DO PROTECTED AREAS INFLUENCE POPULATIONS OF THE THREATENED RED ALGA *PHYLLOPHORA CRISPA* ALONG THE SOUTHWESTERN COAST OF CRIMEA (THE BLACK SEA)?

Vladimir V. Alexandrov*, Nataliya A. Milchakova

A.O. Kovalevsky Institute of Biology of the Southern Seas RAS, Russia
**e-mail: valexandrov@list.ru*

Received: 21.06.2022. Revised: 04.08.2022. Accepted: 14.08.2022.

The perennial sciaphilous alga *Phyllophora crispa* (Phyllophoraceae, Rhodophyta) belongs to the main community-forming species of the Black Sea but due to the catastrophic degradation of its populations, it has been listed as an endangered species in the Red Data Book of the Russian Federation. *Phyllophora crispa* off the southwestern coast of Crimea is preserved within six Protected Areas (PAs) established between 1972 and 2017, which include the narrow 300-m-wide strips of coastal waters ranging from 0.0597 km^2 to 0.208 km^2 in area. Despite a long existence, the degeneration of *P. crispa* assemblages has been shown in some of them that may indicate inefficient measures aimed at conserving bottom communities in Marine PAs (MPAs) and the need to optimise the regional PA network. Therefore, this study addresses assessing the effectiveness of the preservation of *P. crispa* in MPAs along the southwestern coast of Crimea, and it aims at two main objectives: to compare the status of *P. crispa* populations in the study area at present and before the MPA establishment, and to assess their current state in the marine protected and unprotected areas. In 2015–2020, the density, biomass, weight and length of *P. crispa* thalli were determined in five MPAs ranged in age 33–48 years of protection and in 16 unprotected water areas, at depths from 0.5 m to 20 m. A comparison of the obtained and published data for the 5 m to 20 m depth range showed that from 1964–1967 until 2015–2020, the *P. crispa* biomass in the study area decreased on average 2.7-fold, the density 1.5-fold and the thallus weight 2.0-fold. Evaluating the decline of the populations of *P. crispa* along the depth range of 5–15 m showed that it was most pronounced at a 15-m depth, being statistically significant in biomass and thallus weight. At the same time, an analysis of variance showed no effect of protection on the between-year change in *P. crispa* population parameters. In addition, in 2015–2020, there was no statistically significant difference in the average biomass and density of this species inside and outside the MPAs. The average weight and length of thalli did not differ due to protection either, except for a depth of 10 m where values of these parameters were, respectively, 3.3 times and 1.4 times higher inside than outside MPAs. One explanation for the non-significant MPA effect on the state of populations of *P. crispa* could be a lack of statistical power in our study. Another one was the small area and low width of MPAs, which makes the seaweed communities vulnerable to negative impacts near the MPA borders. To improve the effectiveness of the conservation of *P. crispa* in the southwestern Crimea, it was recommended to increase the MPA coverage, create buffer zones around MPAs and take measures resulting in environmental improvement throughout the region.

Key words: biomass, density, effectiveness, long-term changes, macrophytes, Marine Protected Area

Introduction

The perennial sciaphilous alga *Phyllophora crispa* (Hudson) P.S. Dixon (Phyllophoraceae, Rhodophyta) belongs to the main community-forming species of the Black Sea (Kalugina-Gutnik, 1975; Milchakova et al., 2011). The most abundant accumulations (fields) of its unattached form were confined to silty and sandy bottom at depths of 10–60 m, and the attached *Phyllophora* occupied a narrow strip of rocks and boulders at depths of 10–28 m (Kalugina-Gutnik, 1975). Over the last half century, the largest aggregation of the unattached form of *Phyllophora* (Zernov's *Phyllophora* Field) has almost completely disappeared in the northwestern part of the Black Sea, and the production parameters of the coastal communities dominated by the attached form of this species have reduced significantly (Milchakova, 2003; Simakova & Maximova, 2009; Milchakova et al., 2011, 2013; Berov et al., 2018). According to most researchers, this was due to a decrease in water transparency as a consequence of eutrophication (Zaitsev & Mamaev, 1997; Milchakova, 2003; Milchakova et al., 2011, 2013). Due to the catastrophic degradation of the populations of *P. crispa*, it has been listed as an endangered species under the Convention on the Protection of the Black Sea against Pollution (Bucharest Convention, 1992), as a species decreasing in number in the Red Data Book of the Russian Federation (Bardunov & Novikov, 2008), and its habitats on the European Red List of Habitats (Gubbay et al., 2016). This species is also included in regional Red Data Books such as those of the Krasnodarsky Krai (Litvinskaya, 2017), Republic of Crimea (Yena & Fateryga, 2015), and city of Sevastopol (Dovgal & Korzhenevsky, 2018).

It is generally recognised that Marine Protected Areas (MPAs) play a significant role in threatened species conservation and recovery (Roberts et al., 2003), including macroalgae (Gianni et al., 2013). The development of the MPA system is one of the priority tasks of the European Union Marine Strategy (Directive 2008/56/EC), as well as of the «Strategy of Ecological Safety of the Russian Federation for the period up to 2025» (Decree of the President of the Russian Federation of 19.04.2017, №176). It has been shown that creating a MPA leads to an increase in macroalgal diversity (Cacabelos et al., 2020) and abundance (Babcock et al., 1999) or to changes in species dominance (Edgar & Barrett, 1999). However, a decrease in macrophyte density is also possible (Lester et al., 2009), or there were no significant changes in macroalgal assemblages after the establishment of the protection (Benedetti-Cecchi et al., 2003; Barrett et al., 2009; Lester et al., 2009; Turnbull et al., 2021). This is related to the fact that the MPA effectiveness can vary significantly and depends on their size, duration of existence (Claudet et al., 2008), habitat features, protection regime (Benedetti-Cecchi et al., 2003; Ceccherelli et al., 2006; Claudet et al., 2008), biology of target species (Gilby et al., 2015) and environmental quality in the adjacent water areas (Gilby et al., 2015).

In the Black Sea, there are approximately 63 MPAs (Milchakova et al., 2015; Alexandrov et al., 2017; Begun et al., 2012, 2022). *Phyllophora crispa* was found within 30 of them situated in Bulgaria, Romania, Russia, and Ukraine; moreover, some MPAs have been established specifically to protect this species (Tkachenko & Maslov, 2002; Tkachenko & Kovtun, 2014; Milchakova et al., 2015; Skrebovska & Shaposhnikova, 2016; Kolyuchkina et al., 2018; Abaza et al., 2019). The effectiveness of MPAs in the conservation of *P. crispa* has been assessed to date along the Bulgarian coast only, based solely on the data of their connectivity (Berov et al., 2018). However, the influence of MPAs on the condition of populations of this species in the Black Sea still remains unknown.

Phyllophora crispa off the southwestern coast of Crimea (in the Sevastopol Region) exists mainly as an attached form (Fig. 1) and it is protected within the marine portions of six Protected Areas (hereinafter – MPAs) established between 1972 and 2017 (PARF, 2022). Because *P. crispa* is a thick leathery slow-growing shade-adapted species (Kalugina-Gutnik, 1975), and may be considered as a late-successional one (Orlando-Bonaca et al., 2008; but see Orfanidis et al., 2011), having flourished in pristine environments before human induced disturbances and decrease in water transparency (Zaitsev & Mamaev, 1997; Milchakova et al., 2013), its response to protection, which reduces human impacts, is expected to be positive. Nevertheless, despite a long existence of these MPAs, a degeneration of macrophyte assemblages, including those of *P. crispa*, has been found in some of them (Milchakova, 2003; Milchakova et al., 2011, 2019). This may indicate inefficient measures aimed to conserve bottom communities in the coastal Protected Areas (PAs) and the need to optimise the PA network of the Sevastopol Region. Therefore, this study addresses assessing the effectiveness of the preservation of *P. crispa* in MPAs along the southwestern coast of Crimea, and aims at two main objectives: to compare the state of populations of *P. crispa* in the study area at present and before the MPA establishment, and to assess their current state in marine protected and unprotected areas. The primary hypothesis to be tested was: did protection affect the long-term change in the *P. crispa* populations?

Material and Methods

Study area

The study of *P. crispa* (Fig. 1) was conducted in the coastal zone of southwestern Crimea, between Cape Lukull and Cape Sarych (Fig. 2). Because of the steep slope of the bottom near Cape Aya and Cape Kaya-Bashi, the width of the vegetation belt is narrow there, and it ranges from 20 m to 150 m width. The bottom is flatter in other areas, and the width of the macroalgal belt exceeds 300 m. The communities dominated by *Ericaria crinita* (Duby) Molinari & Guiry and *Gongolaria barbata* (Stackhouse) Kuntze were recorded on the blocky substrate and rock outcrops down to 10 m depth, and those with dominance of *P. crispa* were present in the range of 10–20 m depth (Milchakova, 2003; Kovardakov et al., 2004; Milchakova et al., 2011).

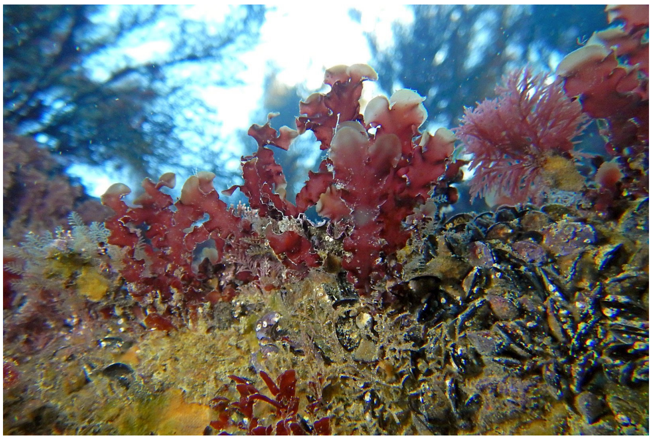

Fig. 1. *Phyllophora crispa* in the Coastal Aquatic Complex near Cape Fiolent Natural Monument (south-western coast of Crimea) at 0.5 m depth (Author: S.S. Raksha).

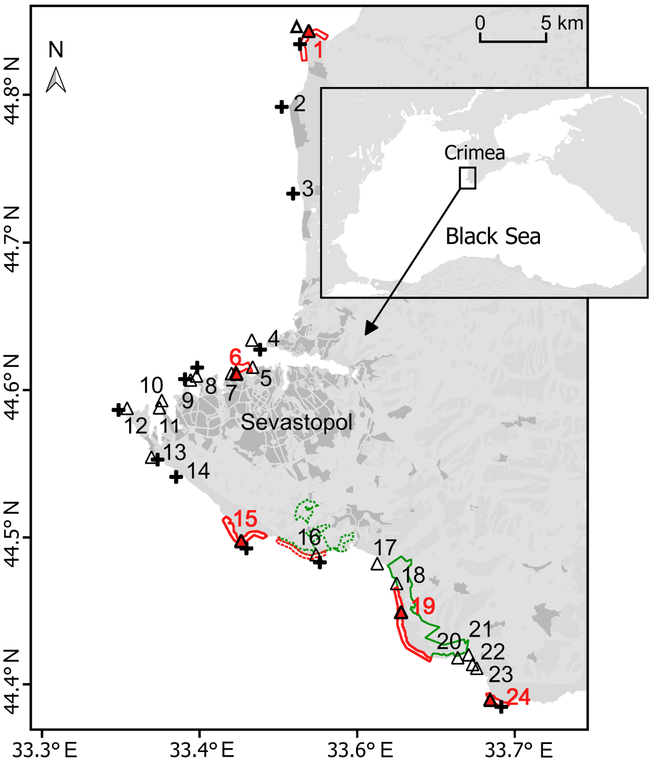

Fig. 2. Sampling transect locations in 1964–1967 (+), in 2015–2020 inside (▲) and outside MPAs (△) along the southwestern coast of Crimea. Designations: 1 – area within and outside Coastal Aquatic Complex near Cape Lukull Natural Monument, 2 – Cape Margopulo, 3 – Osipenko village area, 4 – Cape Kosa Severnaya, 5 – Karantinnaya Bay, 6 – Coastal Aquatic Complex near Tauric Chersonesos Natural Monument, 7 – Pesochaya Bay, 8 – Cape Vostochny, 9 – Cape Peschany, 10 –11 – Cape Manganari, 12 – Cape Khersones, 13–14 – Golubaya Bay, 15 – Coastal Aquatic Complex near Cape Fiolent Natural Monument, 16 – Cape Kaya-Bashi, 17 – Gray Rock, 18 – Ayazma area, 19 – Cape Aya Sanctuary, 20–23 – Laspi Bay, 24 – Coastal Aquatic Complex near Cape Sarych Natural Monument; ▭ – MPAs created before the study; ⬚ – MPAs created after the study; the protected land area is denoted in green.

Sampling was performed in five selected MPAs of southwestern Crimea (Fig. 2) with an age of more than 30 years (33–48 years): within the Coastal Aquatic Complex (CAC) near Cape Lukull Natural Monument (NM) (Site 1, see Fig. 2), CAC near Tauric Chersonesos NM (Site 6, see Fig. 2), CAC near Cape Fiolent NM (Site 15, see Fig. 2), CAC near Cape Sarych NM (Site 24, see Fig. 2), and in the Cape Aya Sanctuary (Site 19, see Fig. 2). Their sizes are 0.1135 km^2, 0.0597 km^2, 0.16 km^2, 0.0588 km^2, and 0.208 km^2, respectively. Collecting of plants and animals and the violation of the integrity of natural complexes are prohibited in the studied MPAs, but transit visits for recreational purposes are allowed there (PARF, 2022). The water area near Cape Kaya-Bashi (Site 16, Fig. 2) was reserved in 2006 for creating Karan'skiy Sanctuary. However, it was included in the list of unprotected areas in our study, since the Karan'skiy Sanctuary had not yet been created by the date of sampling. The CAC near Tauric Chersonesos NM is located in the residential zone close to the Sevastopol Bay and is characterised by a high level of recreational load (Milchakova et al., 2015), over 500 000 m^3/year of untreated wastewater flows into the adjacent water area (Gruzinov et al., 2019). The CAC near Cape Fiolent NM is located far from the wastewater outlets, but sand was previously mined near its borders (Boltachev et al., 2012). The environmental quality near the CAC near Cape Lukull NM is relatively high (Ryabushko et al., 2020). However, high levels of biological oxygen consumption, nitrogen and phosphorus content were periodically recorded in the adjacent areas due to wastewater effluents and river inflow (Gruzinov et al., 2019; Dyakov et al., 2020). The waters around the CAC near Cape Sarych NM are characterised by low nutrient levels (Shchurov et al., 2019); but in recent years, coastal development in this area is increasing and high levels of heavy metals and petroleum hydrocarbons were observed (Sevostyanova et al., 2016; Tikhonova et al., 2020). The marine area of the Cape Aya Sanctuary is the least susceptible to the anthropogenic impact due to the active hydrodynamics of the water masses and the remoteness from the major sources of water pollution (Kovardakov et al., 2004).

Sample collection

In the summer seasons of 2015–2020, macroalgae samples were taken on 21 transects at depths of 0.5 m, 1 m, 3 m, 5 m, 10 m, 15 m and 20 m (Fig. 2). Sampling was performed using quadrate frames 25 × 25 cm and replicated four times at each depth (Kalugina-Gutnik, 1975). *Phyllophora crispa* was found at 61 out of 126 sampling points. Its thallus wet weight (g) and length (cm) were determined for each point; in total, 3676 thalli were handled. The mean values of these parameters, as well as the density (plants/m^2) and biomass (g/m^2, wet weight), were calculated. *Phyllophora crispa* was collected with the permission of the Russian Federation's Federal Agency for Fishery, №61 2017 03 13164. After the samples had been processed all plants were returned to their natural habitat.

Long-term changes of Phyllophora crispa populations inside and outside MPAs

We performed a two-way analysis of variance (ANOVA) (Logan, 2010) to evaluate the effect of the fixed between-subject factor MPA (levels protected and unprotected) and the within-subject factor year (levels 1964–1967 and 2015–2020) on the biomass, density and average thallus weight of *P. crispa*. The main goal of the ANOVA was to test the significance of the effect of interaction between these two factors, i.e. to check whether MPAs influenced the interannual change in values of *P. crispa* parameters. For the analysis we chose the data for the same areas sampled at a depth range 5–20 m both in 1964–1967 and 2015–2020. The sample size for PAs (CAC near Cape Fiolent NM, CAC near Cape Sarych NM, CAC near Cape Lukull NM) and unprotected areas was n = 6 and n = 12 per each period, respectively. The data for 1964–1967 were obtained from publications (Kalugina-Gutnik, 1974, 1975; Kalugina-Gutnik & Kulikova, 1974), but since the thallus weight was not provided in these sources, it was calculated using the biomass-to-density ratio. The statistical significance of differences between means was assessed using a paired two-sample t-test with Holm correction for multiple comparisons (Logan, 2010).

Long-term changes of Phyllophora crispa populations at different depths

The effect of depth on the interannual changes in biomass, density and average thallus weight of *P. crispa* was determined using two-way analysis of variance (Logan, 2010). The fixed between-subject factor depth included the levels of 5 m, 10 m and 15 m, and the within-subject factor year included 1964–1967 (Kalugina-Gutnik, 1974, 1975; Kalugina-Gutnik & Kulikova, 1974) and 2015–2020. The data for the same sites and depths were used for the analysis. The total sample size was n = 16 (n = 5 for 5 m, n = 5 for 10 m and n = 6 for 15 m) for each year. Post-hoc comparisons of means were performed using a two-sample paired t-test followed by the Holm procedure for correction (Logan, 2010). The data for other depths were not used in ANOVA due to low sample sizes and mismatched locations, but for a depth of 20 m, regardless of ANOVA, we computed means for different locations and compared them using the two-sample Mann-Whitney U test (n = 3).

State of Phyllophora crispa populations inside and outside MPAs in 2015–2020 at different depths

To evaluate the state of *P. crispa* the average values of density, biomass, average thallus weight and length inside and outside MPAs were assessed. The comparison of these values in MPAs and in unprotected water areas was carried out using the two-sample Mann-Whitney U-test. To assess the effect of the depth (factor levels 5 m, 10 m and 15 m) and protection (levels protected and unprotected) on *P. crispa*'s population parameters, an ANOVA was performed, followed by multiple comparisons using a one-side t-test with Holm correction, evaluating if parameter values were higher in MPAs than outside. Data for the CAC near Cape Lukull NM (depth 5 m), CAC near Tauric Chersonesos NM (depth 10 m and 15 m), and CAC near Cape Fiolent NM, Cape Aya Sanctuary, CAC near Cape Sarych NM (5 m, 10 m, 15 m), as well as for 11 unprotected areas within the depth range of 0.5–17 m, were used. The sample sizes for the depths of 5 m, 10 m and 15 m were n = 9, n = 11, and n = 10 for unprotected areas, and n = 5, n = 4 and n = 5 for the protected ones, respectively.

Multivariate analysis of the state of the Phyllophora crispa populations

Cluster analysis was used to reveal the groups of populations of *P. crispa* distinguished by their state. We used the data obtained on 21 transects at depths of 0.5–17.0 m in 2015–2020 (sample size n = 61), as well as the results of studies carried out on 12 transects within the depth range of 1–25 m (sample size n = 32) in 1964–1967 (Kalugina-Gutnik, 1974, 1975; Kalugina-Gutnik & Kulikova, 1974). The hierarchical clustering was performed using Ward's method based on a matrix of Euclidean distances (Legendre & Legendre, 1998), calculated from standardised values of biomass, density and thallus weight. The dendrogram obtained was analysed to choose an optimal number of clusters for interpretation. After that the K-means clustering was performed to improve the original partitioning. The clusters were characterised by the mean values of these parameters and the significance of their differences was checked using the Kruskal-Wallis test (Logan, 2010). To evaluate the state of populations of *P. crispa* in 1964–1967 and 2015–2020 a principal component analysis (Legendre & Legendre, 1998) was also applied to the same parameters used in the cluster analysis. A diagram of the distribution of the populations of *P. crispa* and their parameters in the first two components space was created. The trends of long-term changes of the state of *P. crispa* were assessed by the direction of shift of the points corresponding to the same populations in 1964–1967 and 2015–2020. To evaluate the magnitude of between-year changes at different depths or inside and outside MPAs, we computed Euclidean distances between points.

Before statistical analyses the homogeneity of variances and normality of data were checked; if these criteria were not met, log-transformation was used. The calculations were performed in the R statistical computing environment, version 3.6.3 (R Core Team, 2019) using the car (Fox & Weisberg, 2019), FactoMineR (Lê et al., 2008), factoextra (Kassambara & Mundt, 2019), and rstatix (Kassambara, 2021) packages.

Results

Long-term changes of Phyllophora crispa populations inside and outside MPAs

According to the ANOVA results there were no statistically significant main effect of MPA and interaction effect between year and MPA on all the parameters of *P. crispa* (Table 1), only a main effect of year was significant. The average values of parameters of *P. crispa* decreased over the period from 1964–1967 to 2015–2020, both in the areas that became protected and unprotected, with this change being more pronounced for the latter. However, post-hoc tests did not confirm all these differences (p = 0.17–0.73; see grey and red bars in Fig. 3). Exploring the main effect of year has shown significant differences in means over the studied period (calculated for pooled sample of both MPA and unprotected areas; see grey-red bars in Fig. 3): the average values for *P. crispa* biomass decreased 2.7-fold ($t_{(17)} = 2.86$, $p = 0.011$), 1.5-fold reduction was found for density ($t_{(17)} = 2.24$, $p = 0.039$), and 2.0-fold for thallus weight ($t_{(17)} = 2.62$, $p = 0.018$) in the same areas and at the same depths off the southwestern coast of Crimea.

Long-term changes of Phyllophora crispa populations at different depths

We found that biomass, density and thallus weight of *P. crispa* highly depended on the depth, but the effect of the year of sampling was significant only for biomass (Table 2). The interaction of these factors was not significant. The comparison of means showed that both in 1964–1967 and in 2015–2020, the values of all parameters increased with increasing depth (Fig. 4), but in 2015–2020 they were lower than in 1964–1967. The between-year difference in the parameter values was more pronounced at a depth of 15 m, where it was statistically significant for biomass and thallus weight. This difference was even higher at a depth of 20 m, where since 1964–1967 the average biomass decreased from 3396 ± 731 g/m^2 to 339 ± 125 g/m^2, density – from 411 ± 69 plants/m^2 to 173 ± 59 plants/m^2, and thallus weight – from 8.1 ± 0.5 g to 1.9 ± 1.1 g but these changes were not significant (U = 6, p = 0.125) that may be in part attributed to a small sample size (n = 3) that is why these data have not been used in ANOVA.

Table 1. ANOVA results for the response of *Phyllophora crispa* biomass (lnB), density (lnN) and thallus weight (lnW) to the MPA and the year off the southwestern coast of Crimea

Factor	lnB				lnN				lnW			
	DFn	DFd	F	p	DFn	DFd	F	p	DFn	DFd	F	p
MPA	1	16	0.01	0.94	1	16	0.07	0.80	1	16	0.268	0.61
year	1	16	21.29	**0.0003**	1	16	8.41	**0.01**	1	16	8.319	**0.01**
MPA:year	1	16	1.54	0.23	1	16	0.15	0.70	1	16	1.817	0.20

Note: Significant p-values (p < 0.05) are highlighted in bold.

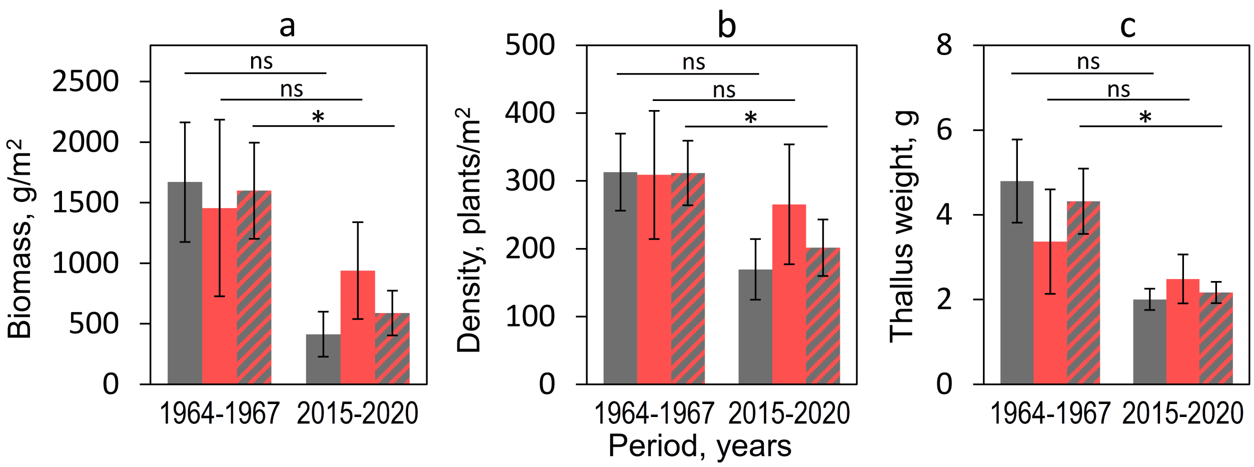

Fig. 3. Average biomass (a), density (b) and thallus weight (c) of *Phyllophora crispa* off the southwestern coast of Crimea at a depth range of 5–20 m in 1964–1967 and 2015–2020 (mean ± error). The p-values of between-year differences of means are given according to a paired t-test: * – p < 0.05, ns – p > 0.05. Grey bars – unprotected areas, red bars – areas that became protected in 1972–1982, grey-red bars – pooled sample of protected and unprotected areas.

Table 2. ANOVA results for the response of biomass (lnB), density (N) and thallus weight (lnW) of *Phyllophora crispa* to depth (5 m, 10 m and 15 m) and year off the southwestern coast of Crimea

Factor	lnB				N				lnW			
	DFn	DFd	F	p	DFn	DFd	F	p	DFn	DFd	F	p
Depth	2	13	12.9	**0.0008**	2	13	4.23	**0.038**	2	13	18	**0.0002**
Year	1	13	11.7	**0.004**	1	13	3.04	0.105	1	13	3.37	*0.089*
Depth:Year	2	13	0.1	0.905	2	13	0.88	0.438	2	13	0.36	0.707

Note: Significant p-values ($p < 0.05$) are highlighted in bold, $p < 0.1$ – in italics.

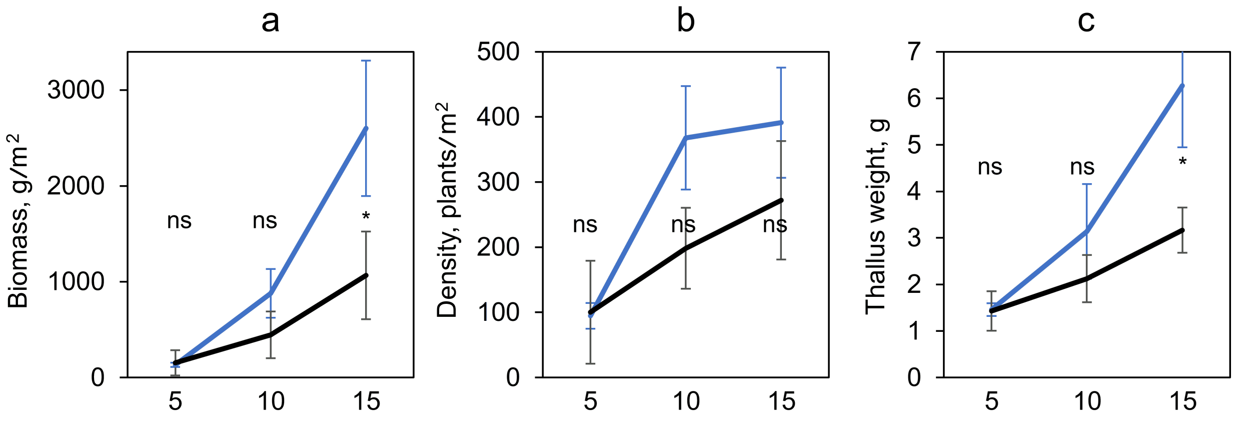

Fig. 4. Average biomass (a), density (b) and thallus weight (c) of *Phyllophora crispa* at different depths in six protected and unprotected areas off the southwestern coast of Crimea in 1964–1967 (▬) and in 2015–2020 (▬) (mean ± error). The p-values of between-year differences of means are given according to a t-test followed by the Holm procedure for correction: * – $p < 0.05$; ns – non-significant.

State of Phyllophora crispa populations inside and outside MPAs in 2015–2020 at different depths

It was found that the biomass of *P. crispa* in MPAs averaged 587 ± 198 g/m^2 and its density was 225 ± 58 plants/m^2. The average values of these parameters were 1.6–1.9-fold lower in the unprotected areas and reached 308 ± 82 g/m^2 and 142 ± 25 plants/m^2, respectively, but these differences were not statistically significant (U = 179–202, p = 0.45–0.85). The values of thallus weight and length of *P. crispa* inside (2.7 ± 0.6 g and 6.4 ± 0.5 cm) and outside the MPAs (2.0 ± 0.3 g and 6.3 ± 0.4 cm) were similar (U = 181–199, p = 0.48–0.79).

ANOVA also revealed no significant effect of MPA on the parameters of *P. crispa* (Table 3), while depth strongly influenced its biomass and thallus weight and length. A significant interaction between these two factors was found for the thallus weight and length indicating that in the MPAs and unprotected areas pattern of changes in the values of these parameters differed.

The post-hoc comparison of means showed that all the parameters of *P. crispa* increased with increasing depth except for the thallus weight and length in MPAs which reached maximum values at 10 m (Fig. 5). In almost the entire range of depths, the values of *P. crispa*'s biomass and density in MPAs were higher than those in unprotected areas, as it was shown above for their average values, but these differences were not statistically significant. The thallus weight and length were significantly higher in MPAs than outside (3.3-fold and 1.4-fold, respectively), but only at a depth of 10 m (Fig. 5). Thus, in MPAs, the values of these parameters enlarged from a depth of 5 m to 10 m, and at 15 m they became lower, whereas in unprotected areas we observed a continuous increase in the values of these parameters over the entire depth range. This difference explains the significant effect of the interaction of the MPA and depth factors in ANOVA.

Multivariate analysis of the state of the Phyllophora crispa populations

After performing a hierarchical cluster analysis based on *P. crispa*'s biomass, density and thallus weight, all the populations were decided to divide into five groups. Initial clustering was improved by the k-means algorithm; the differences between clusters were statistically significant (Table 4). By using a principal component analysis, the distribution of the populations of *P. crispa* and their clusters in the principal components space were obtained (Fig. 6a). Most of the variability of the parameters of *P. crispa* (76.6%) accounted for the first component which was most correlated with biomass (r = 0.97). The second component determined 20.2% of the total variance and is mainly related to the density (r = 0.57) and thallus weight (r = -0.53).

Cluster 1 united the populations characterised by the lowest values of all the parameters (Table 4, Fig. 6a). Cluster 2 included the populations with intermediate density, in which thalli with a minimum weight prevailed. On the contrary, cluster 3 included the populations, where plants had an intermediate weight, but their density, however, was very low. Thus, the state of the populations in clusters 1, 2, and 3 can be generally characterised as depressed. In 1964–1967, such populations comprised 62% of their total number, while they did 94% in 2015–2020 (Fig. 6a).

Cluster 4 united the populations with intermediate values of biomass, density, and thallus weight (Table 4, Fig. 6a). In 1964–1967, the proportion of the populations belonging to this cluster was 14%, but in 2015–2020 only 5%. Cluster 5 is distinguished by the highest values of biomass, density and thallus weight and included only the populations studied in 1964–1967 (with the proportion of 24% of their total number).

The analysis of changes in the position of the same populations in the principal components space from 1964–1967 to 2015–2020 showed that the state of *P. crispa* deteriorated substantially over that period (Fig. 6b). Most of the populations shifted to the lower clusters or stayed within cluster 1 with the largest position shift occurring at a depth of 20 m where it averaged to 4.26 principal component units, whereas for 15 m, 10 m and 5 m, to 2.13, 0.97 and 0.04, respectively. The only exceptions were the two populations within CAC near Sarych NM at depths of 5 and 10 m, which in 1964–1967 belonged to cluster 1 and cluster 2, and by 2015–2020 had shifted to cluster 2 and cluster 4, respectively.

As can be seen from Fig. 6b, the common trend of changes of populations of *P. crispa* for both protected and unprotected areas was biomass reduction, but there were also differences between them. In MPAs, an average shift of *P. crispa*'s population position was lower in magnitude (0.73) and tended more to decrease in density (red arrow lays in opposite direction to blue arrow corresponding to density), whereas outside MPAs the magnitude of changes was higher (1.93) and their direction was more related to decrease in thallus weight. The beginning points of the two arrows averaging the change in the state of the *P. crispa* populations inside and outside the MPAs were at a distance, indicating some initial difference between populations that became protected and remained unprotected. For the latter, values of biomass, density and thallus weight were 1.5, 1.2 and 1.8 times higher respectively, than for those that later became protected.

Table 3. ANOVA results for the response of biomass (lnB), density (lnN), thallus weight (lnW) and length (L) of *Phyllophora crispa* to the depth and protection off the southwestern coast of Crimea

Factor	lnB				lnN				lnW				L			
	SumSq	df	F	p	SumSq	df	F	p	SumSq	df	F	p	SumSq	df	F	p
Depth	41.17	2	11.4	**1.3×10^{-4}**	7.57	2	2.93	*0.07*	13.23	2	14.8	**1.7×10^{-5}**	6648	2	17.6	**3.8×10^{-6}**
MPA	0.99	1	0.5	0.46	0.01	1	0.01	0.94	0.54	1	1.2	0.279	27	1	0.1	0.71
Depth:MPA	2.72	2	0.8	0.48	1.92	2	0.74	0.48	6.67	2	7.5	**0.002**	2356	2	6.3	**0.004**
Residuals	68.77	38			49.00	38			16.95	38			7157	38		

Note: Significant p-values ($p < 0.05$) are highlighted in bold, $p < 0.1$ are italicised.

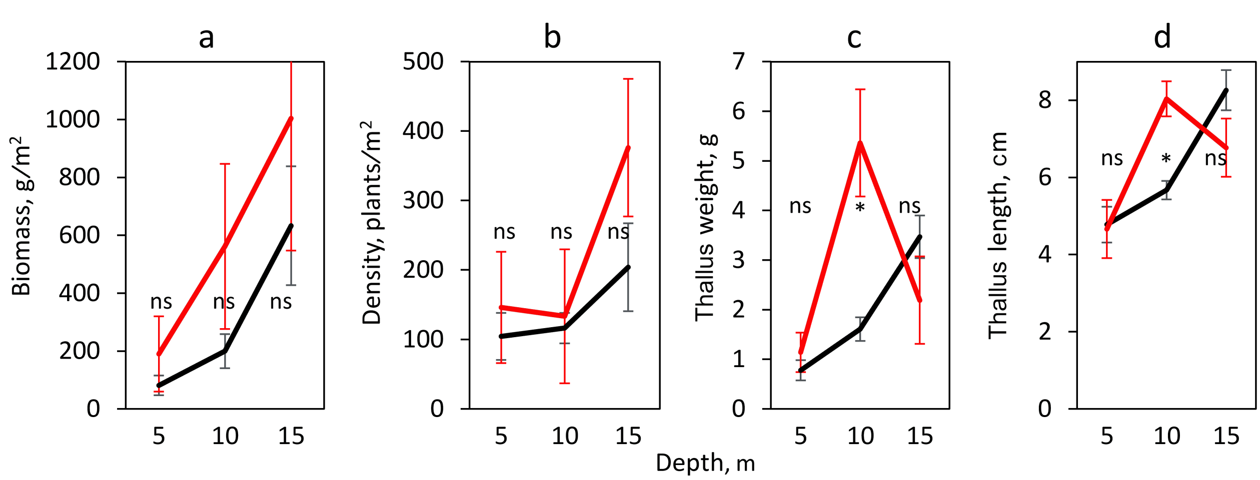

Fig. 5. Average biomass (a), density (b), thallus weight (c) and length (d) of *Phyllophora crispa* at different depths in MPAs (—) and unprotected areas (—) off the southwestern coast of Crimea in 2015–2020 (mean ± SE). The p-values of the one-side t-test followed by the Holm procedure for correction are shown for each depth: * – $p < 0.05$; ns – non-significant.

Table 4. Average biomass B (g/m^2), density N (plants/m^2) and thallus weight W (g) of *Phyllophora crispa* for the selected clusters (mean ± standard error)

Cluster	B (H = 64.4, p = 3.5 × 10^{-13})	N (H = 59.7, p = 3.4 × 10^{-12})	W (H = 60.5, p = 2.3 × 10^{-12})	n
1	86 ± 14	77 ± 10	1.11 ± 0.12	44
2	554 ± 84	370 ± 30	1.48 ± 0.19	15
3	517 ± 77	113 ± 19	4.72 ± 0.36	15
4	2062 ± 154	410 ± 45	5.14 ± 0.59	7
5	3916 ± 208	502 ± 40	8.13 ± 0.87	7

Note: H – value of the Kruskal-Wallis test, n – sample size, p – significance level.

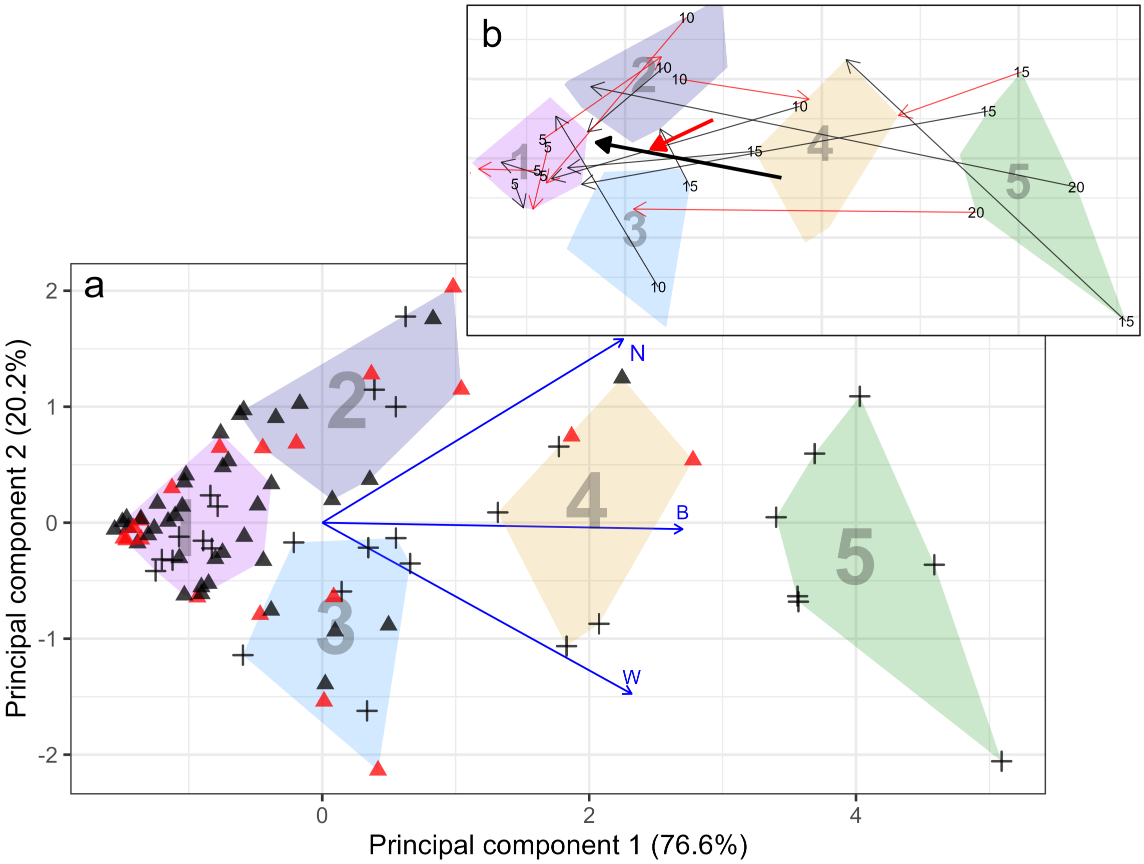

Fig. 6. Populations of *Phyllophora crispa* off the southwestern coast of Crimea in the principal components space. Designations: a) distribution of *P. crispa* populations in 1964–1967 (+) and in 2015–2020 inside (▲) and outside (▲) MPAs; blue arrows denote parameters (B – biomass, N – density, W – thallus weight), polygons – population clusters; b) change in the position of the same *P. crispa* populations in the principal components space from 1964–1967 to 2015–2020; red arrows – populations in MPAs, black arrows – populations in unprotected areas, average shift in the position of the populations is indicated with bold arrows, depths (m) are given near arrow initial points.

Discussion

In this study, no changes in biomass, density and thallus weight of *P. crispa* were found at a depth of 5 m off the southwestern coast of Crimea for a period from 1964–1967 to 2015–2020 while at a depth of 10–20 m degradation of *P. crispa* was observed (Fig. 4). The observed pattern of interannual changes of *P. crispa* biomass along a depth gradient including complete disappearance of this species at 10–25 m depth was previously reported for this region and other parts of the Black Sea (Milchakova, 2003; Simakova & Maximova, 2009; Milchakova et al., 2011; Berov et al., 2018) and attributed to anthropogenic impact, mainly eutrophication (Milchakova, 2003).

Despite the recognition of *P. crispa* as a threatened species (Bucharest Convention, 1992; Bardunov & Novikov, 2008; Yena & Fateryga, 2015; Litvinskaya, 2017; Dovgal & Korzhenevsky, 2018) and its preservation within MPAs, the results of our study showed the effect of protection on this species was negligible (Table 1). In general, we revealed that the state of populations of *P. crispa* was more influenced by depth than protection (see Table 3), as it was reported earlier for other macroalgae species (Ceccherelli et al., 2006; Currie & Sorokin, 2009). A statistically confirmed difference in values of *P. crispa* parameters between protected and unprotected areas was found only when depth was included in the analysis. Specifically, thallus length and weight were significantly greater in the MPAs at a depth of 10 m (Fig. 5c,d) indicating that at least the plant size could benefit from protection. However, because this was observed exclusively at one depth and the pattern of change of these parameters along a depth gradient was unusual (a peak at 10 m instead of 20–40 m (Kalugina-Gutnik, 1975)), we believe that specific habitat conditions in the studied MPAs could influence the results obtained to a larger extent. The habitat difference between protected and unprotected areas could also be responsible for the initial difference between the *P. crispa* populations, although statistical analysis has not confirmed this (Table 1). That as well can be an explanation of different interannual change patterns of *P. crispa* populations inside and outside MPAs (see Fig. 6b).

We also observed a positive change in the *P. crispa* populations within the CAC near Sarych NM (Fig. 6b) that could reflect some environmental improvement in this area. An increase in the *P. crispa* biomass, confined to the shallow zone of 0.5–3.0 m depth, was noted in some areas of the Crimean coast since early 2000s (Milchakova, 2003; Milchakova et al., 2011, 2019). But we found such an increase at a depth of 5–10 m that likely indicates that the water quality improvement extended to deeper water horizons during the last two decades. Nevertheless, because it was found within one area only and at a limited depth range, we believe it was not due to protection but to local conditions. At depths below 10 m, where *P. crispa* was most abundant in 1964–1967, MPAs could not maintain *P. crispa* populations in their pre-protection state (Fig. 6B).

In this study, the lack of a protection effect on *P. crispa* can be partly explained by the fact that the statistical power we have achieved was not sufficient to detect changes in the *P. crispa* population state. For example, although the long-term decrease in *P. crispa* parameter values in MPAs was non-significant, it was not the evidence of successful conservation because the same was found also in unprotected areas. This resulted from low sample sizes, because, for a larger pooled sample of both protected and unprotected areas, a decrease was confirmed statistically (Fig. 3). In 2015–2020, the difference between *P. crispa* abundance inside and outside the MPAs was large in magnitude while still non-significant, probably because of high data variability and deficient sample size. One of the sources of the variability was data pooling for different depths, since, as we have shown, the values of *P. crispa* parameters varied greatly with depth (Table 2, Table 3). This was also an issue in our attempt to confirm the effect of protection on the change of *P. crispa* the condition over the 1964–2020. Particularly, much less interannual biomass decrease inside MPAs compared to unprotected areas (see Fig. 3) might result from underrepresentation of deep-water data (> 5 m) in the analysis: for PAs, they comprised only 50% of the total dataset, while for unprotected areas they did 83%. The lack of statistical power and the need for additional resources were reported to have a role in preventing the detection of consequences of human exclusion for macroalgae in other studies (Fraschetti et al., 2005; Currie & Sorokin, 2009).

The effect of protection was uncertain for other macroalgal species off the coast of Crimea as well. The biomass of *Cladostephus spongiosus* (Huds.) C.Ag., *Laurencia coronopus* J.Ag., and *Osmundea truncata* (Kütz.) K.W.Nam & Maggs was reported to have both increased and decreased in MPAs of different protection categories over the last decades, having increased in unprotected areas (Milchakova, 2012). Such variability in abundance of these algae, regardless of protection, indicates that their condition depends to a greater degree on general environmental trends and local impacts than on their location within an MPA.

The conservation success of *P. crispa* may also depend on the strictness of the protection regime which is known to influence the MPA effectiveness (Benedetti-Cecchi et al., 2003; Claudet et al., 2008; Edgar et al., 2014; Turnbull et al., 2021). The southwestern coast of Crimea lacks state nature reserves, which are the most enforced PAs in the Russian Federation (IUCN category I). All MPAs in this region are of quite low protection level (IUCN category IV–V). They lack no-entry zones and proper enforcement and some types of recreational activities are allowed there as well. The effect of protection strictness on *P. crispa* has not been studied to date, but is probably not highly significant, as bottom communities dominated by this species are available only to divers, who most likely impact other groups of organisms (Milazzo et al., 2002, but see Luna et al., 2009). Potential disturbance to beds of *P. crispa* can also be caused by anchoring of small size recreational vessels, whose passage is permitted, for example, in the Cape Aya Sanctuary. Nevertheless, it is known that infralittoral macroalgal assemblages are affected by this anthropogenic factor to a lesser extent than other macrophyte communities (seagrass or coralligenous) (Milazzo et al., 2002). In general, our results match to those reported by Fraschetti et al. (2005) for common erect macroalgae in the Adriatic Sea, the cover of which did not depend on the level of human activity restrictions.

Capturing of plants and animals is prohibited in the studied MPAs (PARF, 2022). However, we believe this measure does not considerably affect the abundance of *P. crispa*, since its main impact is on commercial species (Claudet et al., 2008), while *P. crispa* has never been of commercial importance along the Crimean coast. Furthermore, its harvesting is prohibited being a protected species (Bucharest Convention, 1992; Bardunov & Novikov, 2008). Nevertheless, no-take protection can influence the state of macrophyte populations indirectly, via trophic cascades triggered by change in abundance of top predators controlling algal feeders (Lester et al., 2009; Shears & Babcock, 2003). Although this effect on *P. crispa* in the Black Sea has not been studied (Berov et al., 2018), up to the present it seems to have been insignificant, since *P. crispa* has not been noted in the herbivorous fish diet (Kalinina, 1963; Shaganov, 2018) and is even less preferable for herbivorous invertebrates than most common *Ericaria crinita* and *Gongolaria barbata* (Makkaveeva, 1964), the amount of which consumed by invertebrates is known to be small (Makkaveeva, 1974). At the same time, an alien herbivorous fish *Sarpa salpa* (Linnaeus, 1758) from the Mediterranean, has naturalised off the southwestern coast of Crimea over the past 20 years, including the area adjacent to Cape Aya Sanctuary (Abliazov et al., 2021), and within CAC near Cape Fiolent NM (Boltachev et al., 2009). *Sarpa salpa* can significantly reduce macrophyte abundance (Gianni et al., 2017), and is likely capable to alter species composition through selective grazing (Verlaque, 1990), especially in MPAs where it is protected from being caught (Raventos et al., 2009; Parravicini et al., 2013). In the Mediterranean, *P. crispa* was reported to constitute only 5.0–6.6% of the *S. salpa* diet (Verlaque, 1990), but in the Black Sea, its feeding preferences and effect on seaweed communities are unknown and need to be investigated. Additional measures to prevent invasive species from being introduced and naturalised are desirable to take as well.

The size of the studied MPAs is significantly smaller than the recommended minimum values of about 20–30 km^2 (McLeod et al., 2009; Saarman et al., 2013), so their conservation value is low. In particular, in Australia, no positive changes in macrophytobenthos were reported within small-area PAs (Edgar & Barrett, 1999). Moreover, the MPAs of the southwestern Crimea are limited to a narrow strip along the coast, about 300 m wide, and are devoid of buffer zones. That makes them vulnerable to negative external impacts (McLeod et al., 2009) such as bottom trawl fishing and sand extraction, which have taken place previously in the vicinity of the studied MPAs (Boltachev, 2006; Boltachev et al., 2012). At present the coastal zone is being intensively developed, recreation is increasing and poorly treated wastewater discharges take place (Gruzinov et al., 2019). These factors lead to the environmental quality deterioration, which causes the degradation of macrophyte communities even within MPAs (Milchakova et al., 2019). Under these conditions, sciaphilic algae are especially threatened, and even after the cessation of eutrophication, their recovery is difficult due to the peculiarities of their biology, in particular low growth rate and long lifespan (Thibaut et al., 2005), which are also typical for *P. crispa* (Kalugina-Gutnik, 1975). Additionally, even well-designed PAs planned to cope with local impacts can be ineffective against large-scale threats such as climate change or eutrophication (Boersma & Parrish, 1999; Parravicini et al., 2013; Gilby et al., 2015). Overcoming these challenges involves adoption of global conservation strategies, such as full protection of 30% of each marine habitat proposed by the IUCN (Wilson et al., 2020), and the marine spatial planning approaches to control the environmental quality not only within MPAs but also outside (Agardy et al., 2011; Vaughan & Agardy, 2020).

Conclusions

It has been found that over the past half century, the state of the threatened red alga *P. crispa* has significantly deteriorated at depths more than 10 m off the southwestern coast of Crimea, which was registered even within MPAs, despite their long existence. At present, the biomass and density of this species are higher inside the MPAs than outside, but this difference is not statistically significant. It is possible, though, that a positive effect of *P. crispa* protection could be confirmed after an increase in the statistical power, which may be achieved through spreading the scope of further investigations to the entire Crimean coast to include more MPAs into the analysis. This would make it also possible to collect enough data for comparing the effect of full vs. partial protection. Moreover, it is desirable to focus further research on the depth range of 15–20 m where, according to our data, the most pronounced interannual changes of the *P. crispa* populations occurred.

Statistically insignificant effect of MPAs on *P. crispa* may be caused by their low efficiency in preventing decrease in environmental quality within their boundaries caused by natural or human-induced disturbances in the adjacent unprotected areas. We believe that in order to reduce the vulnerability of the MPAs to local negative impacts, it is reasonable to enlarge them and change their regional status to a federal one, which will make it possible to create marine buffer zones around these PAs. To mitigate large-scale threats to macroalgal-dominated habitats it is also necessary to incorporate local MPA planning into national and international strategies of environmental management resulting in water quality improvement throughout the region.

Acknowledgements

The study was supported by the Federal Research Center A.O. Kovalevsky Institute of Biology of the Southern Seas RAS (IBSS) under the state assignment №121030100028-0. Authors are grateful to the IBSS Phytoresources Laboratory staff, divers D.V. Shapkin and A.N. Tereshchenko for their help in material collection and processing, and to S.S. Raksha for the photo of *Phyllophora crispa*.

References

Abaza V., Dumitrache C., Filimon A., Marin O. 2019. Present ecological status of the main benthic habitats from the Romanian Black Sea Shelf. *Marine Research Journal* 49(1): 74–91. DOI: 10.55268/CM.2019.49.74

Abliazov E.R., Boltachev A.R., Karpova E.P., Pashkov A.N., Danilyuk O.N. 2021. Ichthyofauna of the Black Sea coastal zone in the Laspi Bay area (Crimea). *Marine Biological Journal* 6(2): 3–17. DOI: 10.21072/mbj.2021.06.2.01

Agardy T., di Sciara N.G., Christie P. 2011. Mind the gap: Addressing the shortcomings of marine protected areas through large scale marine spatial planning. *Marine Policy* 35(2): 226–232. DOI: 10.1016/j.marpol.2010.10.006

Alexandrov B., Minicheva G., Zaitsev Y. 2017. Black Sea Network of Marine Protected areas: European approaches and adaptation to expansion and monitoring in Ukraine. In: P.D. Goriup (Ed.): *Management of Marine Protected Areas: A Network Perspective*. New York: John Wiley & Sons Ltd. P. 227–246. DOI: 10.1002/9781119075806.ch12

Babcock R.C., Kelly S., Shears N.T., Walker J.W., Willis T.J. 1999. Changes in community structure in temperate marine reserves. *Marine Ecology Progress Series* 189: 125–134. DOI: 10.3354/meps189125

Bardunov L.V., Novikov V.S. (Eds.). 2008. *Red Data Book of the Russian Federation. Plants and Fungi*. Moscow: KMK Scientific Press Ltd. 885 p. [In Russian]

Barrett M.S., Buxton C.D., Edgar G.J. 2009. Changes in invertebrate and macroalgal populations in Tasmanian marine reserves in the decade following protection. *Journal of Experimental Marine Biology and Ecology* 370(1–2): 104–119. DOI: 10.1016/J.JEMBE.2008.12.005

Begun T., Muresan M., Zaharia T., Dencheva K., Sezgin M., Bat L., Velikova V. 2012. *Conservation and Protection of the Black Sea Biodiversity. Review of the existing and planned protected areas in the Black Sea (Bulgaria, Romania, Turkey) with a special focus on possible deficiencies regarding law enforcement and implementation of management plans*. EC DG Env. MISIS Project Deliverables. 110 p.

Begun T., Teacă A., Mureşan M., Quijón P.A., Menabit S., Surugiu V. 2022. Habitat and Macrozoobenthic Diversity in Marine Protected Areas of the Southern Romanian Black Sea Coast. *Frontiers in Marine Science* 9: 845507. DOI: 10.3389/fmars.2022.845507

Benedetti-Cecchi L., Bertocci I., Micheli F., Maggi E., Fosella T., Vaselli S. 2003. Implications of spatial heterogeneity for management of marine protected areas (MPAs): examples from assemblages of rocky coasts in the northwest Mediterranean. *Marine Environmental Research* 55(5): 429–458. DOI: 10.1016/S0141-1136(02)00310-0

Berov B., Todorova M., Dimitrov L., Rinde E., Karamfilov V. 2018. Distribution and abundance of phytobenthic communities: Implications for connectivity and ecosystem functioning in a Black Sea marine protected area. *Estuarine, Coastal and Shelf Science* 200: 234–247. DOI: 10.1016/j.ecss.2017.11.020

Boersma P.D., Parrish J.K. 1999. Limiting abuse: marine protected areas, a limited solution. *Ecological Economics* 31(2): 287–304. DOI: 10.1016/S0921-8009(99)00085-3

Boltachev A.R. 2006. Trawl fishery and its effect on the bottom biocenoses in the Black Sea. *Marine Ecological Journal* 5(3): 45–56. [In Russian]

Boltachev A.R., Karpova E.P., Danilyuk O.N. 2009. Findings of new and rare fish species in the coastal zone of the Crimea (the Black Sea). *Journal of Ichthyology* 49(4): 277–291. DOI: 10.1134/S0032945209040018

Boltachev A.R., Sergeeva N.G., Zagorodnyaya Yu.A., Klimova T.N. 2012. Sand mining on the shelf near Sevastopol as a threat to coastal ecosystem and biological diversity of the Black Sea. In: N.V. Bagrov (Ed.): *Biodiversity and Sustainable Development*. Simferopol. P. 39–42. [In Russian]

Bucharest Convention. 1992. *Convention on the protection of the Black Sea against pollution. ANNEX 2 Provisional list of species of the Black Sea importance*. Available from http://www.blacksea-commission.org/Official%20Documents/The%20Convention/full%20text/

Cacabelos E., Martins G.M., Faria J., Prestes A.C.L., Costa T., Moreu I., Neto A.I. 2020. Limited effects of marine protected areas on the distribution of invasive species, despite positive effects on diversity in shallow-water marine communities. *Biological Invasions* 22: 1169–1179. DOI: 10.1007/s10530-019-02171-x

Ceccherelli G., Casu D., Pala D., Pinna S., Sechi N. 2006. Evaluating the effects of protection on two benthic habitats at Tavolara-Punta Coda Cavallo MPA (North-East Sardinia, Italy). *Marine Environmental Research* 61(2): 171–185. DOI: 10.1016/j.marenvres.2005.09.002

Claudet J., Osenberg C.W., Benedetti-Cecchi L., Domenici P., García-Charton J.A., Pérez-Ruzafa A., Badalamenti F., Bayle-Sempere J., Brito A., Bulleri F., Culioli J.M., Dimech M., Falcón J.M., Guala I., Milazzo M., Sánchez-Meca J., Somerfield P.J., Stobart B., Vandeperre F., Valle C., Planes S. 2008. Marine reserves: size and age do matter. *Ecology Letters* 11(5): 481–489. DOI: 10.1111/J.1461-0248.2008.01166.X

Currie D., Sorokin S. 2009. Evaluating the effects of reserve closure on algae, invertebrate and fish assemblages at a temperate South Australian marine reserve. *Journal of* *the Marine Biological Association of the United Kingdom* 89(4): 651–661. DOI: 10.1017/S0025315409000125

Dovgal I.V., Korzhenevsky V.V. (Eds.). 2018. *Red Data Book of the city of Sevastopol*. Kaliningrad; Sevastopol: ROST-DOAFK. 432 p. [In Russian]

Dyakov N.N., Malchenko Y.A., Lipchenko A.E., Bobrova S.A., Timoshenko T.Y. 2020. Hydrological and hydrochemical characteristics of Crimean coastal waters and necessary measures to reduce the pollution level of recreation zones. *Proceedings of the State Institute of Oceanography* 221: 163–194. [In Russian]

Edgar G.J., Barrett N.S. 1999. Effects of the declaration of marine reserves on Tasmanian reef fishes, invertebrates and plants. *Journal of Experimental Marine Biology and Ecology* 242(1): 107–144. DOI: 10.1016/S0022-0981(99)00098-2

Edgar G., Stuart-Smith R., Willis T., Kininmonth S., Baker S.C., Banks S., Barrett N.S., Becerro M.A., Bernard A.T.F., Berkhout J., Buxton C.D., Campbell S.J., Cooper A.T., Davey M., Edgar S.C., Försterra G., Galván D.E., Irigoyen A.J., Kushner D.J., Moura R., Parnell P.E.D., Shears N.T., Soler G., Strain E.M.A., Thomson J.R. 2014. Global conservation outcomes depend on marine protected areas with five key features. *Nature* 506(7487): 216–220. DOI: 10.1038/nature13022

Fox J., Weisberg S. 2019. *An R companion to applied regression*. 3rd ed. Thousand Oaks, CA: Sage. 608 p.

Fraschetti S., Terlizzi A., Bussotti S., Guarnieri G., D'Ambrosio P., Boero F. 2005. Conservation of Mediterranean seascapes: analyses of existing protection schemes. *Marine Environmental Research* 59(4): 309–332. DOI: 10.1016/j.marenvres.2004.05.007

Gianni F., Bartolini F., Airoldi L., Ballesteros E., Francour P., Guidetti P., Meinesz A., Thibaut T., Mangialajo L. 2013. Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of marine protected areas. *Advances in Oceanography and Limnology* 4(2): 83–101. DOI: 10.1080/19475721.2013.845604

Gianni F., Bartolini F., Pey A., Laurent M., Martins G.M., Airoldi L., Mangialajo L. 2017. Threats to large brown algal forests in temperate seas: the overlooked role of native herbivorous fish. *Scientific Reports* 7(1): 6012. DOI: 10.1038/s41598-017-06394-7

Gilby B.L., Maxwell P.S., Tibbetts I.R., Stevens T. 2015. Bottom-up factors for algal productivity outweigh no-fishing marine protected area effects in a marginal coral reef system. *Ecosystems* 18(6): 1056–1069. DOI: 10.1007/S10021-015-9883-8

Gruzinov V.M., Dyakov N.N., Mezenceva I.V., Malchenko Y.A., Zhohova N.V., Korshenko A.N. 2019. Sources of coastal water pollution near Sevastopol. *Oceanology* 59(4): 523–532. DOI: 10.1134/S0001437019040076

Gubbay S., Sanders N., Haynes T., Janssen J.A.M., Rodwell J.R., Nieto A., García Criado M., Beal S., Borg J., Kennedy M., Micu D., Otero M., Saunders G., Calix M. 2016. *European Red List of habitats. Part 1: Marine habitats*. Luxembourg: Publications Office of the European Union. 46 p. DOI: 10.2779/032638

Kalinina E.M. 1963. Growth and nutrition of the Black Sea greenfinches of the genera *Crenilabrus* and *Symphodus*. *Proceedings of the Sevastopol Biological Station* 16: 323–336. [In Russian]

Kalugina-Gutnik A.A. 1974. Bottom vegetation of the Sevastopol Bay. *Biologiya Morya* 32: 133–164. [In Russian]

Kalugina-Gutnik A.A. 1975. *Phytobenthos of the Black Sea*. Kiev: Naukova Dumka. 248 p. [In Russian]

Kalugina-Gutnik A.A., Kulikova N.M. 1974. Bottom vegetation near the western coast of Crimea. *Biologiya Morya* 3: 111–129. [In Russian]

Kassambara A. 2021. *rstatix: pipe-friendly framework for basic statistical tests. R package version 0.7.0*. Available from https://CRAN.R-project.org/package=rstatix

Kassambara A., Mundt F. 2019. *factoextra: extract and visualize the results of multivariate data analyses. R package version 106*. Available from https://CRAN.R-project.org/package=factoextra

Kolyuchkina G.A., Syomin V.L., Simakova U.V., Mokievsky V.O. 2018. Presentability of the Utrish Nature Reserve's benthic communities for the North Caucasian Black Sea Coast. *Nature Conservation Research* 3(4): 1–16. DOI: 10.24189/ncr.2018.065

Kovardakov S.A., Kovrigina N.P., Izmest'yeva M.A. 2004. Bottom phytocenosis in the water area from Cape Balaklavsky to Cape Aya and its contribution to the processes of self-purification. *Monitoring Systems of Environment* 7: 250–257. [In Russian]

Lê S., Josse J., Husson F. 2008. FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software* 25(1): 1–18. DOI: 10.18637/jss.v025.i01

Legendre P., Legendre L. 1998. *Numerical Ecology*. 2nd ed. Amsterdam: Elsevier. 853 p.

Lester S., Halpern B., Grorud-Colvert K., Lubchenco J., Ruttenberg B., Gaines S., Airamé S., Warner R. 2009. Biological effects within no-take marine reserves: a global synthesis. *Marine Ecology Progress Series* 384: 33–46. DOI: 10.3354/meps08029

Litvinskaya S.A. (Ed.). 2017. *Red Data Book of the Krasnodarsky Krai. Plants and Fungi*. 3rd ed. Krasnodar: Administration of the Krasnodarsky Krai. 850 p. [In Russian]

Logan M. 2010. *Biostatistical design and analysis using R: a practical guide*. Chichester: Wiley-Blackwell. 546 p.

Luna B., Pérez C.V., Sánchez-Lizaso J.L. 2009. Benthic impacts of recreational divers in a Mediterranean marine protected area. *ICES Journal of Marine Science* 66(3): 517–523. DOI: 10.1093/icesjms/fsp020

Makkaveeva E.B. 1964. Macrofauna of the seaweed biocenosis in the western Crimean coast. *Proceedings of the Sevastopol Biological Station* 15: 180–195. [In Russian]

Makkaveeva E.B. 1974. Production of the most abundant species of *Cystoseira* biocenosis. In: A.S. Konstantinov, Yu.G. Aleev (Eds.): *Biological productivity of the southern seas*. Kiev: Naukova Dumka. P. 57–70. [In Russian]

McLeod E., Salm R., Green A., Almany J. 2009. Designing marine protected area networks to address the impacts of climate change. *Frontiers in Ecology and the Environment* 7(7): 362–370. DOI: 10.1890/070211

Milazzo M., Chemello R., Badalamenti F., Camarda R., Riggio S. 2002. The impact of human recreational activities in marine protected areas: What lessons should be learnt in the Mediterranean Sea? *Marine Ecology* 23(s1): 280–290. DOI: 10.1111/j.1439-0485.2002.tb00026.x

Milchakova N.A. 2003. Macrophytobenthos. In: V.N. Eremeev, A.V. Gaevskaya (Eds.): *Modern condition of biological diversity of Crimea (the Black Sea sector).* Sevastopol: Ekosi-Gidrophisika. P. 152–208. [In Russian]

Milchakova N.A. 2012. Cenopopulation dynamics and problems of macrophytes protection in the Ukrainian shelf of the Black Sea. In: S.L. Mosyakin (Ed.): *The plant kingdom in the Red Data Book of Ukraine: Implementing the global strategy for plant conservation*. Kyiv: Palyvoda A.V. P. 209–212. [In Russian]

Milchakova N.A., Mironova N.V., Ryabogina V.G. 2011. Biological resources of the Black Sea and the Sea of Azov. In: V.N. Eremeev, A.V. Gaevskaya, G.E. Shulman, Yu.A. Zagorodnyaya (Eds.): *Biological resources of the Black Sea and of Azov*. Sevastopol: Ekosi-Gidrophisika. P. 117–139. [In Russian]

Milchakova N.A., Mironova N.V., Alexandrov V.V. 2013. Dynamics, causes of degradation and the prognosis of restoration Zernov *Phyllophora* field (the Black Sea). In: G. de Lange, M. Gačić, A. Romaña, M. da Costa, F. Boero, C. Turan (Eds.): *Rapports et Procès-Verbaux des Réunions de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée*. Vol. 40. Monaco: CIESM. P. 622.

Milchakova N.A., Alexandrov V.V., Bondareva L.V., Pankeyeva T.V., Chernysheva E.B. 2015. *Marine protected areas of the Crimea*. Simferopol: N. Orianda. 312 p. [In Russian]

Milchakova N.A., Alexandrov V.V., Ryabogina V.G. 2019. State of key phytocenoses of marine protected areas and problems their conservation (southwestern Crimea, Black Sea). *Plant Biology and Horticulture: theory, innovation* 149: 113–123. DOI: 10.36305/0201-7997-2019-149-113-123 [In Russian]

Orfanidis S., Panayotidis P., Ugland K. 2011. Ecological Evaluation Index continuous formula (EEI-c) application: a step forward for functional groups, the formula and reference condition values. *Mediterranean Marine Science* 12(1): 199–232. DOI: 10.12681/mms.60

Orlando-Bonaca M., Lipej L., Orfanidis S. 2008. Benthic macrophytes as a tool for delineating, monitoring and assessing ecological status: the case of Slovenian coastal waters. *Marine Pollution Bulletin* 56(4): 666–676. DOI: 10.1016/j.marpolbul.2007.12.018

PARF. 2022. *Information-Analytical System «Protected Areas of the Russian Federation»*. Available from http://oopt.aari.ru/ [In Russian]

Parravicini V., Micheli F., Montefalcone M., Morri C., Villa E., Castellano M., Povero P., Bianchi C.N. 2013. Conserving biodiversity in a human-dominated world: degradation of marine sessile communities within a protected area with conflicting human uses. *PLoS ONE* 8(10): e75767. DOI: 10.1371/journal.pone.0075767

R Core Team. 2019. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available from https://www.R-project.org/

Raventos N., Ferrari B., Planes S. 2009. Differences in population parameters and behaviour of the herbivorous fish *Sarpa salpa* between protected and unprotected seagrass meadows in the north-western Mediterranean. *Journal of the Marine Biological Association of the United Kingdom* 89(6): 1153–1159. DOI: 10.1017/S0025315409000423

Roberts C.M., Andelman S., Branch G., Bustamante R.H., Castilla J.C., Dugan J., Halpern B.S., Lafferty K.D., Leslie H., Lubchenco J., Mcardle D., Possingham H.P., Ruckelshaus M., Warner R.R. 2003. Ecological criteria for evaluating candidate sites for marine reserves. *Ecological Applications* 13(sp1): 199–214. DOI: 10.1890/1051-0761(2003)013[0199:ECFECS]2.0.CO;2

Ryabushko V.I., Shchurov S.V., Kovrigina N.P., Lisitskaya E.V., Pospelova N.V. 2020. Comprehensive Research of the Environmental Status of Coastal Waters of Sevastopol (Western Crimea, Black Sea). *Ecological Safety of the Coastal and Shelf Zones of the Sea* 1: 103–118. DOI: 10.22449/2413-5577-2020-1-103-118 [In Russian]

Saarman E., Gleason M., Ugoretz J., Airamé S., Carr M., Fox E., Frimodig A., Mason T., Vasques J. 2013. The role of science in supporting marine protected area network planning and design in California. *Ocean and Coastal Management* 74: 45–56. DOI: 10.1016/j.ocecoaman.2012.08.021

Sevostyanova M.V., Pavlenko L.F., Korablina I.V. 2016. Current level of the Black Sea pollution by oil and heavy metals. In: A.V. Gaevskaya (Ed.): *Marine biological research: achievements and perspectives*. Vol. 3. Sevastopol: EKOSI-Gidrofizika. P. 210–213. [In Russian]

Shaganov V.V. 2018. *Fish of the stony sublittoral of the South-Eastern Crimea: composition, distribution by biotopes, ecology features*. PhD Thesis. Kerch: Kerch State Marine Technological University. 133 p. [In Russian]

Shchurov S.V., Kovrigina N.P., Ladygina L.V. 2019. Seasonal variation of abiotic factors of environment and phytoplankton in the Laspi Bay mussel farm area (2010–2011). *Scientific Notes of V.I. Vernadsky Crimean Federal University. Geography. Geology* 5(2): 184–201. [In Russian]

Shears N.T., Babcock R.C. 2003. Continuing trophic cascade effects after 25 years of no-take marine reserve protection. *Marine Ecology Progress Series* 246: 1–16. DOI: 10.3354/meps246001

Simakova U.V., Maximova O.V. 2009. Present-day state of the attached *Phyllophora* community in the vicinity of the Novorossijsk. In: *Proceedings of the 9th International Conference on the Mediterranean Coastal Environment, Medcoast 2009 (Sochi, 10–14 November 2009)*. Vol. 1. Sochi. P. 317–322.

Skrebovska S.V., Shaposhnikova A.O. 2016. Macrophytic algae of western part of Dzharylgach Gulf in the Black Sea. *Chornomorski Botanical Journal* 12(1): 72–77. [In Ukrainian]

Thibaut Th., Pinedo S., Torras X., Ballesteros E. 2005. Long-term decline of the populations of Fucales (*Cystoseira* spp. and *Sargassum* spp.) in the Alberes coast (France, North-western Mediterranean). *Marine Pollution Bulletin* 50(12): 1472–1489. DOI: 10.1016/j.marpolbul.2005.06.014

Tikhonova E.A., Soloveva O.V., Mironov O.A., Burdiyan N.V. 2020. Sanitary and Biological Characteristics of the Laspi Reserve Coastal Waters (the Black Sea). *Ecological Safety of Coastal and Shelf Zones of Sea* 3: 95–106. DOI: 10.22449/2413-5577-2020-3-95-106 [In Russian]

Tkachenko F.P., Kovtun O.O. 2014. Contemporary condition of seaweeds flora of Zmeiny island costal zone (Black Sea). *Chornomorski Botanical Journal* 10(1): 37–47. [In Ukrainian]

Tkachenko F.P., Maslov I.I. 2002. Marine macrophytobenthos of Chernomorsky Biosphere Reservation. *Ecologiya Morya* 62: 34–40. [In Russian]

Turnbull J.W., Johnston E.L., Clark G.F. 2021. Evaluating the social and ecological effectiveness of partially protected marine areas. *Conservation Biology* 35(3): 921–932. DOI: 10.1111/cobi.13677

Vaughan D., Agardy N. 2020. Marine protected areas and marine spatial planning – allocation of resource use and environmental protection. In: J. Humphreys, R.W.E. Clark (Eds.): *Marine Protected Areas: Science, Policy and Management*. Amsterdam-Oxford-Cambridge: Elsevier. P. 13–35. DOI: 10.1016/B978-0-08-102698-4.00002-2

Verlaque M. 1990. Relationships between *Sarpa salpa* (Linnaeus, 1758) (Téléostéen, Sparidae), other browser fishes, and the Mediterranean algal phytobenthos. *Oceanologica Acta* 13(3): 373–388.

Wilson K.L., Tittensor D.P., Worm B., Lotze H.K. 2020. Incorporating climate change adaptation into marine protected area planning. *Global Change Biology* 26(6): 3251–3267. DOI: 10.1111/gcb.15094

Yena A.V., Fateryga A.V. (Eds.). 2015. *Red Data Book of the Republic of Crimea. Plants, algae and fungi*. Simferopol: ARIAL. 480 p. [In Russian]

Zaitsev Yu.P., Mamaev V.O. 1997. *Marine biological diversity in the Black Sea: a study of change and decline*. New York: United Nations Publications. 208 p.

ВЛИЯЮТ ЛИ ООПТ НА ПОПУЛЯЦИИ ОХРАНЯЕМОЙ КРАСНОЙ МАКРОВОДОРОСЛИ *PHYLLOPHORA CRISPA* ЮГО-ЗАПАДНОГО ПОБЕРЕЖЬЯ КРЫМА (ЧЕРНОЕ МОРЕ)?

В. В. Александров*, Н. А. Мильчакова

Институт биологии южных морей имени А.О. Ковалевского РАН, Россия
**e-mail: valexandrov@list.ru*

Многолетняя сциафильная водоросль *Phyllophora crispa* (Phyllophoraceae, Rhodophyta) относится к основным ценозообразующим видам Черного моря. В связи с катастрофической деградацией ее популяций, она была внесена в Красную книгу Российской Федерации. *Phyllophora crispa* у берегов юго-западного Крыма встречается в акватории шести особо охраняемых природных территорий (ООПТ), созданных в период с 1972 по 2017 гг., ширина акватории которых составляет около 300 м, а площадь колеблется от 0.0597 км^2 до 0.208 км^2. Несмотря на длительный срок существования, в некоторых из них ранее была выявлена деградация сообществ *P. crispa*, что может свидетельствовать о неэффективности мер по охране донных сообществ на ООПТ и необходимости оптимизации природоохранной сети региона. Учитывая это, целью настоящего исследования являлась оценка эффективности охраны *P. crispa* в ООПТ юго-западного Крыма. В задачи работы входило сравнить состояние популяций этого вида в исследуемом регионе в настоящее время и в период до создания ООПТ, а также оценить их современное состояние в охраняемых и неохраняемых акваториях. Исследования выполнены на глубинах от 0.5 м до 20 м в 2015–2020 гг. Определяли численность, биомассу, массу и длину талломов *P. crispa* в пяти ООПТ, срок существования которых составлял 33–48 лет, и в 16 неохраняемых акваториях. Сравнение собственных и литературных данных показало, что за период с 1964–1967 гг. по 2015–2020 гг. на глубинах 5–20 м биомасса *P. crispa* в регионе уменьшилась в среднем в 2.7 раза, численность – в 1.5 раза, масса слоевищ – в 2.0 раза. Установлено, что в диапазоне глубин 5–15 м наиболее выраженное ухудшение состояния популяций *P. crispa* произошло на 15 м, где снижение биомассы популяций и массы талломов этого вида было статистически значимым. При этом согласно результатам дисперсионного анализа охранный статус акваторий не влиял на многолетние изменения значений параметров популяций *P. crispa*. Кроме того, выявлено, что в 2015–2020 гг. различия средней биомассы и численности этого вида в ООПТ и за их пределами были статистически недостоверными. Масса и длина талломов в охраняемых и неохраняемых акваториях также существенно не различались, исключая глубину 10 м, на которой значения этих показателей в ООПТ были соответственно в 3.3 и 1.4 раза выше, чем на прочих участках. Одной из причин недостоверного различия показателей состояния популяций *P. crispa* в ООПТ и за их пределами могла быть недостаточная статистическая мощность исследования. Другой причиной, вероятно, является малая площадь и незначительная ширина акватории ООПТ, что делает сообщества макрофитов уязвимыми к негативным факторам, действующим вблизи границ объектов. Для повышения эффективности охраны *P. crispa* в юго-западном Крыму рекомендовано увеличить площадь охраняемых акваторий, создать буферные зоны и принять меры по улучшению качества вод во всем регионе.

Ключевые слова: биомасса, многолетние изменения, макрофиты, морская ООПТ, плотность, эффективность