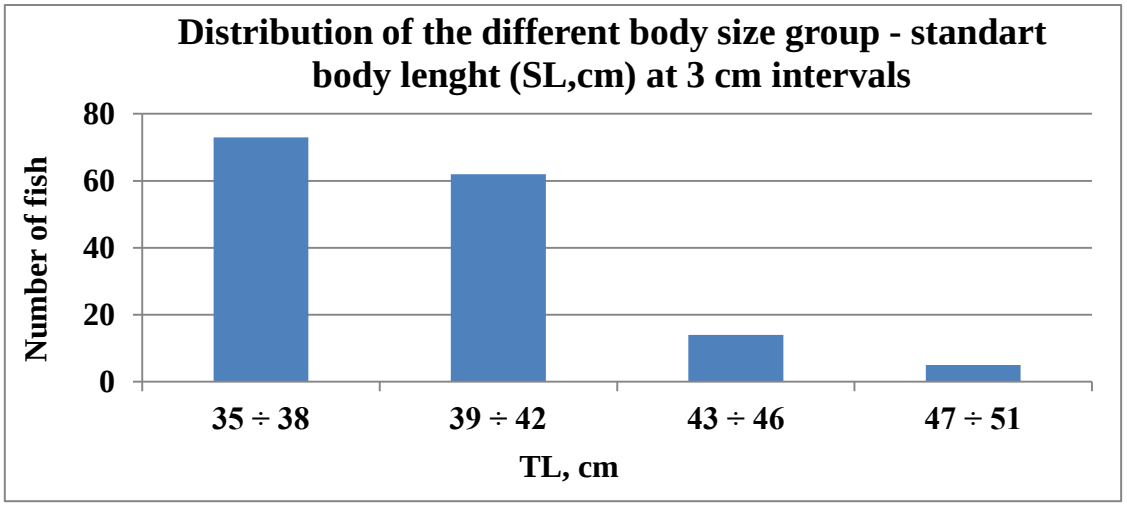


Fig. 5. Distribution of individuals in different size groups, according to the standard body length (SL, cm)

The most numerous is the group of turbot with standard body length (SL, cm) within 35-38 cm range–47.40%, followedby the 39-42 cm group (40.26%).

Individuals with standard body length in the range of 35-38 cm are 73 specimens. Fish with standard body length of 39-42 cm are 62 specimens.

The range of the total length of the body (TL, cm) is 45.30-52.00 cm, and the weight within the range of 1.25-4.10 kg. The dominant groups are those of 45.50-52.00 cm and weight of 2 – 3 kg.

The correlation of size and weight structure of the turbot landing is presented in **Fig. 6**.

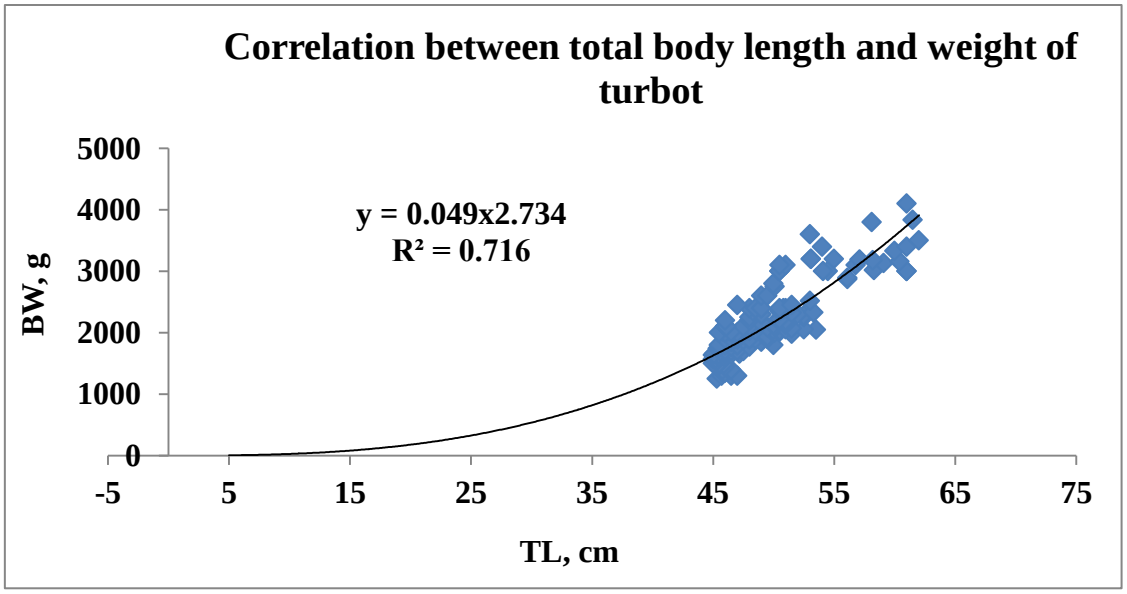


Fig.6. Correlation between total body length (TL, cm) and weight (W, g) of turbot during spring-summer season 2016

3.4. Characterization of the reproductive biology of turbot

3.4.1. Gender composition

The percentage distribution of gender composition of the fish is shown in **Fig. 7**. From a total of 154 specimens of turbot, 27 are females and 23 are males. The ratio between female and male is 54:46%, or 1, 17:1.

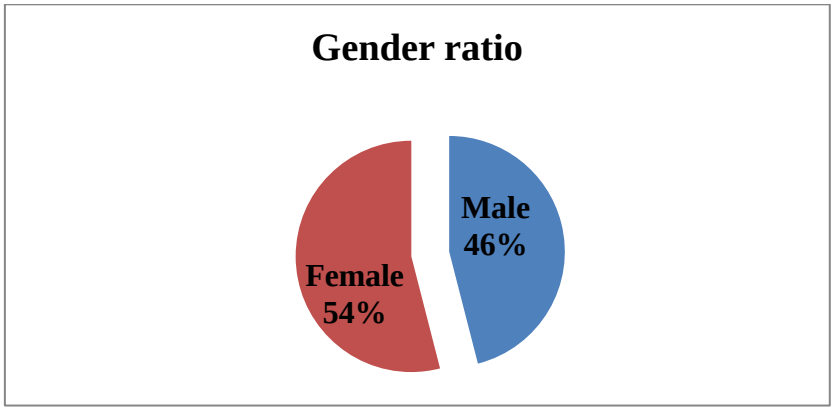


Fig. 7.Gender ratio

3.4.2. Gonadosomatic index (GSI, %)

The average weight of female fish, measured in March 2016, is 2.82 and it is within the range 2.53-3.23 kg. The average total length is 51cm and it is within the range 49.5-55.50 cm. The average age of the fish is 5 years. The ovary weight is between 95.3 and 240 g. (**Table 3**).

Table 3. Values of body weight, total and standard body length, gender, age and ovary weight for female turbot in March 2016

№	BW (kg)	TL (cm)	SL (cm)	Sex	Age	OW (kg)
1	2.53	51.00	40.50	F	5	0.103
2	2.55	53.00	42.50	F	5	0.180
3	2.60	50.50	40.50	F	5	0.154
4	2.64	52.50	43.40	F	5	0.135
5	2.69	49.50	41.30	F	4	0.069
6	2.70	50.50	41.50	F	5	0.125
7	3.03	52.20	42.40	F	5	0.095
8	3.13	53.00	43.20	F	5	0.179
9	3.14	50.70	42.00	F	5	0.210

10	3.23	55.50	45.50	F	6	0.240

The gonadosomatic index (GSI. %) for March 2016 has an average of 5.25% with the maximum and minimum values being 7.44% and 2.58% respectively (**Table 4**).

Table 4. Values of body weight, GSI(%) and age in female turbot in March 2016

March 2016			
Number of individuals	**BW (kg)**	**GSI (%)**	**Age (years)**
1	2.53	7.06	5
2	2.55	7.44	5
3	2.60	5.14	5
4	2.64	6.71	5
5	2.69	3.15	4
6	2.70	5.96	5
7	3.03	5.74	5
8	3.13	2.58	5
9	3.14	4.08	5
10	3.23	4.66	6
Average value	**2.82**	**5.25**	**5**

The average weight of female fish measured in May 2016 is 2.54 kg and it is within the range of 1.47-5.98 kg. The average length is 45 cm and it is within the range 38.00 - 64.00 cm. The average age of the fish is 5 years. The ovary weight is between 40.00 and 340 g. (**Table 5**).

Table 5. Values of weight, total and standard body length, gender, age and ovary weight in female turbot in May 2016

№	**BW (g)**	**TL (cm)**	**SL (cm)**	**Sex**	**Age (years)**	**OW (g)**
1	1.950	39.00	36.00	F	3	0.170
2	2.830	47.00	40.50	F	5	0.340
3	2.300	42.00	37.00	F	4	0.160
4	1.870	40.00	36.00	F	3	0.240
5	1.540	39.50	36.00	F	3	0.040

6	2.670	48.00	43.50	F	5	0.080
7	5.980	64.00	58.00	F	9	0.320
8	1.650	39.00	37.00	F	4	0.140
9	5.260	60.50	52.00	F	8	0.250
10	2.640	45.00	39.50	F	5	0.210
11	1.560	38.00	34.00	F	4	0.060
12	2.650	44.50	43.00	F	5	0.200
13	2.180	44.50	39.00	F	5	0.140
14	1.940	41.50	33.80	F	4	0.170
15	1.990	43.00	36.50	F	4	0.120
16	1.840	43.00	35.00	F	3	0.280
17	3.820	54.00	49.00	F	6	0.130
18	2.430	46.50	43.00	F	5	0.120
19	3.290	49.50	46.00	F	6	0.130
20	2.580	49.50	42.00	F	5	0.140
21	2.410	46.50	40.00	F	4	0.090
22	2.630	46.00	42.00	F	4	0.210
23	1.660	40.50	36.00	F	4	0.070
24	1.470	38.00	34.00	F	3	0.110
25	1.810	39.00	37.00	F	3	0.130
26	2.630	45.00	42.00	F	5	0.180
27	2.870	47.00	42.00	F	5	0.310
28	3.640	51.50	47.00	F	6	0.160

The gonadosomatic index (GSI, %) in May 2016, has an average of 12.93% with maximum and minimum values being 42.17% and 3.40% respectively (**Table 6**).

Table 6. Values of body weight, GSI(%) and age in female turbot in May 2016

May 2016			
Number of individuals	**BW (kg)**	**GSI, %**	**Age (years)**
1	1.47	7.48	3

2	1.54	6.03	3
3	1.56	4.94	4
4	1.65	7.18	4
5	1.66	3.95	4
6	1.81	3.40	3
7	1.84	8.48	3
8	1.87	6.42	3
9	1.94	5.43	4
10	1.95	6.96	3
11	1.99	8.76	4
12	2.18	8.72	5
13	2.30	7.55	4
14	2.41	7.98	4
15	2.43	7.95	5
16	2.58	12.83	5
17	2.63	4.75	5
18	2.63	15.22	4
19	2.64	10.80	5
20	2.65	5.35	5
21	2.67	12.93	5
22	2.83	12.01	5
23	2.87	25.97	5
24	3.29	38.46	6
25	3.82	42.17	6
26	5.26	29.96	8
27	5.98	37.34	9
Average value	**2.54**	**12.93**	**5**

The average weight of female fish, measured in July 2016, is 2.01 kg and it is within the range of 1.49-3.84 kg. The average age of the fish is 4 years. The gonadosomatic index (GSI, %), for July 2016 has an average value of 2.52% with maximum and minimum values being 6.9% and 1.3% (**Table 7**).

Table 7. Values of body weight, GSI (%) and age in female turbot in July 2016

July 2016			
Number of individuals	**BW (kg)**	**GSI (%)**	**Age (years)**
1	1.49	1.90	3
2	1.50	2.43	3
3	1.50	3.35	3
4	1.57	3.10	3
5	1.64	1.38	3
6	1.65	3.59	3
7	1.66	1.74	3
8	1.66	3.84	3
9	1.70	1.42	3
10	1.72	6.91	3
11	1.74	2.17	3
12	1.88	2.73	3
13	1.91	1.66	3
14	1.94	2.40	4
15	2.01	2.20	4
16	2.02	3.01	4
17	2.03	1.92	4
18	2.04	1.92	4
19	2.05	2.96	4
20	2.06	1.53	4
21	2.07	1.52	4
22	2.15	3.03	4
23	2.18	1.58	5
24	2.22	2.66	5
25	2.87	3.69	5
26	3.33	2.18	6
27	3.84	1.30	7
Average value	**2.01**	**2.52**	**4**

The dynamics of GSI (%) during spring – summer period is presented in **Table 8**.

Table 8. Values of GSI, % in females during the spring-summer period 2016

Month	March	May	July
BW (kg)	2.82	2.54	2.01
GSI (%)	5.25	12.93	2.52
age	5.00	4.59	3.81

The gonadosomatic index (GSI, %) for males, caught in July 2016, has an average value of 0.42%. The maximum and minimum recorded values are respectively 0.72% and 0.04% (**Fig. 8**).

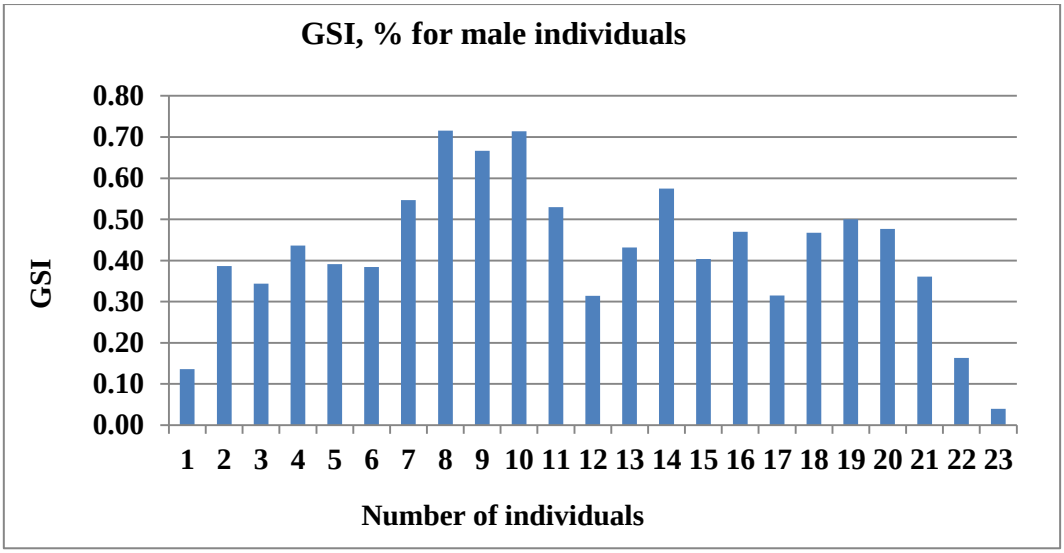
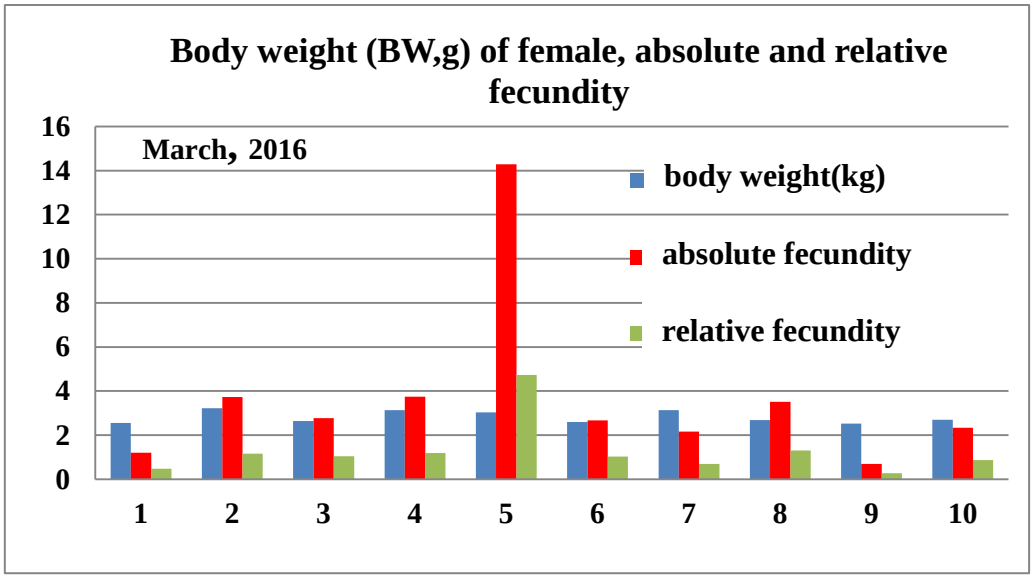


Fig. 8. Values of GSI, % for male individuals

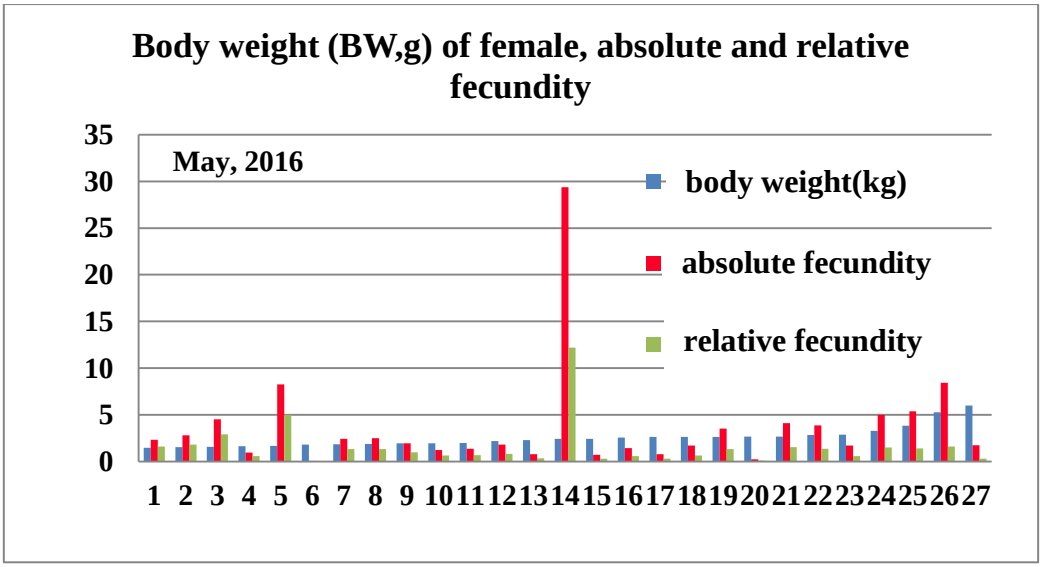
3.4.3. Fecundity of female fish

In March 2016 the fecundity of 10 specimens is determined, with an average body weight of 2.82 kg, total body length of 54 cm, and average age of 5 years (**Fig. 9**).

The absolute fecundity measured has an average of 3 711 242.6 eggs and the relative fecundity of number of 1 276 621.508 eggs.



Фиг.9.Body weight (BW) of female, absolute and relative fecundity in March 2016



Фиг.10.Body weight (BW,g) of female, absolute and relative fecundity for May 2016

In May 2016 the fecundity of 27 specimens is determined, with an average body weight of 2.54 kg, total body length of 45 cm and average age of 5 years (**Fig.10**).

The measured absolute fecundity has an average of 3 664945.0 eggs and the relative fecundity has an average of 1 546 324.0 eggs. The established fecundity is almost a twice lower than the one in previous studies - 5-6000 000 eggs. Such fecundity is observed in fish weighing over 5-6 kg, whose presence in the catches is low. Fecundity increases with the increasing of body weight (**Fig. 11** and **Fig. 12**). The lower values of fecundity can be explained by the lower weight of the individuals studied, but the lower weight is within the average weight values obtained on the basis of all 1626 studied specimens - 2.54 kg.

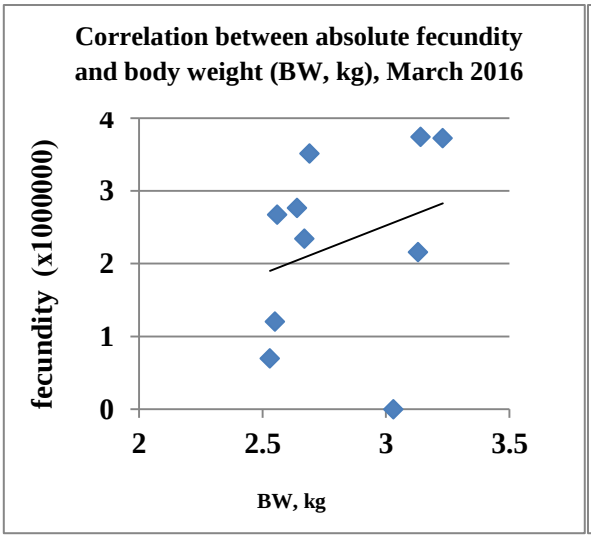


Fig. 11.Correlation between fecundity and weight, March 2016.

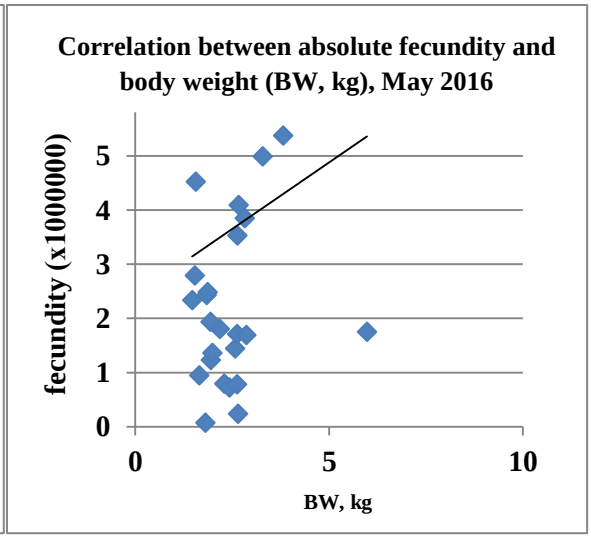


Fig.12.Correlation between fecundity and weight, May 2016.

3.4.4. Determination of the maturity stage of the gonads

In the ovary and testis of the studied individuals, III-IV degree of maturity is established in March, and IV-V is reached in May. The development of the gonads of the turbot during the reproduction season has a group-synchronous character. At least two populations of oocytes can be differentiated in the ovary at the same time during reproduction - a synchronous cohort of oocytes with definitive size, ovulating or ready for ovulation, and a more heterogeneous cohort of smaller vitellogenic oocytes, of which the first is to be filled in.

During the reproductive period (in May) in the ovary there are several stages of oocyte development, which is a reflection of the group-portional nature of their ovulation. There are at least 5 structural elements - empty follicular envelopes of ovulated oocytes, atretic oocytes, ovulation-ready oocytes in definitive size, and smaller vitellogenic oocytes forming the next ovulatory wave. In July the reproductive organs reach VI-II degree of maturity. A post-ovulatory ovary, containing empty follicular envelopes, atretic follicles in different stage of resorption, and a number of previtellogenic oocytes, which will form the new generation of folicles for the next reproduction period, is observed.

3.4.5. Age structure and growth

To determine the age composition of the turbot at the Bulgarian Black Sea coast during the spring - summer season of 2016, 50 pairs of otoliths are investigated. The age structure of the 27 female specimens examined ranged from 3 to 7 year olds, dominant are the 3 and 4 years old fish (**Fig. 13**). They make up for 81% of the total number. Most fish are at the age of 3

years - 48%, followed by 4-year-olds, which are 33%. Five year olds are 11% and those at 6 and 7 years old are a small percentage of the statistical sample.

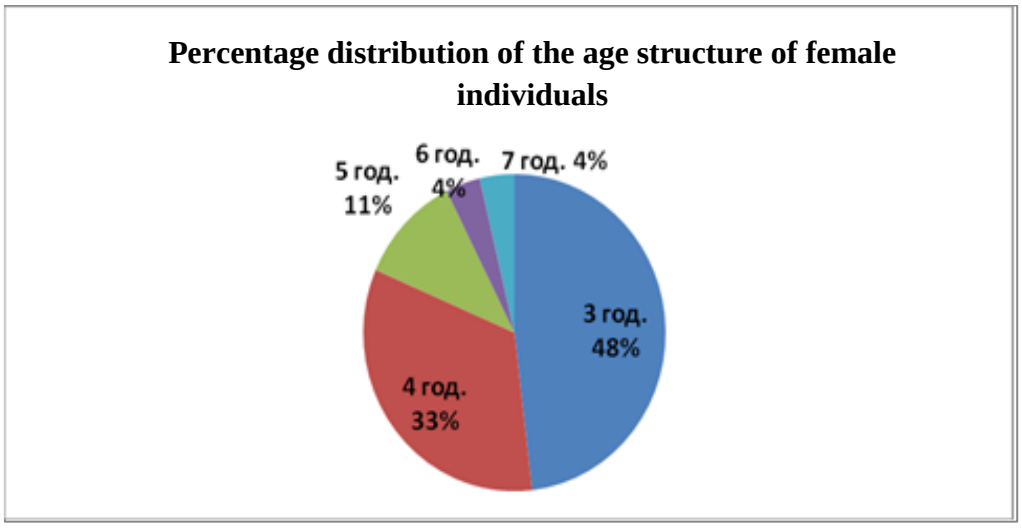


Fig. 13.Percentage distribution of the age structure of female individuals

The age composition of the investigated 23 male turbot specimens included 3, 4, 5 and 6-year old individuals, with 4-year olds prevailing (**Fig. 14**). The percentage participation of sexually mature males at this age is about 35%, followed by 5 years old at 31% and 6 year olds are 4% of the total number of turbot landings.

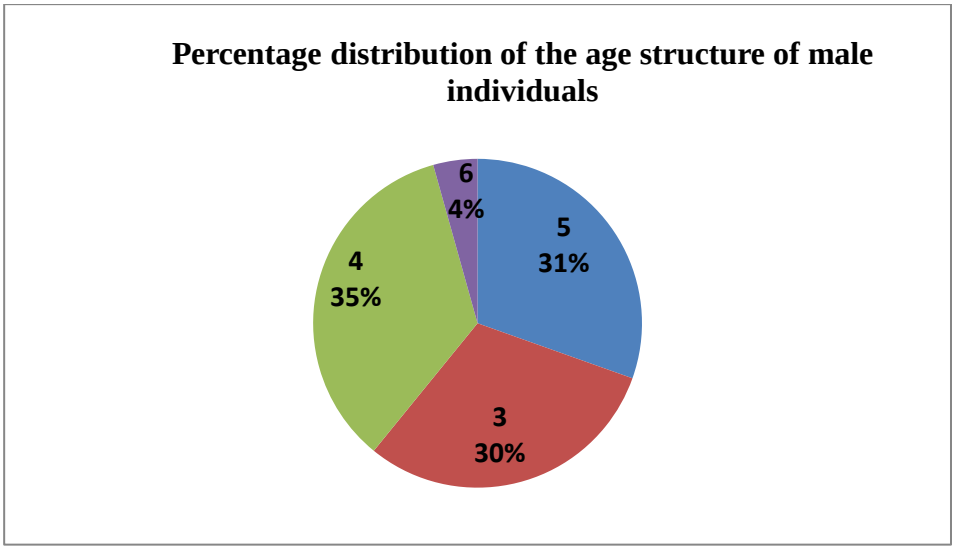


Fig. 14.Percentage distribution of the age structure of male individuals

The analysis of the results shows that the percentage participation of the mmature adults at age of 3 is 28%, at age of 4 - about 24%. Turbots at age of 5 are 20% and over 5 represent a negligible percentage of the statistical sample (**Figures 13 and 14**).

In **Fig. 15** and **16** is shown that after 45 cm the female specimens grow more in length with the age than the males.

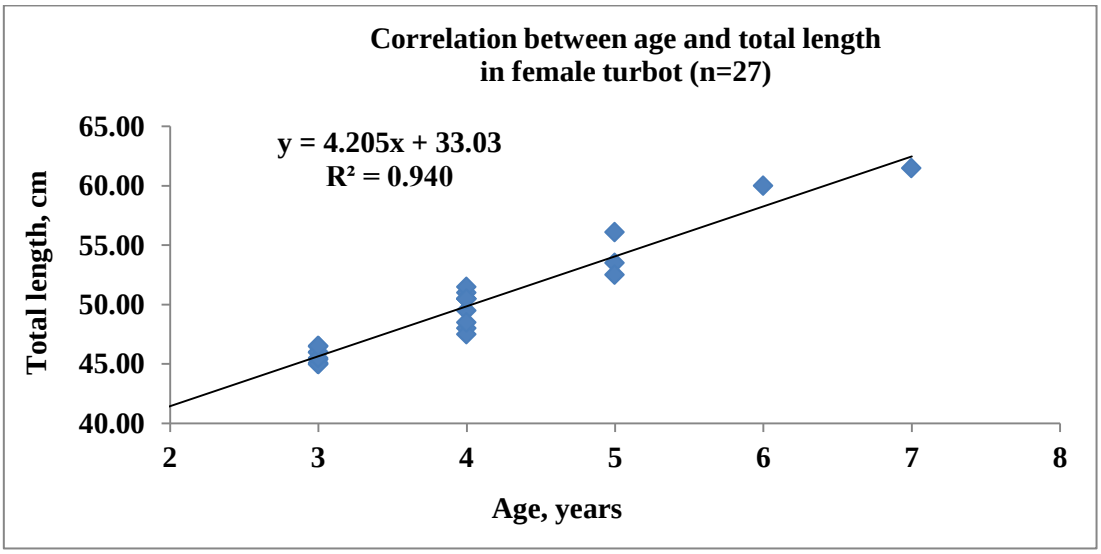


Fig. 15.Correlation between age and length of female turbot

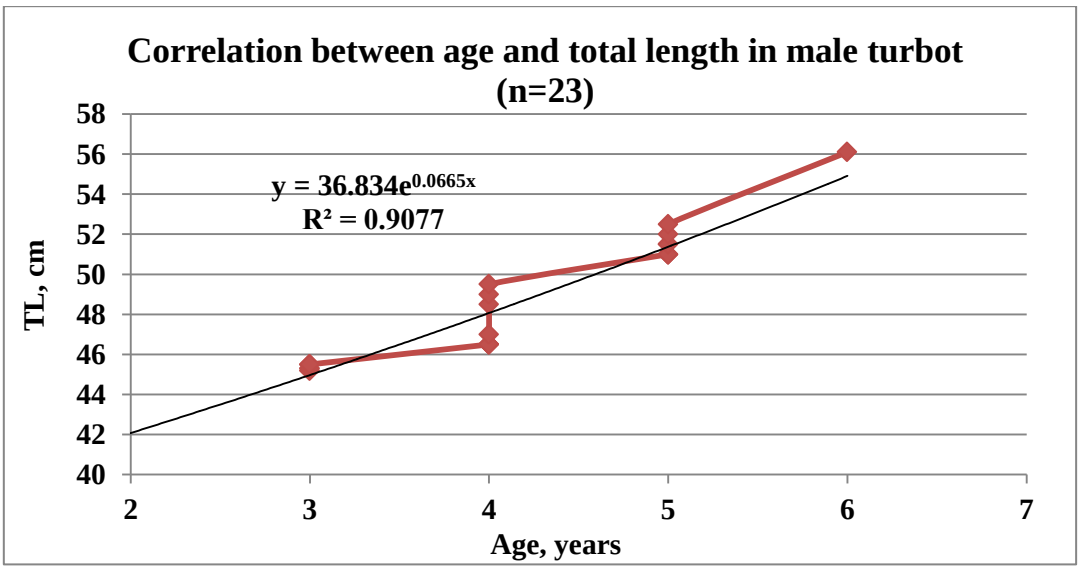


Fig. 16. Correlation between age and total length in male turbot (n=23)

The correlation between the total body length and age in males and females is shown in **Figures 15** and **16**. Individuals with total body length up to 49.00 cm are dominantly male and with length of 53.5 cm the percentage participation of the females increases. The group with total body length of over 60 cm is represented only by females.

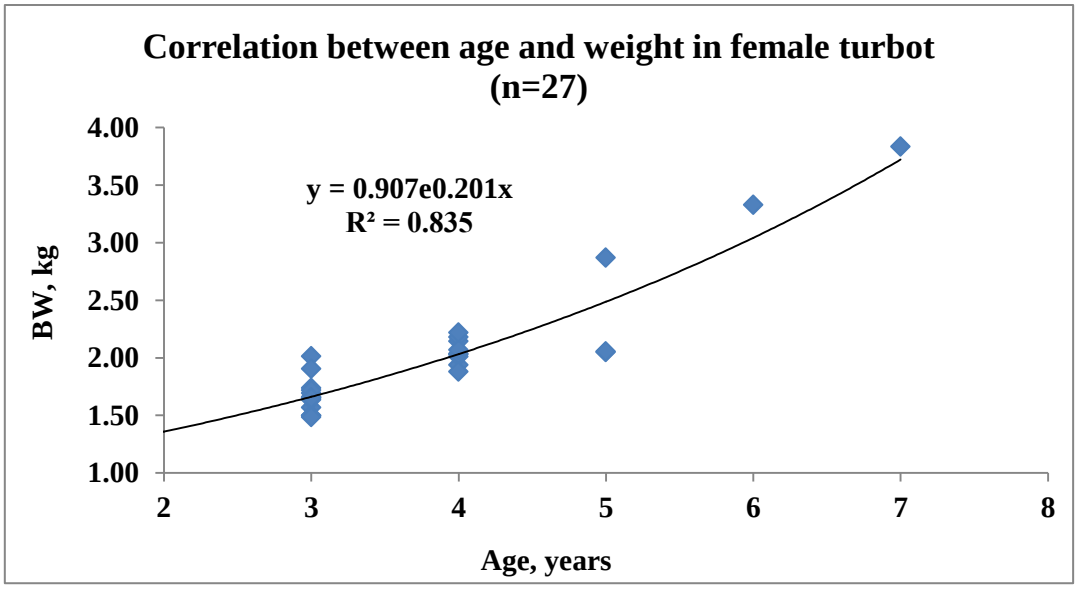


Fig. 17. Correlation between age and weight in female turbot

Figures 17 and 18 show the weight increase of the turbot depending on age and gender. From the figures shown it can be seen that after 45 cm the female specimens grow faster and reach a higher weight with age than the males.

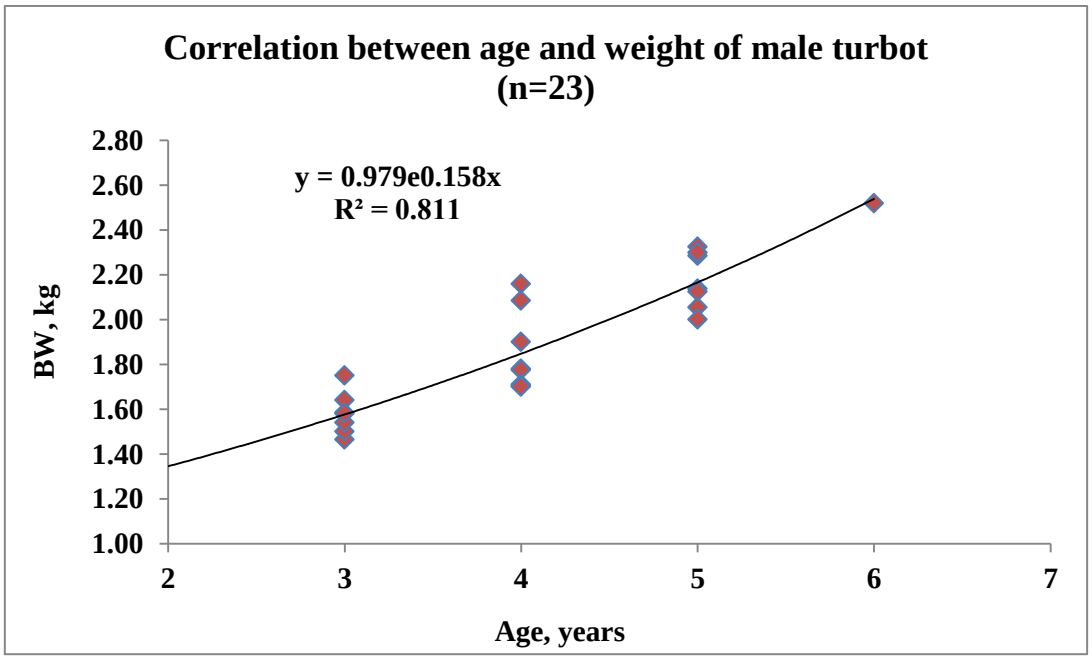


Fig. 18. Correlation between age and weight of male turbot

4. Conclusions and Recommendations

Based on the results of the biological monitoring carried out on turbot catches at the Bulgarian Black Sea coast in spring and summer of 2016 we can draw the following conclusions and recommendations:

• During the spring and summer of 2016 the fishing ships catch an average of 7 specimens of turbot and an average of 15 kg per day. The maximum number of fish from the landings of the monitored ports is 50 fish and the minimum is 1 fish.

• A total of 22 landings of the monitored ports are measured. 154 specimens of turbot are investigated with an average weight of 2.15 kg, an average total body length of 49.36 cm and an average standard body length of 39.64 cm. The maximum measured weight is 4.10 kg and the minimum measured is 1.25kg.

• 68 turbot or 44% have weight up to 2 kg and 61 or 40% have weight of 3 kg. Therefore 84% of all turbot have weight of 3 kg.

• The highest percentage share is that of the weight group from 2.0 to 3.0 kg – 62%. followed by the weight group of 2.00 kg – 44%. With 40% is represented the group of 3.00 kg, with 15% the group of 4.00 kg and 1% represents the 4.00 kg to 5.00 kg group.

• Female individuals after reaching 45 cm grow in length and weight faster than males.

• The gonadosomatic index (GSI, %) for females in March has an average value of 5.25%. with the highest value being 7.44% and the lowest – 2.58%. GSI, % for May 2016 has an average value of 12.93% with maximum and minimum values being 42.17% and 3.40%. The gonadosomatic index (GSI, %) for July 2016 has an average value of 2.52% with maximum and minimum values being 6.9% and 1.3%

• The gonadosomatic index (GSI. %) for males caught in July 2016 has an average value of 0.42%. The maximum and minimum recorded values are respectively 0.72% and 0.04%

• With an average body weight of 2.5-2.8 kg for female specimens an average absolute fecundity of 3.7 Mill. eggs is established and an average relative fecundity of 1.4 Mill. eggs, which is almost a twice lower than the one found in previous years - 5-6 Mill. Such fecundity is seen in fish weighing over 5-6 kg, whose presence in the catches is low.

• The lower values of fecundity compared to the data obtained by other authors can be explained by the lower weight of the studied specimens, but this is within the average weight values obtained on the basis of all 1626 studied individuals.

• 3, 4 and 5 year old fish with a body weight of 2.5 - 3.0 kg and a total length of 45 - 50 cm have the most significant impact on stock replenishment.

• The ovary and testis of the studied individuals in March reached III-IV degree of maturity and in May - IV-V degree. In July the reproductive organs reached VI-II degree of maturity

• The age composition of the investigated 23 male turbot specimens included 3, 4, 5 and 6-year old individuals, with 4-year olds prevailing. Percentage participation of sexually mature males at this age is about 35%, followed by 5 years old at 31% and 6 year olds are 4% of the total number of turbot landings. The analysis of the results shows that the percentage participation of the mature adults at age of 3 is 28%, at age of 4 - about 24%. Turbots at age of 5 are 20% and over 5 represent a negligible percentage of the statistical sample.

• According to references the age limit reached by turbot is 12-13 years, but no individuals at this age are found during the study. The maximum age is 7-year-old females, which account for only 4% of the total number of specimens.

• The percentage ratio between females and males is 54:46% or 1.17:1

• In the group of these individuals with an absolute body length of up to 50.50 cm the male specimens prevail, at the length of 53.50 cm the percentage of female participation increases, and at a length above 60.00 cm are only the females.

We are grateful to NAFA, Burgas, Agricultural Academy, Sofia and the branch fishing sector for their assistance during the research activities under contract № D - 59 / 05.07.16 by NAFA.