Morphometric variability in round goby *Neogobius melanostomus* (Perciformes: Gobiidae) from the Sea of Azov

Морфологическая изменчивость бычка-кругляка *Neogobius melanostomus* (Perciformes: Gobiidae) Азовского моря

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A total of 38 morphometric characters of the round goby *Neogobius melanostomus melanostomus* (Pallas, 1814) were studied in its native range in the Sea of Azov. The aim was to assess joint effect of sexual dimorphism and size variability on overall variability within groups of samples (populations) using appropriate methods of traditional statistical analysis (one-dimensional and multivariate statistics). Sex and size-dependent variability was studied based on model samples of males and females of different size. Most of the studied morphometric characters of round goby from the Sea of Azov demonstrated statistically significant sex- and size-dependent in-group variability. The pattern of the variability suggests that, for a comparison of round goby from different sea regions and between populations, separate samples of males and females within the range 9–13 cm SL should be examined in order to minimise the effect of the size and sex factors. The approach of searching for the most informative size range could be useful not only for further studies of infraspecific variation but for comparisons between morphologically close gobiin species.

С целью оценки совместного влияния полового диморфизма и размеров рыб, используя соответствующие методы статистического анализа (одномерные и многомерные) изучена морфологическая изменчивость бычка-кругляка *Neogobius melanostomus melanostomus* (Pallas, 1814) из Азовского моря – нативного ареала вида. Всего изучено 38 пластических признаков самок и самцов разных размеров. Подавляющее большинство изученных морфологических признаков бычка-кругляка Азовского моря демонстрируют статистически значимую половую и размерную изменчивость. Характер морфологической изменчивости показывает, что при сравнительных исследованиях, с целью минимизации влияния размеров и пола рыб, анализируемые выборки должны включать рыб одного пола в интервале длин (SL) 9–13 см. Аналогичный подход, основанный на выявлении наиболее информативного размерного интервала, может быть использован не только для изучения внутривидовой изменчивости, но и в таксономических исследованиях близких видов бычковых рыб.

Key words: variability, sex, size, statistical analysis, round goby, Sea of Azov

Ключевые слова: изменчивость, пол, размеры, статистический анализ, бычок-кругляк, Азовское море

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INTRODUCTION

The native range of the round goby *Neogobius melanostomus melanostomus* (Pallas, 1814) includes basins of the Black and Marmara seas and the Sea of Azov where it inhabited mostly lower reaches of rivers and the whole brackish Sea of Azov. In the latter, the round goby is one of the most abundant and commercially valuable species (up to a third of annual fish harvest in 2008–2015). *Neogobius melanostomus affinis* (Eichwald, 1831) distributed in the Caspian Sea basin, mostly inhabits marine and estuarine habitats.

Round goby are spread across the sea from almost freshwater areas with a salinity of about 0.5‰ (eastern Gulf of Taganrog) to over 15‰ (near the Kerch Strait). The most dense aggregations of round goby in summer and autumn, when they intensively forage, occur in areas with high biomass of zoobenthos with a predominance of bivalve molluscs in the western part of the sea. Round goby do not perform large-scale migrations and only move to coastal areas in spring and away from the coast to slightly deeper areas in autumn.

In the second half of the last century, the round goby began to expand the area of distribution upstream the rivers Danube, Dniester, Southern Bug, Dnieper, Don, and Volga (Smirnov, 2001; Pinchuk et al., 2003; Bogutskaya et al., 2004). In 1990, with the ballast water of ships the round goby was introduced into the Baltic Sea (Skòra, Stolarski, 1993), and began to actively spread, first, in the Baltic Sea (Ojaveer, 2006) and then in the coastal zone of the North Sea. The round goby was introduced with the ballast water of ships into the North American continent where it was first collected in 1990 and then became successfully established and currently occurs in the basin of all the Great Lakes (Jude et al., 1992; Corkum et al., 2004; Kornis et al., 2012). So far, the round goby has become an object of intensive research due to a significant expansion of its nonindigenous range and the impact on native aquatic ecosystems.

Morphology of the round goby in its native range is not sufficiently studied; in particular, age-and-size variability and sexual dimorphism are poorly known which is critical to ensure comparability across samples and populations. Some aspects of morphological variability and sex-dependent morphometric differences in round goby have been studied earlier. Thus, it was shown that some morphometric characters correlated with body size and sex in the round goby from the Dnieper-Bug estuary (Bil'ko, 1971; Bil'ko, Vybornaya, 1972). Smirnov (1986) reported the presence of sexual dimorphism in this species in the Molochny Liman estuary and the Danubian coast of the Black Sea but found no difference between males and females in the Sasyk Lagoon.

The absence of sex-dependent differences was also reported for the round goby outside its native range, e.g., the Kuibyshev and Saratov reservoirs (Shemonaev, 2006) and the middle reaches of the Danube River (Simonović et al., 2001). These conclusions were based solely on the results of statistical analyses using Student's test but without providing two mandatory conditions for its applicability (Lakin, 1980; Orlov, 2002): 1) the use of Student's test assumes a normal distribution in samples; this assumption can only be violated with large samples, and 2) compared samples need to have the same variance. However, the publications mentioned above did not discuss the question of applicability of Student's t-test, even when very small samples were compared.

Data on sexual dimorphism and size variability of morphometric characters in the round goby from the Sea of Azov were first published only recently. Zabroda (2009) and Zabroda & Diripasko (2009) reported on size-dependent variability in males and compared morphometric characters in males from two different localities in view of the revealed features of size variability. These data demonstrated further needs for a comprehensive and integrated approach to assess joint effect of sexual dimorphism and size variability on overall variability within groups of samples (populations) and variation between groups using appropriate methods of statistical analysis. So, the aim of this study was to analyse sex and size variability based on model samples of round goby males and females of different size.

MATERIAL AND METHODS

The material, containing 50 females and 65 males, was collected in early spring (when matured ovaries do not affect female body shape) in 2009 from catches of gobies done by mechanised dredges in North-Western region (at a location at 46°10´N, 35°20´E) of the Sea of Azov.

As round goby males attain larger maximum size than that in females, the statistical analyses were performed on both males and females within one and the same standard length (SL) range, 5–15 cm. Males of the entire SL range (5–18 cm) were also analysed.

A total of 40 measurement were done (Fig. 1) using a calliper to a nearest 0.1 mm. For the subsequent statistical processing, relative morphometric characters as percentage of SL and head length (HL) were used.

For statistical processing of data, methods of one-dimensional and multivariate statistics were used.

In order to be able to compare the results of different methods, both parametric and nonparametric statistical tests were applied. As the first step, a Shapiro-Wilk normality test was performed to check if the variables (morphometric characters) follow a normal distribution, a mandatory condition to be met for using parametric tests.

Nonparametric Mann-Whitney test (*U*-test) and parametric Student's test (*t*-test) were used to infer about statistical significance of differences between the morphometric characters.

Statistical correlation between the characters and SL was measured using two coefficients: 1. Pearson's correlation coefficient for normally distributed variables and 2. Spearman's rank correlation coefficient for unknown or non-normal distributions because it is less sensitive to non-normality (Sachs, 1976; Rebrova, 2002). Correlation coefficient (Pearson's *r*) absolute values from 0.50 to 0.75 indicate moderate to good correlation, and *r* values from 0.75 to 1 indicate very good to excellent correlation between variables (Dawson & Trapp, 2004).

To evaluate statistical dependence of morphometric characters on SL and sex, the following methods were also used:

1. Multivariate analysis of variance (MANOVA). Despite the fact that this method is, in fact, parametric and does not have any immediate nonparametric analogues, it is applicable as being not particularly robust to departures from multivariate normality (Anderson, 2001). The MANOVA analysis was preceded by testing for distribution normality of either of morphometric variables and these data were taken into account when interpreting the results.

2. Univariate analysis of variance (ANOVA) which analyses the differences among group means and their associated procedures such as descriptive statistics and correlations between variables among and between groups (in our case, size groups with a 2 cm SL interval). Since this method of test statistics are applicable for parametric statistical hypotheses, the Kruskal-Wallis test, which is a nonparametric analogue of ANOVA, was also used.

For the purpose of classification of samples, cluster analyses (using the Complete Linkage method and the Euclidean distance as a proximity/similarity measure) were implemented using Microsoft Excel and Statistica 6.0 software.

Abbreviations: n, number of specimens; SD, standard deviation; *r*, Pearson's correlation coefficient: *R*, Spearman's rank correlation coefficient; *U*-test, Mann–Whitney's nonparametric test; *t*-test, Student's test. For abbreviations of measurements see Fig. 1.

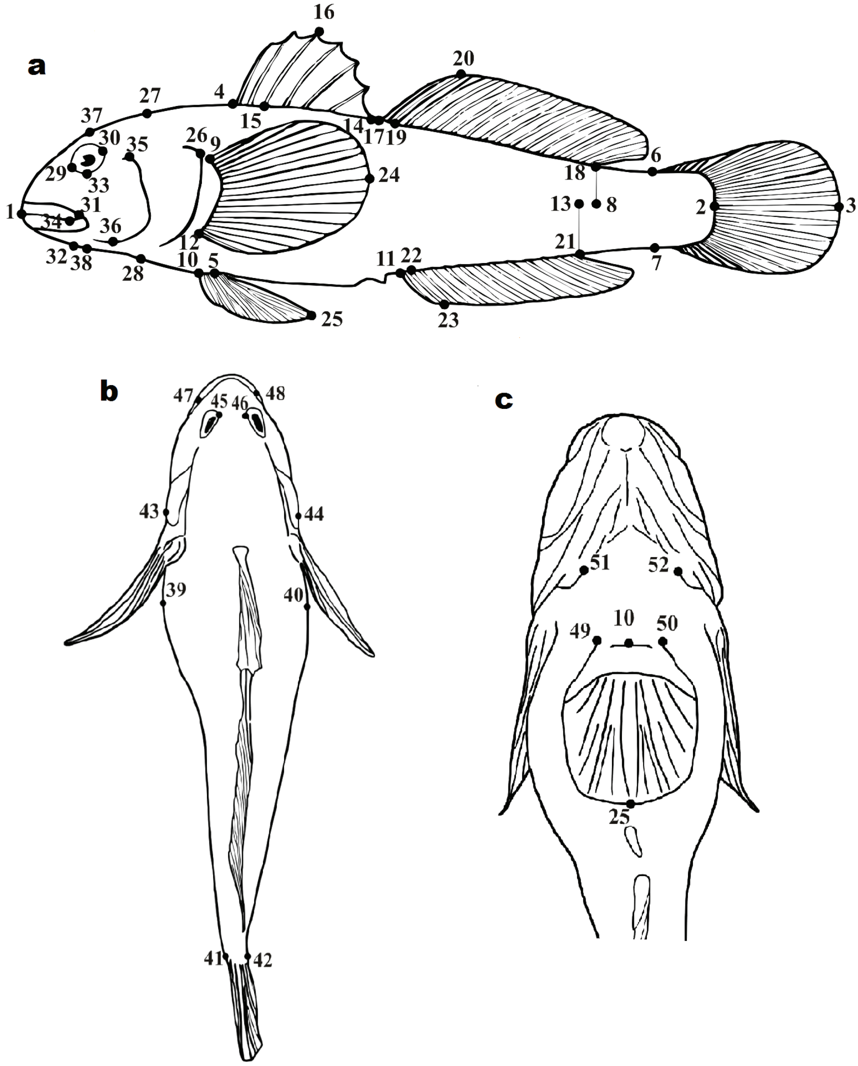

Fig. 1. Scheme of morphometric measurements round goby: **a)** side view, **b)** top view, **c)** a bottom view. 1–2, standard length (SL); 1–3, total length (TL); 1–4, predorsal distance (aD); 1–9, prepectoral distance (aP); 1–10, prepelvic distance (aV); 1–11, preanal distance (aA); 1–26, head length (HL); 1–29, preorbital distance (ao); 1–31, upper jaw length (lm); 1–32, lower jaw length (lmd); 2–3, caudal fin length (lC); 2–8, postdorsal distance (pD); 2–13, caudal peduncle length (pl); 4–5, maximum body depth (H); 4–14, length of first dorsal fin base (l1D); 6–7, minimum body depth (caudal peduncle depth) (h); 9–10, pectoral-pelvic distance 1 (from upper end of pectoral fin base) ($P^{(sup)}$–V); 9–12, width of pectoral fin base (iP); 9–24, pectoral fin length (lP); 10–11, pelvic-anal distance (V–A); 10–12, pectoral-pelvic distance 2 (from lower end of pectoral fin base) ($P_{(inf)}$–V); 10–25, pelvic fin length (lV); 11–21, length of anal fin base (lA); 15–16, first dorsal fin depth (h1D); 17–18, length of second dorsal fin base (l2D); 19–20, second dorsal fin depth (h2D); 22–23, anal fin depth (hA); 26–30, postorbital distance (op); 27–28, head depth at nape (hcz); 29–30, horizontal eye diameter (o); 33–34, distance between eye and corner of mouth (or); 35–36, cheek depth (hop); 37–38, head depth through middle of eye (hco); 39–40, maximum body width (iH); 41–42, minimum body width (caudal peduncle width) (ih); 43–44, head width (ic); 45–46, interorbital distance (io); 47–48, mouth width (ir); 49–50, width of pelvic fin base (iV); 51–52, isthmus width (ist).

RESULTS

Data of statistical analyses applied for two samples, males (49 specimens) and females (50 specimens), both groups including fish 5–15 cm long, are shown in Tables 1 and 2. Results of the analysis of size-dependent morphometric variability of males within the entire range of SL (5–18 cm) are given in Table 3.

The results of the Shapiro-Wilk test showed that most of the examined characters are normally distributed; the characters demonstrating some departures from the normal distribution either in males or in females are marked with # in Tables 1–3.

The degrees of correlation estimated by the Pearson's correlation coefficient and Spearman's rank coefficient are very similar. The exceptions were two characters in males (Table 3): minimum body width and second dorsal fin depth.

For the minimum body width index which departs from the normal distribution in males, the Pearson's coefficient is statistically significant (p=0.05) while the Spearman's coefficient is not. For the second dorsal fin depth index which is normally distributed, the results are opposite.

For most of the examined characters, a statistically significant correlation of moderate to good power (*r* absolute values 0.5–0.75) was revealed while some characters are strongly ($r \geq 0.75$) correlated with SL (Tables 1–3). The direction of correlation (either positive or negative) of all characters with statistically significant correlation coefficients is the same in the males and in the females. However, characters strongly correlated with SL are not entirely the same in the females and in the males; in the females, ten of them are strongly correlated with SL: five describing the body shape (% SL) and five describing the head shape (% HL), but in the males (similar in both groups, 5–15 cm and 5–18 cm), eight characters are strongly correlated with SL: one describing the body shape and seven describing the head shape (Tables 1, 3). The results of the variance analyses (ANOVA and Kruskal-Wallis) of round goby males including the large-sized specimens were similar except for a single character (predorsal distance) (Table 3).

The evaluation of differences in characters depending on sex of fish using the two tests resulted in similar values (Table 1). Only two characters (maximum body depth and lower distance between pectoral and pelvic fins) were found to be different with regard to the level of significance obtained from the *U*-test and the *t*-test: the *t*-test revealed no statistically significant differences ($p > 0.05$) while the *U*-test showed statistically significant differences ($p \leq 0.05$) (Table 1). In total, the males and females differ by 21 from 38 examined characters.

Thus, most of the studied morphometric characters of round goby in the examined samples from the north-western region of the Sea of Azov demonstrate statistically significant sex- and size-dependent in-group variability.

The results of the MANOVA (Table 2) showed that the vast majority of the morphometric characters depend on both sex and SL. Only three of them (pD, l2D, and op) out of 38 analysed, are not confirmed to be significantly dependent on the size of fish and/or sex (Table 2).

The variability dependent on both size and sex of fish based on the entire set of examined morphometric characters is shown by a dendrogramme resulting from a cluster analysis (Fig. 2). A separate branch of the graph shows a group of juvenile round goby of 5–7 cm SL of both sexes. A distinct cluster is formed by large males with SL exceeding 13 cm (three size groups: 13–15, 15–17, and 17–19 cm). This cluster does not include females of 13–15 cm SL that means that they are more similar smaller individuals of both sexes than to males of the same size. Also, the females and males in the size group 7–9 cm are much more similar to each other than to individuals of their respective sex from the adjacent size groups. The most similar are the males from the size groups 9–11 cm and 11–13 cm and the females from the same size groups (Fig. 2).

Table 1. Measurements of females and males of round goby from north-western Sea of Azov, correlation with size and evaluation of significant differences between females and males.

Character	Females (n=50)						Males (n=49)						Actual level of significance (p)	
	Mean	SD	Min	Max	*r*	*R*	Mean	SD	Min	Max	*r*	*R*	*U*-test	*t*-test
SL, cm	9.63	2.700	5.1	14.3	–	–	10.67	2.720	5.4	14.8	–	–	0.063	0.058
% SL														
H	23.48	1.889	18.04	26.58	0.763	0.775	23.00	1.513	19.38	27.97	0.553	0.485	**0.048**	0.168
h	11.28	0.628	9.45	12.67	0.695	0.711	11.83	0.731	10.45	13.85	0.509	0.539	**0.000**	**0.000**
iH	19.35	1.606	15.19	21.90	0.812	0.840	18.51	1.235	15.85	20.78	0.551	0.493	**0.002**	**0.004**
ih	5.05	0.387	4.36	5.71	-0.441	-0.441	#5.22	0.479	4.40	6.33	-0.328	-0.315	0.127	0.057
aD	32.83	1.208	30.37	35.25	0.558	0.576	32.72	1.115	29.38	35.61	0.184	0.110	0.552	0.623
pD	19.44	1.168	16.85	22.20	-0.223	-0.211	19.78	1.104	17.07	22.22	-0.190	-0.145	0.150	0.146
aP	29.02	0.820	26.90	30.58	0.223	0.246	29.61	1.084	26.82	31.73	0.499	0.452	**0.002**	**0.003**
aV	29.31	1.099	26.67	31.58	0.495	0.459	30.31	1.319	27.30	32.65	0.468	0.452	**0.000**	**0.000**
aA	55.68	2.039	51.18	59.66	0.535	0.491	55.80	1.769	51.93	59.53	0.212	0.230	0.872	0.751
P^{sup}-V	16.20	1.532	12.41	19.08	0.855	0.883	#16.57	1.444	13.39	18.50	0.720	0.672	0.212	0.223
P_{inf}-V	6.04	0.787	3.89	7.48	0.829	0.819	5.79	0.559	4.39	7.11	0.320	0.351	**0.044**	0.077
V-A	27.52	1.996	22.55	32.03	0.540	0.537	27.10	1.876	22.04	31.02	0.091	0.026	0.281	0.280
pl	20.97	0.967	18.89	22.68	-0.384	-0.382	21.08	1.398	18.80	24.80	-0.179	-0.140	0.869	0.643
l1D	17.32	1.058	14.44	19.25	0.508	0.503	16.95	1.160	13.54	19.77	0.495	0.436	0.103	0.104
h1D	14.59	0.965	12.24	16.30	-0.661	-0.646	15.74	0.920	14.15	17.72	-0.465	-0.454	**0.000**	**0.000**
l2D	32.81	1.309	30.59	35.79	0.287	0.299	32.79	1.417	30.00	36.83	0.239	0.252	0.793	0.927
h2D	15.79	1.159	13.22	18.70	-0.645	-0.641	15.75	0.861	13.78	18.05	-0.134	-0.186	0.475	0.845
lA	24.97	1.192	22.15	26.96	-0.083	-0.126	25.45	1.339	23.19	28.81	0.347	0.315	0.176	0.066
hA	11.18	0.834	9.62	13.33	-0.454	-0.394	#11.54	0.809	10.15	14.07	-0.458	-0.365	**0.048**	**0.034**

Table 1. *Continued.*

Character	Females (n=50)						Males (n=49)						Actual level of significance (p)	
	Mean	SD	Min	Max	*r*	*R*	Mean	SD	Min	Max	*r*	*R*	*U*-test	*t*-test
lP	30.29	1.441	26.56	33.14	-0.684	-0.700	30.30	1.570	26.85	34.33	-0.292	-0.302	0.761	0.986
iP	12.05	0.678	10.00	13.42	0.579	0.597	12.44	0.898	10.45	14.41	0.793	0.790	**0.025**	**0.016**
lV	23.49	1.359	20.00	25.98	-0.567	-0.537	#23.68	1.118	22.10	27.22	-0.593	-0.548	0.911	0.465
iV	6.86	0.518	5.64	7.83	0.727	0.737	7.16	0.466	6.06	8.20	0.664	0.643	**0.007**	**0.004**
lC	21.47	1.416	18.72	25.16	-0.749	-0.769	21.47	1.110	19.66	24.03	-0.444	-0.422	0.801	0.971
HL	26.91	0.924	25.00	28.93	-0.068	-0.076	27.75	1.051	25.66	30.19	0.469	0.540	**0.000**	**0.000**
% HL														
hcz	82.03	6.718	64.38	92.62	0.837	0.855	81.21	4.003	69.94	87.83	0.685	0.626	0.304	0.467
ic	88.38	8.172	65.75	100.93	0.823	0.828	#92.54	6.327	76.69	101.46	0.765	0.734	**0.007**	0.006
ao	31.18	2.856	23.65	38.17	0.675	0.691	#31.30	2.323	23.93	34.92	0.590	0.564	0.571	**0.826**
O	22.19	1.937	18.35	27.63	-0.731	-0.722	20.87	2.058	16.95	24.75	-0.844	-0.841	**0.003**	**0.001**
op	50.80	1.668	46.30	54.01	0.248	0.218	51.59	1.653	46.01	54.79	0.273	0.259	**0.011**	**0.020**
io	#16.50	2.636	9.87	20.75	0.821	0.830	18.07	2.494	10.65	23.36	0.841	0.846	**0.008**	**0.003**
lm	30.47	2.717	24.24	35.16	0.687	0.701	#32.92	3.370	24.54	38.17	0.824	0.807	**0.000**	**0.000**
lmd	#38.98	2.983	29.45	43.57	0.501	0.449	42.44	3.819	34.46	49.86	0.758	0.777	**0.000**	**0.000**
or	24.36	1.914	20.95	27.67	0.575	0.547	25.83	2.380	21.26	31.18	0.613	0.613	**0.002**	**0.001**
hop	#42.01	3.196	34.91	47.12	0.655	0.656	43.76	3.363	35.80	49.87	0.768	0.753	**0.011**	**0.009**
ir	47.07	4.223	37.67	56.12	0.829	0.827	53.67	5.506	41.72	63.73	0.893	0.874	**0.000**	**0.000**
ist	#40.49	3.603	32.80	46.19	0.810	0.807	40.57	2.627	34.32	45.80	0.467	0.461	0.647	0.895
hco	58.30	3.426	50.57	64.63	0.691	0.698	60.72	3.587	50.92	68.30	0.691	0.665	**0.001**	**0.001**

Note. #, characters of non-normal distribution. Statistically significant correlation (p = 0.05) for *r* and *R* highlighted; actual level of significance (*U*-test and *t*-test) less than 0.05 given in bold.

Table 2. Morphometric characters of round goby from north-western Sea of Azov and results of correlation and MANOVA analyses to assess dependence on sex and SL factors.

Character	Males and females (n = 99)				Correlation analysis		MANOVA analysis, p actual		
	Mean	SD	Min	Max	*r*	*R*	Sex	SL	Sex and SL
SL, cm	10.14	2.747	5.1	14.8	–	–	–	–	
% SL									
H	23.24	1.721	18.04	27.97	0.621	0.588	**0.001**	**0.000**	0.419
h	11.55	0.731	9.45	13.85	0.612	0.619	**0.001**	**0.000**	0.707
iH	18.93	1.489	15.19	21.90	0.598	0.574	**0.000**	**0.000**	0.351
ih	#5.14	0.441	4.36	6.33	-0.326	-0.335	**0.007**	**0.000**	**0.023**
aD	32.78	1.158	29.38	35.61	0.362	0.337	0.237	**0.000**	0.144
pD	19.61	1.143	16.85	22.22	-0.173	-0.159	0.079	0.398	0.689
aP	29.31	0.999	26.82	31.73	0.409	0.400	**0.018**	**0.000**	0.068
aV	29.81	1.306	26.67	32.65	0.507	0.481	**0.000**	**0.001**	0.104
aA	55.74	1.902	51.18	59.66	0.384	0.362	0.809	**0.018**	0.303
$P^{(sup)}$-V	#16.38	1.493	12.41	19.08	0.792	0.780	0.617	**0.000**	0.433
$P_{(inf)}$-V	5.91	0.691	3.89	7.48	0.555	0.527	**0.000**	**0.000**	**0.003**
V-A	27.32	1.939	22.04	32.03	0.295	0.260	0.068	**0.005**	0.084
pl	21.02	1.195	18.80	24.80	-0.246	-0.238	0.593	**0.025**	0.573
l1D	17.13	1.119	13.54	19.77	0.454	0.422	**0.002**	**0.000**	0.417
h1D	15.16	1.102	12.24	17.72	-0.004	-0.017	**0.000**	0.520	**0.000**
l2D	32.80	1.356	30.00	36.83	0.256	0.274	0.516	0.111	0.499
h2D	15.77	1.017	13.22	18.70	-0.310	-0.281	0.683	**0.016**	**0.002**
lA	25.21	1.283	22.15	28.81	0.173	0.125	0.164	0.297	**0.043**
hA	#11.36	0.836	9.62	14.07	-0.397	-0.344	**0.002**	**0.000**	0.950
lP	30.29	1.499	26.56	34.33	-0.471	-0.504	0.503	**0.000**	0.128
iP	12.24	0.815	10.00	14.41	0.707	0.695	0.142	**0.000**	**0.000**
lV	#23.59	1.243	20.00	27.22	-0.549	-0.541	**0.037**	**0.000**	0.830
iV	7.01	0.512	5.64	8.20	0.709	0.702	**0.007**	**0.000**	0.426
lC	21.47	1.267	18.72	25.16	-0.599	-0.588	0.246	**0.000**	0.145
HL	27.33	1.070	25.00	30.19	0.269	0.290	**0.000**	**0.010**	**0.023**
% HL									
hcz	81.62	5.530	64.38	92.62	0.726	0.718	**0.002**	**0.000**	0.085
ic	#90.44	7.575	65.75	101.46	0.799	0.796	**0.031**	**0.000**	0.586
ao	#31.24	2.593	23.65	38.17	0.626	0.644	0.341	**0.000**	0.758
o	22.54	2.094	16.95	27.63	-0.794	-0.792	**0.005**	**0.000**	0.386
op	51.19	1.700	46.01	54.79	0.293	0.270	0.097	0.111	0.323

Table 2. *Continued.*

Character	Males and females (n = 99)				Correlation analysis		MANOVA analysis, p actual		
	Mean	SD	Min	Max	*r*	*R*	Sex	SL	Sex and SL
io	17.28	2.672	9.87	23.36	0.835	0.841	**0.016**	**0.000**	0.852
lm	#31.68	3.280	24.24	38.17	0.762	0.767	**0.001**	**0.000**	0.132
lmd	40.69	3.822	29.45	49.86	0.646	0.627	**0.000**	**0.000**	**0.019**
or	25.09	2.268	20.95	31.18	0.612	0.595	**0.012**	**0.000**	0.796
hop	42.87	3.379	34.91	49.87	0.725	0.700	0.155	**0.000**	0.501
ir	50.34	5.895	37.67	63.73	0.804	0.800	**0.000**	**0.000**	0.304
ist	40.53	3.142	32.80	46.19	0.648	0.626	0.130	**0.000**	0.090
hco	59.50	3.694	50.57	68.30	0.703	0.689	**0.009**	**0.000**	0.845

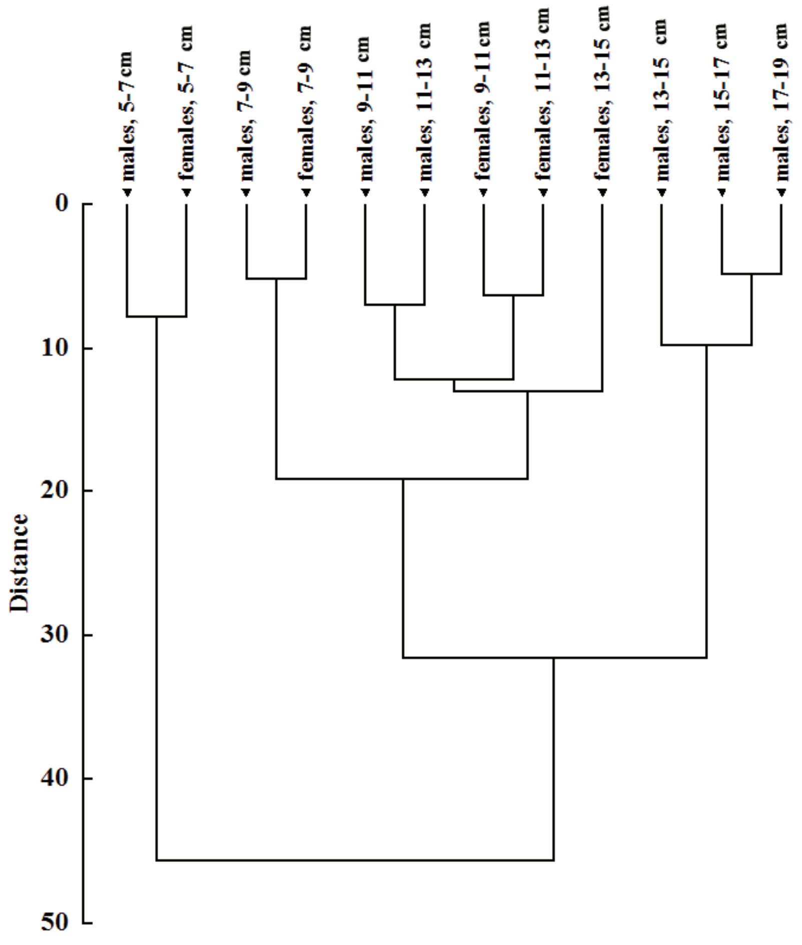

Fig. 2. Dendrogramme resulting from cluster analysis of morphometric characters in round goby males and females of different SL from north-western Sea of Azov using complete linkage method.

Table 3. Morphometric characters of round goby males (including largest specimens over 15 cm long) from north-western Sea of Azov and results of correlation and variance analyses (ANOVA and Kruskal-Wallis) to assess dependence on standard length factor.

Character	Males (n = 65)				Correlation analysis		Variance analyses, p actual	
	Mean	SD	Min	Max	*r*	*R*	ANOVA	Kruskal-Wallis
SL, cm	12.10	3.476	5.4	18.0	–	–	–	–
% SL								
H	23.10	1.436	19.38	27.97	0.408	0.326	**0.000**	**0.004**
h	12.01	0.751	10.45	13.85	0.588	0.602	**0.000**	**0.000**
iH	18.71	1.247	15.85	21.21	0.513	0.443	**0.000**	**0.001**
ih	#5.20	0.464	4.40	6.33	-0.251	-0.157	**0.008**	**0.024**
aD	32.61	1.065	29.38	35.61	-0.015	-0.084	**0.017**	0.072
pD	19.56	1.181	16.76	22.22	-0.381	-0.370	**0.019**	**0.047**
aP	29.74	1.072	26.82	31.73	0.458	0.401	**0.005**	**0.035**
aV	30.44	1.267	27.30	32.96	0.421	0.405	**0.012**	**0.018**
aA	55.93	1.705	51.93	59.74	0.203	0.189	0.766	0.776
$P^{(sup)}$-V	#16.84	1.379	13.39	19.41	0.689	0.617	**0.000**	**0.000**
$P_{(inf)}$-V	5.90	0.570	4.39	7.11	0.434	0.435	**0.002**	**0.006**
V-A	27.13	1.849	22.04	32.31	0.049	-0.018	0.727	0.806
pl	20.87	1.398	17.86	24.80	-0.292	-0.260	0.131	0.153
l1D	17.15	1.303	13.54	20.07	0.419	0.386	**0.000**	**0.002**
h1D	15.84	0.977	13.99	17.72	-0.373	-0.387	**0.005**	**0.007**
l2D	33.02	1.583	29.94	37.69	0.328	0.357	0.248	0.117
h2D	15.55	0.900	13.29	18.05	-0.239	-0.253	**0.015**	**0.008**
lA	25.69	1.354	23.19	28.97	0.420	0.426	0.052	0.071
hA	#11.29	0.859	9.71	14.07	-0.631	-0.628	**0.000**	**0.000**
lP	30.18	1.618	26.23	34.33	-0.268	-0.267	0.075	0.076
iP	#12.69	0.920	10.45	14.41	0.810	0.812	**0.000**	**0.000**
lV	23.30	1.285	20.83	27.22	-0.689	-0.659	**0.000**	**0.000**
iV	7.26	0.481	6.06	8.20	0.665	0.636	**0.000**	**0.000**
lC	21.39	1.128	18.89	24.03	-0.348	-0.319	0.094	0.129
HL	27.86	1.021	25.66	30.19	0.424	0.455	**0.002**	**0.003**
% HL								
hcz	82.49	4.364	69.94	90.83	0.745	0.723	**0.000**	**0.000**
ic	#94.30	6.465	76.69	104.26	0.797	0.779	**0.000**	**0.000**
ao	#31.84	2.270	23.93	34.92	0.659	0.662	**0.000**	**0.000**
o	19.97	2.463	14.26	24.75	-0.881	-0.886	**0.000**	**0.000**

Table 3. *Continued.*

Character	Males (n = 65)				Correlation analysis		Variance analyses, p actual	
	Mean	SD	Min	Max	*r*	*R*	ANOVA	Kruskal-Wallis
op	#51.77	1.651	46.01	54.79	0.292	0.305	0.134	0.273
io	18.88	2.672	10.65	24.55	0.862	0.860	**0.000**	**0.000**
lm	#33.84	3.376	24.54	38.25	0.840	0.843	**0.000**	**0.000**
lmd	43.68	4.115	34.46	51.19	0.807	0.816	**0.000**	**0.000**
or	26.43	2.409	21.26	31.18	0.675	0.667	**0.000**	**0.000**
hop	45.39	4.171	35.80	53.36	0.866	0.867	**0.000**	**0.000**
ir	#56.25	6.670	41.72	67.07	0.939	0.931	**0.000**	**0.000**
ist	41.12	2.729	34.32	47.09	0.512	0.484	**0.000**	**0.003**
hco	61.64	3.696	50.92	68.87	0.704	0.689	**0.000**	**0.000**

DISCUSSION

As shown above, the characters that are strongly correlated (≥ 0.75) with SL are not the same in males and females. Thus, the females have five strongly size-dependent characters describing the general body shape and the shape of the fins (maximum body depth, maximum body width, pectoral-pelvic distances 1 and 2, and caudal fin length) while the males only one (width of pectoral fin base). It can be hypothesised that such a correlation between the width of the pectoral-fin base and SL in males is caused by their spawning behaviour – a male, guarding the nest, actively aerates the eggs moving the pectoral fins.

The head parameters strongly correlated with SL are more numerous in the males (seven vs. five in the females). Three characters are shared by the males and females (maximum head width, interorbital distance, and mouth width); two characters are specific for the males (horizontal eye diameter, cheek depth, upper jaw length, and lower jaw length) and two characters are found only in the females (head depth at nape and isthmus width). It is probable that a strong correlation between the cheek depth and length of jaws in males and their body length, are also relevant to the spawning behaviour–while constructing the nest, the male carefully cleans it from the "garbage" moving it outside the nest with the aid of the mouth. Later, when guarding the laid eggs, the male scares other fish demonstrating aggressiveness by inflating the cheeks.

The statistical methods used in the study proved to be appropriate for discovering variability of different nature.

The applicability of methods of parametric statistics is determined primarily by the normal distribution of the random variable distribution though most of actual distributions of measurements of biological objects are not normal (Orlov, 2002). So, results of studies using parametric statistics, especially the frequently used *t*-test, for non-normal distributions and small compared samples, should be accepted critically.

From the theoretical point, thousands of observations are needed for any reliable verification of the normality. However, if the homogeneity of two samples is to be studied using a *t*-test (with an a priori assumption of the equality of variances), then the effect of departures from the normality decreases with the increase of the sample size (Orlov, 2002). In practice, the concept of "large" and "small" samples are used in biological and fisheries research (Aksyutina, 1968; Lakin, 1980), a large enough sample containing over 30 observations when, with certain reservations, parametric statistics can be applied.

The methods of parametric and nonparametric statistics used in the present study resulted in very similar estimates that is apparently connected with a sufficiently large size of the analysed samples. In general, when the normal character distribution is considered, the parametric tests are more powerful than the nonparametric ones. So, in all cases when compared samples are taken from normally distributed populations, it could be recommended to prefer the parametric tests. In case of considerable departures from the normality, nonparametric tests should be used.

For the analysis of size-dependent variability, as shown by our results, it was not sufficient to base conclusions on correlation test statistics only. A comparison of the data based on the analysis of variance with the results of the correlation analysis revealed certain differences. Even in cases of a statistically significant correlation, but at relatively not very high values of the correlation coefficient (less than 0.5), the analysis of variance, in many cases, does not confirm the existence of such a relationship (Tables 2, 3). In other words, a statistically significant but weak correlation revealed in the data set for each individual character, is not necessarily evident from the in-group analysis of the character. For example, the results of the correlation analysis of size-dependent variability in round goby males 5–18 cm long showed the statistically significant correlation for caudal peduncle length ($r = -0.292$), length of second dorsal fin base ($r = 0.328$), length of anal fin base ($r = 0.420$), pectoral fin length ($r = -0.268$), caudal fin length ($r = -0.348$), and postorbital distance ($r = 0.292$) but the results of the ANOVA demonstrate the absence of correlation of these morphometric characters with SL.

This clearly supports the known idea (e.g., Aivazyan et al., 1985) that the correlation analysis though allows to reveal the presence and strength of statistical relationships between two variables but is not sufficient to prove the existence of a causal link between characters (SL and relative morphometric characters in our case). A more reliable conclusion can be only obtained when a variance analysis is also applied because it estimates in-group variability of the factors and their joint effect. This is especially true for ambiguous results, for example, 1) if results of the used statistical tests (e.g. *U*-test and *t*-test) do not coincide as in the case of maximum body depth and pectoral-pelvic distance 2 when the females and males were compared (Table 2), or 2) analysing correlation between variables (characters), especially in the range of low values of the correlation coefficient.

It should be noted that in recent years, some authors (Kováč, Syriová, 2005; Ľavrinčíková et al., 2005) successfully applied a regression analysis using geometric morphometric methods for the study of developmental variability in gobies, including the round goby.

Summarising conclusions on the methodology used for a morphometric analysis are as follows. 1. In order to reach a correct assessment, a study of morphological variability, especially of sex- and size-dependent differences, should use appropriate methods selected with a particular attention to the actual characteristics of data to be analysed (distribution of variables, sample size). 2. Evaluation of correlation should be based not only on values of a correlation coefficient calculated for the entire set of data per character but, also, on results of an in-group analyses of the pattern and extent of correlation within the range of data proper, e.g., within size groups or classes. This approach is most fully realised in the methods of variance analyses. The approach of searching for the most informative size range could be useful for further studies of infraspecific variation and comparison between morphologically close gobiin species.

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