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Ecological factors limiting the distribution of the seagrasses of the genus Zostera in the sublittoral zone of the Burgas Bay (the Black Sea): importance of wind waves and epiphyte abundance

A U T O R E F E R A T

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The dissertation was elaborated within the framework of a free doctorate in the department "Biology and Ecology of the Sea" at the Institute of Oceanology – BAS.

The dissertation 247pages long, contains 10 chapters, 170 figures, 32 tables and 6 appendices. The list of cited literature includes 211 titles of which 38 in Cyrillic, 173 in Latin and 4 websites.

The dissertation public defence will be held on ……………… at ………… at the Insitute of oceanology.
Scientific jury:

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The materials on the defense are available to those interested in the library of the Institute of Oceanology "Prof. Fritijof Nansen”, Varna, 40 Parvi May Str.

To my parents

I thank to God for the love and help during my way.
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From the Psalm 22 (23)

“The Lord is my shepherd, I shall not want.
He makes me lie down in green pastures.
He leads me beside still waters;
He restores my soul.
He leads me in the paths of righteousness for His name's sake.
Even though I walk through the valley of the shadow of death,
I fear no evil, for Thou art with me. …”

I. Introduction

One of the fundamental characteristics of the life strategy of benthic organisms is the colonization of an accessible substrate and the preservation of the conquered territory. Through the conquered space, they gain practical access to all the vital conditions for existence. The retreat of the benthic populations from the boundaries of their habitat, as a result of living conditions deterioration, is the final stage in the process of adaptation to suboptimal environmental conditions.

The spatial boundaries of the habitats of aquatic angiosperms in natural conditions are determined by: the shallow-water depth of distribution and the deep-water depth of distribution, between which the habitable part of the benthic is located (Koch, 2006). With the exception of cases of freshwater discharges (Mathiesen and Nielsen, 1956, cited in Send-Jensen, 1977), under unaffected conditions, the upper depth of higher aquatic vegetation is most often determined by the effects of waves, and the lower limit by the available light.

The Bulgarian Black Sea coast, as a whole, is exposed to wind waves (Vlachev et al., 2014). But in the Burgas Bay there are areas protected from the wave action and it is suitable for studying the effect of wind waves on seagrass.

In the process of reduction of the available light below the compensation point of the grasses and of shallowing their lower limit, both light attenuation through the water column and the shading by the epiphytes are important.

The available examples show ambiguity in the scientific judgement of the indicator applicability of epiphytes for assessment of the level of eutrophication. The different level of their reaction is indicative for existance of local and seasonal specificity in their responce which necessitates an assessment of their indicator applicability for each specific case.

II. Literature review

The literature review includes a summary of research conducted worldwide and in the Black Sea on the importance of the two environmental factors wind waves and epiphytic abundance for the spatial distribution of seagrass.

The literature reference focuses on modeling as an approach to studying the wave impact on seagrass. In the models for spatial distribution of seagrass, the pair "sea wave - light climate" almost invariably participates in support of the thesis that the two factors outline the roughest and widest boundaries of the spatial ecological niche of the species inhabiting soft bottoms (Koch, 2006). От прегледа става ясно, че получените оценки за вълновия ефект се основават не на преки измервания, а на статистическо моделиране (Infantes et al., 2009, Sundblad et al., 2014, Schubert et al., 2015). The review shows that the obtained estimates for the wave effect are not based on direct measurements, but on statistical modeling (Infantes et al., 2009, Sundblad et al., 2014, Schubert et al., 2015). The set of explanatory (independent) variables varies depending on the complexity of the model, and in addition to the two environmental factors cited, the following may be included: season of the year (Fonseca et al., 2002), bottom slope (Brown, 2015); sediment content, bottom currents, tides, bottom tangential stress, organic carbon content, downtime, tide duration, proximity to estuaries, solid river runoff, bottom temperature, summer surface temperature (Grech and Coles, 2010) and others.

In the statistical modeling of the seagrass-wave relation, the latter is represented by various characteristics correlating with its strength and intensity, of which the most commonly used are: exposure indices and wave parameters. The more specificity in the wave propagation reflect the exposure indices, the better the predictive results of the model are (Sundblat et al., 2014). The significant wave height is the most often used parameter (Infantes et al., 2009; Callaghan et al., 2015). Among the most applied statistical models are: Baezian network (Grech and Coles, 2010), generalized linear modeling (Fonseca et al., 2002; Callaghan et al., 2015), generalized additive modeling (Schubert et al., 2015), modeling of the suitability of the habitat, MAXENT, modeling platforms, which include several statistical approaches, etc. Positive or negative effects of waves on angiosperm species show local specificity. Where conditions are highly protected and are subjected to freshwater inflow, waves reduce the risk of bottom hypoxia and anoxia and correlate positively with macrophyte status (Rubegni et al., 2013). If the conditions are more open - the waves have a limiting effect (Infantes et al., 2009).

From the review it becomes clear that for the Bulgarian Black Sea coast, there are few studies on the effect of waves on seagrass communities and the forecasted aquatory of segrasses biome (Jayathilake and Costello, 2018) is probably overestimated.

The literature review also summarizes the studies of eutrophication on seagrass and the role of the epiphytic "response" in this process. In the presence of suitable hydrodynamic conditions and substrate, the growth rates of seagrass depend strongly on light and nutrients (Short et al., 1995). The cultural eutrophication affects the values of both factors, causing a cascade of effects, through a variety of direct and indirect mechanism.. The main types of effects can be summarized according to the mechanism of their action on the respective biological level of the organization: physiological and biochemical, individual, population and at the community level (Martines - Crego et al., 2008).

Light stress upon seagrass caused by abundant development of microalgae (including phytoplankton) and macroalgae (eg Sand - Jensen, 1975; Bulthuis and Woelkerling, 1983; Borum et al., 1984; Silberstein et al., 1986 ; Cambridge et al., 1986; Krause-Jensen et al., 1996; Coffaro and Bocci, 1997; Brush and Nixon, 2002; Hauxwell et al., 2001; Sugimoto et al., 2007), is cited as one of the most frequent seagrass suppression mechanisms (Hauxwell et al., 2001; McGlathery, 2001; Nelson, 2009; Moreno-Marin et al., 2016; Han et al., 2016). Twilley et al., 1985 summarize that epiphytes affect the productivity, synthesis and accumulation of reserve substances and the survival of angiosperms through the effects of available photosynthetically active radiation (PAR) (e.g. Send-Jensen K., 1977; Philips et al., 1978; Balthuis and Woelkerling, 1983; Cambridge et al., 1986; Silberstein et al., 1986), reduction of diffuse transport of inorganic carbon (Send-Jensen, 1977), nitrogen (Cornelisen and Thomas, 2004) and phosphorus (Johnstone, 1979). Epiphytes can affect the gas exchange that takes place through the leaf surface of grasses (Brodersen et al., 2015). Harlin (1975) points out that epiphytes can also be used as an indicator of environmental conditions. Studies have shown two aspects of their response to increased levels of trophic state: changes in abundance (Mathiesen and Nielsen, 1956, cited in Send-Jensen, 1977, Cambridge et al., 1986; Silberstein et al., 1986; Neverauskas, 1987a, Neverauskas, 1987b, Brandt and Koch, 2003, Franchovitch and Fourqurean, 2007) and changes in qualitative composition (Cambridge et al., 1986).

There are also studies that report the low sensitivity of epiphytes to increased nutrients (Fourqurean et al., 2010; Terrados and Pons, 2008). Therefore the differences in the indicator value of the epiphyte load, accumulated on the seagrass leaves show a strong local specificity, determined by the hydrodynamic regime, the grazing pressure and other factors.

III. Aim and tasks:

III. 1. Aim

The aim of this study is:

- to assess the importance of wind waves for the spacial distribution of seagrasses in the Burgas Bay,

- to assess the potential of the epiphytic load, as an indicator of the segarass status, under eutrophication gradient, and

- to develop an indicator to assess the risk of light stress on seagrass caused by the accumulated epiphytic load.

III. 2. Woking hypothesis

The literature review and the established gaps in information about the Bulgarian coast allow for formulation of the following working hypotheses:

Hypothesis 1:

The wind wave limits the distribution of seagrass in the Burgas Bay, determining the upper (shallow) limit of field distribution.

Antithesis:

The distribution of grasslands does not depend on the excitement, but is determined by other environmental factors.

Hypothesis 2:

The periphyton community in the conditions of eutrophication gradient has different qualitative composition and quantity.

Antithesis: The accumulated epiphytic loads on the leaves of seagrass, in conditions of eutrophication gradient, do not differ in their qualitative composition and quantity.

Hypothesis 3:

The attenuation of light from the periphyton off the Bulgarian Black Sea coast has a pattern different from that established for other regions of the world ocean.

Antithesis: The periphyton causes a reduction in light by the same pattern that has been found in other parts of the world ocean.

III. 3. Tasks

In order to achieve this goal, the following tasks were formulated and performed:

1) To determine by modeling the effect of sea waves on the distribution boundaries of seagrass, in areas of the coast with different exposure. To determine the directions of the sea waves with a limiting effect in the studied areas.

2) To establish the qualitative composition of the mass components of the periphyton, inhabiting artificial and natural (seagrass) substrates.

3) To describe the stages of the succession of the periphyton community under the conditions of eutrophication gradient. To evaluate the importance of substrate persistence for the quality and quantity of accumulated periphyton.

4) To investigate experimentally the relationship between the abundance of overgrowth in the different stages of the succession of the periphyton community and the passed PAR.

Matherial and methods

IV.1. Phisycogeographyc characteristic of the area

On a relatively small area in the Burgas Bay there is presented a great variety of habitats, due to the combination of natural characteristics (relief, water bodies and land cover of the catchment area, wind regime, coastal exposure and underwater relief) and anthropogenic impact (land use, discharges, regulation of freshwater and solid inflow, hydrotechnical engineering). This allows seagrass meadows to inhabit under a great variety of conditions. Most of the WWTPs discharging into the sea have been reconstructed and modernized, and have treatment process to remove nitrogen and phosphorus. Most of the rivers flowing into the sea have relatively small water quantities and therefore have local impacts. The most significant point sources of freshwater inflow are the channel connecting the lake Mandra with the Foros Bay and the Ropotamo River, as they viasually have the highest outflow.

The review of the biological communities status has shown that macrozoobenthos has the best balanced community structure. The greatest deviation from the classical taxonomic structure, typical for the period unaffected by eutrophication, is typical for the bay phytoplankton and for the zooplankton but to a lesser degree. The bay macrophytes in some areas retain a partially balanced structure, while in others a process of degradation has taken place, accompanied by a shift in biological dominants and loss of valuable communities dominated by sensitive species. In thease areas local factors maintain "poor" and "bad" ecological status. Their impact has a limited spatial effect and does not affect zoobenthos and planktonic coenoses in the deep water region, but due to the small range of macrophyte habitats, the negative impact of these "local" sources is significant and requires special attention.

IV.2. Significance of wind waves for the distribution of seagrass in the Burgas Bay

IV.2.1 Determination of the parameters of the sea wind wave

In order to characterize the peculiarities of the wind regime, model data for wind direction and speed at a height of 10 m above sea level were used, for the period from 01.01.2012 to 06.09.2017, from the model NOMADS, NOAA http: //www.nomad3. ncep.noaa.gov/, accessible, through the Department of Remote Sensing Methods at the Marine Hydrophysical Institute, Russian Federation, for a point with coordinates: 28°0'0''E, 42°50'0''N. The estimation of the most probable wind speed and the probability of observing wind with a speed higher than a given threshold is made with a Weibull distribution. The parameters of the function are calculated by the method of Epf (energy pattern factor)(Kidmo et al., 2015). The wind speed with the highest repeatability is selected as the input parameter when calculating the "deep water" conditions. Wind fetch, ie. the free space in a given direction where the wind can move unobstructed over the water surface (Roghweder et al., 2012) are calculated with a resolution of 10 m. The values of the wave parameters in the conditions of “deep water" are calculated according to the recommendations in Kabatchenko et al. (2018). The obtained wave parameters in the “deep water” conditions were used as input data for the OLUCA-MC module of the software product SMC v. 2.5.

IV.2.2 Relationship between the bottom orbital velocity and the upper boundary of the fields

The modeling of the relationship between the bottom orbital velocity and the presence of seagrass is done using a generalized linear model, using the Real Statistics Resource Pack software application (Release 5.4). Copyright (2013 - 2018) Charles Zaiontz. www.real-statistics.com.

IV.2.3 Mapping of the boundaries of the seagrass meadows

The information about the location of the upper border of the grass fields was obtained with the help of GPS GarminEchoMap CHIRP 42 with single beam echo sounder, frequency 200kHz and validated with a underwater camera Aqua Vu micro micro 5 DVR - for the regions: Nessebar Bay, Foros Bay, Chengene Scaffolding, Sozopol Bay. At a water layer depth of less than 0.60 m, the boundary is determined by walking and detecting points with a manual Garmin GPSmap 64. In Vromos Bay, the location of the upper boundary is determined by pictures from GOOGLE Earth.

IV.3. Qualtitative and quantity of the periphyton on artificial and natural substrate

IV.3.1. Sampling and analisys of seagrass samples

Marine macrophyte samples were taken according to the methodology described in Dencheva and Doncheva (2014). The species identification of macroalgae was performed according to Dimitrova-Konaklieva (2000), Zinova (1967), Kosinskaya (1948), Brodie et al. (2006). The species identification of Zostera sp. is according to Den Hartog and Kuo in Short and Duarte (2001). The rate of leaf exchange was determined on the basis of the relationship between the relative surface area of the leaves and their productivity (Haylov et al., 1992 and Duarte, 1991).

IV.3.2 Experimental set - up of the field experiment for periphyton fouling

Artificial substrate collectors were used to assess periphyton fouling (Stanckelis et al., 1999). The regions for their placement were selected in order to cover the gradient in trophic state, which causes a "response" in the seagrass at the population level (Karamfilov et al., 2019) (Fig. 1).

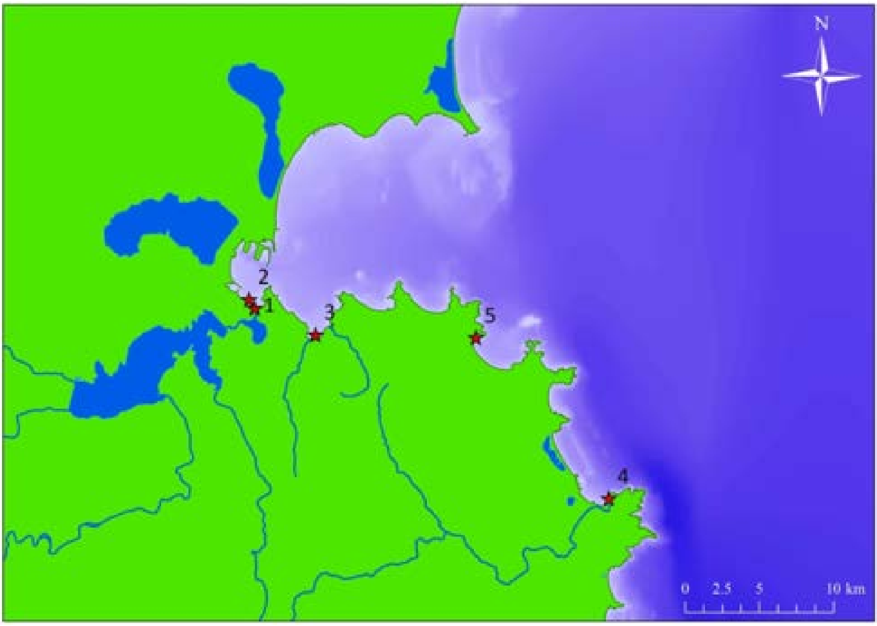


Fig.1 Area of experimental set-up. (Numbers indicate the locations of the artificial substrate collectors. The names of the areas are presented in Table 1.)

Table 1. Samples for qualitaty and quantity of the periphyton

№	Region	Period	Exposure, days	Sample number
Quality:				
1	Foros - in	07.05.16 – 22.9.2016 11.09.17 – 22.9.17	4,8,22 и 63	12
2	Foros - out	07.05.16 – 22.9.2016 11.09.17 – 22.9.17	4,8,22 и 63	12
3	Marinka	07.05.16 – 22.9.2016 11.09.17 – 22.9.17	4,8,22 и 63	12
4	Gradina	11.09.17 – 22.9.17	4,8,22*	3
5	Ropotamo	07.05.16 – 22.9.2016 11.09.17 – 22.9.17	4,8,22 и 63	12
Quanitity:				
1	All	07.05.2016 – 22.9.2016 17.06.2017 – 22.9.2017	4,8, 15, 22, 45, 63	223

*__Note__: The collector left for 63 days in “Gradina” area were not found.

Samples of natural and artificial substrates were processed according to the methodological recommendations in Taylor et al. (2007). A minimum of 400 cells are counted to assess the proportion of species in the qualitative composition.

The names of all species of organisms are according to the WORMS database (World Register of Marine Species: http://www.marinespecies.org/.

The measurement of PAR attenuation when passing through the epiphytic layer accumulated on the collectors was performed according to the methodology described in Stanckelis et al. (1999) and Franckovitch et al. (2005).

IV.3.3 Statistical analisys of the results

The analysis of the diversity and abundance data of diatoms is performed with PRIMER – E V.6 Ltd, Plymouth. The Shannon-Wiener species diversity index and the Pielou evenness index (Clarcke and Warwick, 2006) were used to characterize diatom species diversity. The variation in the overall taxonomic distinction (presence/absence of species in the samples) was calculated according to (Clarke and Warwick, 2006).

Ordination analysis of diatom communities was performed by applying principal component analysis ("R" software, "Factoextra"). The identification of potential indicator species was made on the basis of an assessment of the contribution of the species to the difference between the samples (SIMPER, Clarke and Warwick, 2006) and through the indval approach (Dufrene and Legendre, 1997). The Indicatorscpecies module, R-Package "Vegan" was used to calculate indval. The similarity analysis between the samples in the degree of shading was performed with ANOSIM, (Clarke and Warwick, 2006).

In search for dependencies between the distribution of abiotic factors and diatoms, an approach was used to measure the correspondence in the similarity matrices for the biotic and abiotic characteristics of the experiment (BEST, Clarke and Warwick, 2006), with the software product Primer -E, LTD – v. 6.

The examination of the statistical hypotheses in the dissertation is performed at a level of statistical significance of $p = 0.05$.

The following assumptions were made in this study:

1) When calculating the wave parameters in the deep water, it is assumed that the wind does not change in direction or speed for the period of time between the individual reports.

2) When calculating the transformation of the monochromatic wave in the conditions of "shallow water" it is assumed that there are no tidal phenomena and the flow velocity is equal to zero. It is assumed that there is no "attenuation" of the wave as it propagates through the grass field.

3) It is assumed that the epiphytic and periphyton film are composed of optically homogeneous layers (Francovitch et al., 2005).

4) It is assumed that the periphyton on the artificial substrate does not differ from that on the natural substrate, in terms of its optical activity (Nelson, 2017).

IV. Results and discussion

V.1. Seagrass meadows distribution in the shallowwater zone of the Burgas Bay

Seagrasses occupies shallow coastal areas in front of the Bulgarian coast with wave exposure ranging from semi-protected to highly protected type of coast, according to the CERC scale, 1977 (Valchev et al., 2014). The available natural fields are mostly concentrated in the waters of the Burgas Bay.

Table2. Characteristics of the fields mapped in the summer of 2018.

№	Meadow	Region	Area, km ^{2}	Min depth, m	Max depth, m
1	Sveti Vlas	Nesebar Bay	0,35	2,50	9,60
2	Elenite	Nesebar Bay	>0,65	3,0	7,60
3	Foros	Foros Bay	0,58	0,35	3,00-3,50
4	Chengene skele	Chengene ckele Bay	0,32	0,15-0,20	2,50-5,50
5	Gradina	Sozopol Bay	0,25	<1,00-3,50-4,00	>9,00

V.2. ***Importance of wind waves for limitation of seagrass meadows distribution in the Burgas Bay***

V.2.1 **Analysis of the wind regime and the wave exposure. Wave parameters in "deep water" conditions.**

To test the hypothesis that the combination of the predominant coast orientation and the dominant wind regime, through wind waves, limits the development of grasses and the formation of extensive fields, an analysis of wind speed and direction data is made at a point with coordinates: 28.00 ° E, 42.50 ° N.

During the studied period in the open water area adjacent to the Burgas Bay, winds with orientation from northeast - north prevailed (azimuths from 0 to 90). The cases of "calm weather to light breeze" (0-5.4 m/sec) were the most frequent. In the category "gentle to moderate breeze", the winds from the north (azimuth 0°) and northeast (azimuth 40 °) prevail, respectively 2.3 and 2.5% of the cases. The lowest frequency have winds from southeast and east (0.2% of azimuth 110°, 0.3% azimuth 90°, 100°, 120°). In the "strong to frech breeze" category, winds from 0 ° to 50 ° azimuth prevailed, with a recurrence of less than 1 percent of cases. Given the general orientation of the coast, potentially limiting impact for seagrass is expected to have waves caused by winds from azimuths from 0° to 180°. The analysis of wind speed data (Weibull distribution) has shown that the most likely speeds are entirely in the "light breeze" category, with the exception of 170 ° and 180 ° azimuths, which are in the "gentle breeze" range. Winds from the north, northeast and east are more likely, but at relatively lower speeds. Winds from the southeast and south (azimuths 140-180) are less likely, but when observed - speeds are higher. The cumulative probability of winds of the category "gentle breeze" and "strong brezze" i.e. speed > 7 m/sec, generally increases from azimuth 0 to azimuth 180, except for azimuths 10, 20, 30 and 50, where the probability is zero. The maximum values of the wind fetch for the Bulgarian coast have azimuths from 30 ° to 110 ° (Figures 2 and 3).

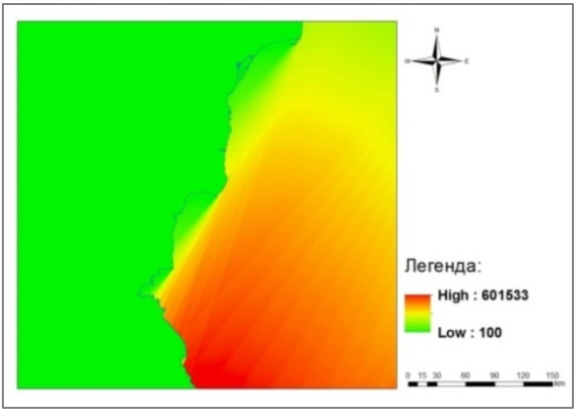
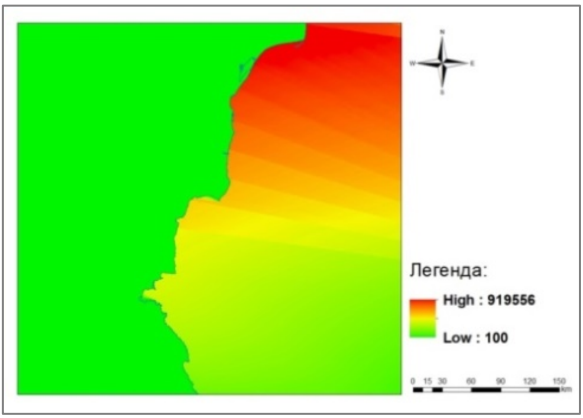


Fig. 2 Fetch (m) azimuth 30°.



Фиг. 3 Fetch (m), azimuth 110°.

The relatively low wind speeds of the most frequently observed winds cause waves with small height, period and length to be the most common ones, although long fetches are available in the Burgas Bay. The maximum average values of the wave parameters in the deep water zone are: average height - 0.46 m, average length - 18.82 m, average period - 3.2 sec, from azimuth 160°, at wind speed 6.4 m/sec (the Nesebar Bay). The minimum average values are - average height - 0.11 m, average length - 4.01 m, average period - 1.6 sec, azimuth 50°, wind - 2.9 m/sec (the Sozopol Bay).

V.2.2. Wave propagation in "shallow water" conditions and modeling of the relationship between the bottom orbital velocity and the upper seagrass meadow limit

The combination between the configuration of the shore and the slope of the bottom creates an individual wave microclimate due to the phenomena of wave refraction and reflection. In the range of the capes and the nearby waters, waves have enough speed and energy to impact the bottom thus limiting the persistence of seagrasses. In the areas where capes create wave shadow waves propagate with less speed, height and length and, accordingly, their effect on the bottom is weaker: "quiet" conditions are created for settlement and accomodation of seagrass.

The bottom orbital velocity is the factor that combines the effect of the fetch, the predominant direction of the waves and their characteristics, the effect of the bottom and the configuration of the shore. It is suitable for seeking dependence on the spatial distribution of seagrass (Infantes et al., 2009).

he Bay of Burgas offers a "diverce picture" of the habitats of marine angiosperms, in which the factor of wind turbulence has a different effect and determines different limits of their distribution. The selected areas for modeling are „a sample“ covering different types of conditions: the most wave exposed area - Nessebar Bay (north coast), an area with an island - Sozopol Bay, an area with significant freshwater inflow – Foros and the most protected area - Chengene skele-the mouth of the Marinka River).

The results of the statistical modeling are presented in Table 3:

Table 3. Results of statistical modeling between the bottom orbital velocity and the upper limit of seagrass field. The coefficients b0 and b1 are the coefficients of the straight line found by the method of the maximum likelihood, AUC (area under the ROC curve).

№	Azimuth	Coefficient b0	Coefficient b1	Percent of correct forecastats	AUC	Discriminating ability (Hosmer and Lemeshow, 2000)
Несебърски залив						
1	90	5,49	-207,88	91	0,97	excellent
2	100	5,22	-65,88	90	0,90	very good
3	110	4,02	-249,02	92	0,92	excellent
4	120	4,15	-233,24	92	0,98	excellent
5	130	5,66	-93,46	92	0,98	excellent
6	140	6,09	-76,30	92	0,98	excellent
7	150	5,24	-91,53	91	0,98	excellent
8	160	7,46	-47,00	90	0,98	excellent
9	170	4,56	-135,57	92	0,98	excellent
10	180	5,16	-110,73	91	0,98	excellent
Sveti Vlas						
1	90	0,40	-58,68	65	0,76	satisfactory

№	Azimuth	Coefficient b0	Coefficient b1	Percent of correct forecastats	AUC	Discriminating ability (Hosmer and Lemeshow, 2000)
2	100	6,90	-78,58	91	0,97	excellent
3	110	2,18	-134,63	86	0,93	excellent
4	120	2,17	-120,45	85	0,93	excellent
5	130	2,92	-50,76	83	0,92	excellent
6	140	3,08	-41,21	82	0,91	excellent
7	150	2,63	-49,36	82	0,91	excellent
8	160	3,51	-24,40	79	0,89	very good
9	170	-0,08	-19,25	63	0,50	poor
10	180	0,06	-32,24	44	0,53	poor
The Sozopol Bay						
1	20	0,81	-38,9	68	0,50	poor
2	30	1,24	-41,9	75	0,61	poor
3	40	1,95	-55,5	79	0,75	satisfactory
4	50	1,43	-209,9	75	0,76	satisfactory
5	60	1,56	-68,2	80	0,75	satisfactory
6	70	2,04	-25,8	82	0,70	satisfactory
7	80	1,43	-6,17	72	0,62	poor
8	90	0,02	6,33	63	0,62	poor
The Foros Bay						
1	40	1,15	-51,918	79	0,72	satisfactory

№	Azimuth	Coefficient b0	Coefficient b1	Percent of correct forecastats	AUC	Discriminating ability (Hosmer and Lemeshow, 2000)
2	60	0,47	-0,51	59	0,62	poor
3	70	1,53	-209,9	80	0,79	satisfactory
4	80	0,87	-2,61	70	0,75	satisfactory
5	90	1,03	-10,04	74	0,65	poor
Chengene skele – mouth of the Marinka River						
1	20	-0,25	-0,70	56	0,18	poor
2	30	-0,22	-1,68	56	0,26	poor
3	40	-0,22	-0,58	56	0,28	poor
4	60	-0,13	-4,22	56	0,36	poor
5	70	-0,17	-1,00	56	0,34	poor
6	80	0	0	44	0,77	-
7	90	0	0	44	0,92	-
The Vromos Bay						
1	20	3,12	-82,3	81	0,94	excellent
2	30	4,17	-155,5	85	0,97	excellent
3	40	4,43	-200,3	88	0,97	excellent
4	60	3,47	-175,6	85	0,96	excellent
5	70	8,35	-180,9	91	0,98	excellent
6	80	2,25	-553,6	72	0,74	satisfactory
7	90	0	0	60	0,38	poor

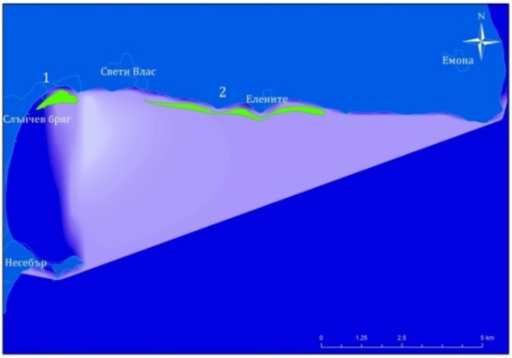


Fig.4 Wave height when waves approaching from 170 ° azimuth. The Nessebar Peninsula creates a wave "shadow". Field "St. Vlas "- 1, the fields" Elenite "- 2. The maximum wave height is in light tone, and the minimum - in dark. The seagrass fields are in green.

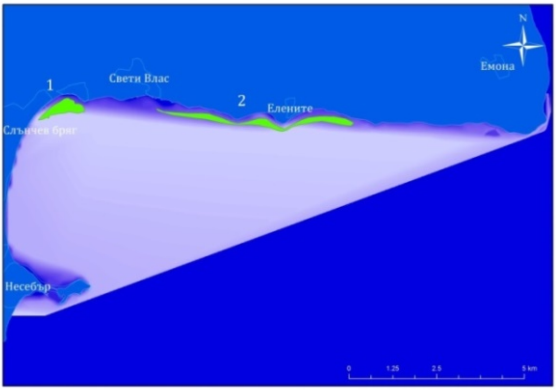


Fig.5 Wave height when waves approaching from 100°azimuth. The whole Nessebar Bay is exposed and for all meadows the logistics model shows excellent discriminatory ability. The maximum wave height is in light tone, and the minimum - in dark. The seagrass fields are in green.

Waves approching from azimuths from 90° to 180° limit (show very good and excellent discriminative ability of the model) segrasses and the obtained statistical models can explain the upper limit of their distribution in the Elenite meadows (Table 3). The Sveti Vlas field is limited by waves coming from azimuths from 90° to 160 °. Here, the most frequently observed velocities of the wave from 170° and 180°

azimuths cannot explain the observed spatial distribution of seagrasses. The reason for this is the "wave shadow" that creates the Nessebar peninsula (Fig. 4).

The Gradina field is limited by waves corresponding to azimuths of 40°, 50°, 60°, 70° (satisfactory discriminative ability of the model). The Sveti Ivan Island creates a wave shadow under certain conditions. Because it is too small it cannot protect the entire coast. Most likely, this is the reason why two fields have been formed in the Sozopol Bay: "Garden" and "Sozopol - South", and not one continuous meadow. The Foros Bay is open to the northeast - east, but the azimuths with a high frequency of wind repeatability 0°, 10°, 20°, 30°, 40°, 50° and 60° have a short fetch and the possibility to generate high energy waves is minimal. The absence of seagrass in the area adjacent to the mouth of the canal connecting Lake Mandra with the bay cannot be explained by the limiting (destructive) action of the waves (Fig. 6).

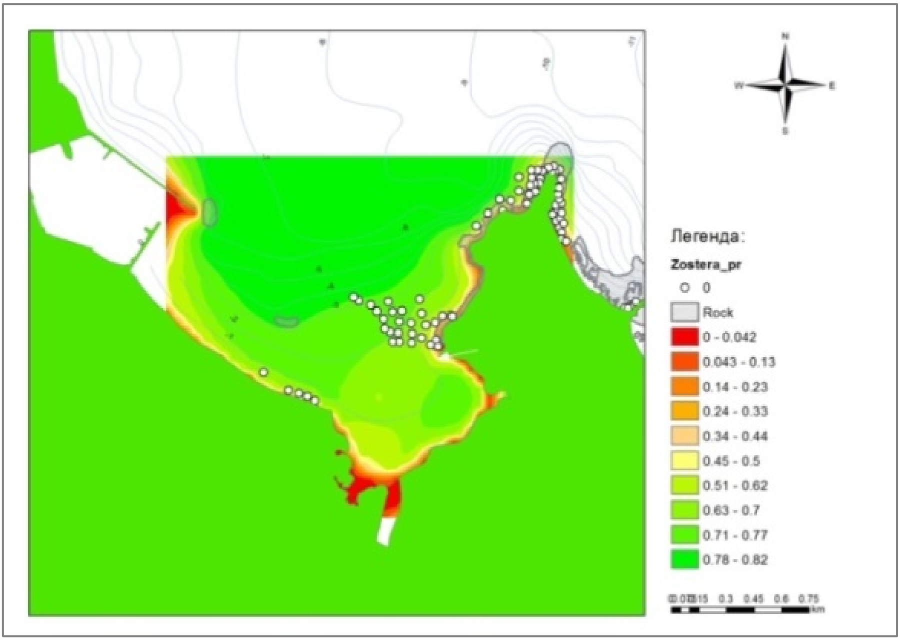


Fig.6 Application of the statistical model for estimating the probability of the presence seagrass (70 ° azimuth). An arrow indicates the place from where the water from the lake flows to the bay. Areas of absence of grass are indicated by white spots

In the adjacent area of the Marinka River outflow (Chengene Skele) the data on the spatial distribution of angiosperms cannot be well described from the obtained statistical models (Table 3). The observed distibution of the seagrasses cannot be explained by the effect of the bottom speed, as their upper boundary (0.20-0.30 m depth) is not limited by the waves, but is determined by the level of the water layer.

The innermost, shallow area is very sensitive to drying due to the action of the winds blowing from the coast and reduced river inflow during the summer months.

The Vromos Bay is partially open to the northeast. From the north it is protected by the Pomorie Peninsula and the short fetch limits the waves growth. At the most frequent wind speed, their height is only 0.14 m. From the east it is protected by the Cape Accra. The waves approaching from the northeast (azimuths of 20° to 80°) may explain the observed distribution of seagrass in the bay: the models are characterized by excellent to satisfactory discriminative ability and a sufficiently high percentage of accurate forecasts. When approaching from the east, the Cape Accra creates a wave shadow over the entire bay and cannot affect the observed distribution of seagrass here (Table 3).

The obtained dependancy of the bottom orbital velocity by a wave caused by the most frequently observed wind speed and the upper limit of the grass fields show different values of the discriminating bottom orbital speed from 0.16 m/sec ("Elenite", azimuth 160°) to 0.01 m/sec and are regionally specific (Infantes et al., 2009). These are statistically derived threshold values, not the physical values that seagrasses can tolerate. In other studies (review in Koch, 2006; Infantes et al., 2009), the threshold value is higher due to the fact that the authors used significant wave height (Infantes et al., 2009; Van Katwjick et al. ., 2000), which is higher than the average wave height. However, the average wave parameters used in this study correspond well with the average significant wave height obtained during the summer season for the western half of the Black Sea (Arkhipkin et al., 2014).

*3. **Estimation of the rate of leaf exchange of the genus Zostera and its significance for the peculiarities of the epiphytic community***

The ecological life strategy of the basiphyte species significantly affects the epiphytic community formed on it, as the percistence (continuity) of the colonized surface determines the duration of existence, appearance and structure of the epiphytic community (Borowitzka et al., 2006). The relationship between: 1) leaf lifespan and 2) abundance and composition of the epiphytic community, through the mechanism of PAR and UV-protection (light stress) caused by epiphytes, plays a crucial role in survival of seagrass species under high levels of nutrients.

The data on the morpho-dynamic characteristics of the seagrasses obtained in the present study during the summer period (June, July, August, September) show that the plastochron interval (PI - the time between the appearance of two consecutive leaves) of the vegetative stems of *Z. noltei* is an average of 1.38 days (± 0.04), with an average number of leaves per shoot equal to 4. The duration of existence of one leaf is an average of 5.5 days (± 0.15), (Table 4). For *Z. marina* (5 leaves per shoot) the duration of existence of the leaves is 16.3, reaching a maximum of 28 days in September (PI = 3.23 days). характеристики на тревите получени в

Table 4. Morphological and dynamic characteristics of the leaves of Zostera sp. from the Bulgarian coast (after Khailov et al. 1992 and Duarte, 1991); dw-dry weight, ww-wet weight, rs - relative surface, PI - plastrochronous interval, LLS-leaf life span

Species	number	Leaf surface, mm^2/ shoot	dw, mg/ shoot	ww, mg/ shoot	RS, mm^2/mg ww	PI, days	LLS, days
Z. noltei	188	1320,3± 63	38,61± 1,96	141,53 ± 7,18	19,36± 0,63	1,38± 0,04	5,5± 0,15
Z. marina	35	-	206,69 ± 88,68	-	-	3,23± 0,69	16,3± 3,43

Studies by other authors show that species of the genus Zostera are characterized by short to medium leaf life span, and that the rate of leaf exchange depends on seasonal changes in environmental conditions, and for temperate latitudes the increase in temperature (spring - summer period) affects inversely the span of the leaf substrate (review in Borowitzka et al., 2006; Borum, 1984; Philips et al., 2006; Ribaudo et al., 2016). The results for the Bulgarian coast are in accordance with the studies cited above and show one of the shortest values of leaf duration. In terms of epiphytes, Zostera spp. provide a high level of disturbance: epiphytes have a very short time "window" in which they must colonize the substrate, reach sexual maturity and reproduce.

Any ecological factor that affects the rate of leaf metabolism indirectly affects the epiphytic communities. As for *Z. noltei*, one of these factors is the parasite *Plasmodiophora bicaudata* J. Feldmann, 1941. (Fig. 7), which slows down leaf metabolism thus helping accumulation of epiphytes on infected plants.

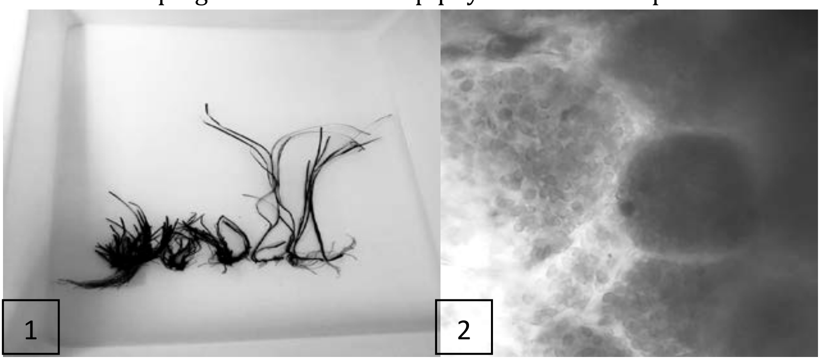


Fig. 7 Infected plants: *1 (left) u healthy ones (wright) and 2: spores of the parasite within the host rhizome cells*

V.4. Qualitative composition of the periphyton community during the summer season. Succession of the periphyton.

V.4.1 **Species diversity of epiphytes inhabiting natural and artificial substrate**

A total of 11 species have been identified on the leaves of *Z. noltei* (intraspecific taxa are not included). The short duration of leaf existence during the summer period does not allow the formation of abundant epiphyton. The species richness is low and is represented mainly by low-profile diatoms, red and green algae (Fig. 8). Diatoms are dominated by *Cocconeis scutellum var. scutellu*m и var. *intermedia*, C. *placentula, G. olivaceum var. minutissima.*

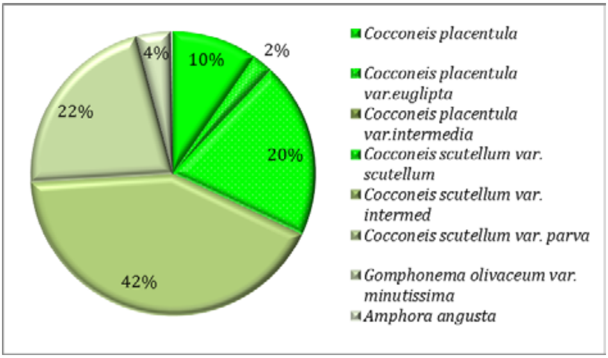


Fig.8 Qualitative composition of diatoms on the leaves of Z. noltei - July, 2016

A total of 63 species (intraspecific taxa not included) have been identified on PET (polyethylene terephthalate) strips placed in the marine environment for different periods. The representatives of the kingdom of Chromista have a predominant share (51). Their dominance in the composition of the periphyton film is due to the class Bacillariophyceae - the richest in species and intraspecific forms class in periphyton (50). Most of the observed species belong to the weakly mobile or immobile guild *sensu* Passy (2007) – low-profile, encrusting forms firmly attached to the substrate. The abundant species of diatoms belong here: Cocconeis, Amphora, Halamphora, Gomphoneis, as well as non-diatom algae and animal species: Ulvella, Myrionema, Hydrolithon, Colaconema, Cyanophyceae, Botrillus, Conopeum. The motile guild (Passy, 2007) in diatoms consists of 13 species: *B. paxillifera*, Diploneis sp., *L. lyra*, Pseudo-nitzschia sp., Navicula sp., Nitzschia sp., *P. elongatum*, *F. forcipata*. High-profile (Passy, 2007) species are 14 (13 diatoms Licmophora, Rhoicosphenia, Achnanthes longipes, A. brevipes, Mastogloia (in mucilage tubes), Melosira, Synedra and one cyanophyceae - *C. scopulorum*).

V. 4.2 Evaluation of the significance of the experimental site and the constancy of the substrate for the features of the periphyton

Pusico-geographical gradients in the studied area

In the selected study area, there is a trophic gradient causing a population-level “response” in *Z. noltei* seagrass (Karamfilov et al., 2019). The observed gradient is related to the nature of land use and other human activities in the catchment (Karamfilov et al., 2019). The trophic status of a given region is a function not only of the nutrient load, but of the self-purifying ability as well. The latter is directly dependent on the residence time of the water and the wave exposure (Table 5).

Table 5. Physico-geographical factors corresponding to vulnerability to load from terrestrial sources

Region	Integral wave fetch, conditional units	Watershed area, km^2	Index of vulnerability to land based sources (IVLS) = watershed/integral fetch
Foros inner part	41	2398	58,49
Foros external part	1990	2398	1,21
Marinka	498	31	0,06
Ropotamo	12762	246	0,02

Results of a field experiment for artificial substrate fouling

The sites of placement of the experimental substrates are at different distances from the relevant point source, which determines the difference in the effect of its impact. The greatest species richness of the periphyton is observed for the longest period of exposure in the most heavily loaded area - Foros inner part (Fig. 9).

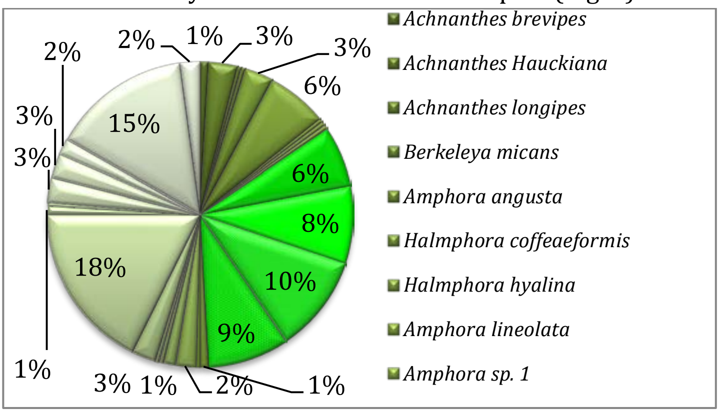


Fig. 9 Qualitative composition of the periphyton on PET tape - foros inner part, 63 days

The analysis of the data from the set experiment with artificial substrate shows both general features in the development of the succession process and some specific features:

- *In all substrates there is a specific spatial distribution of the amount of periphyton, which changes in the course of fouling - the process starts from the edge of the substrate and continues to its central part.* This type of edge-to-center colonization is especially important for basiphytes such as seagrass. The colonization of the epiphytes as described assumes the latest fouling and shading of the central part of the petiole. Thus, the shape of the leaves, among other adaptive functions, can be considered as "passive" protection against shading.
- *The duration of existence of the substrate determines the characteristic of the epiphytic community, whose development is limited in time.* It is represented by species that appear during the initial stages of colonization of a new substrate - there is a primary ecological succession. During the succession, the species and functional diversity of the periphyton gradually increases. The value of the biodiversity indices (Shannon – Weaver, H) and evenness (Pielou, J) is naturally increasing. The importance of the motile and high-profile guilds increases, at the expense of the low-profile guild, but the latter it retains leading role in all regions except Ropotamo. The results obtained are in agreement with the hypothesis that in the initial stages, the spread of propagules plays a leading role in the formation of the community and dictates the types of organisms that are able to colonize. As the succession process progresses, the role of biotic factors (competition and mutualism) increases progressively, leading to a gradual increase in functional and taxonomic diversity (Chang and HilleRisLambers, 2016).
- *Significance of the duration of colonization for the qualitative composition of the periphyto.*

The analysis of the contribution of the species to the difference in the factor "colonization period in days" (SIMPER) shows that there is a significant difference between the selected colonization periods: 4, 8, 22 and 63 days for all studied areas (> 50%). Naturally, the difference increases from 4 to 63 days. The qualitative composition of diatoms is most similar for the period between 4 and 8 days of colonization, regardless of the area of the experiment (difference 56,81%). According to the results of the SIMPER analysis some relatively good indicators (discriminatory species) for the "colonization period" factor emerge: *C. placentula*, *C. scutellum, var. scutellum, C. scutellum var. intermedia*. The application of the indval (Dufrene and Legendre, 1997) approach shows the presence of a total of 6 indicator species (Table 6).

Table 6. Results of the analysis for statistically significant indicator species by the method indval (Dufrene and Legendre (1997) multipattern analyzes, package "Vegan", "R"), A - specificity of the indicator, B - sensitivity of the indicator.

Indicator species for colonization duration:	A	B	Statistic (√indval)	p-value (significance level)
Group: 63 days				
T. parva	0.8837	0.75	0.814	0.036 *
Grup: 22+63 дни				
Pseudonitzschia1	0.9549	1	0.977	0.008 **
H.cofeaeformis	0.9188	1	0.959	0.010 *
L.Ehrenbergii	1	0.875	0.935	0.004 **
A.Hauckiana	0.9687	0.875	0.921	0.014 *
G. olivaceum var. minutissima	1	0.75	0.866	0.048 *
S.grandis	0.9813	0.75	0.858	0.038 *
Code for statistical significance: 0 ***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1				

The indicator species identified according to the indval approach (Dufrene and Legendre, 1997) belong mainly to the group of mobile or high-profile diatoms. As expected, they were defined as good indicators of a later stage of community succession, corresponding to 22 and 63 days of substrate persistence (low level of disturbance). With a maximum level of specificity emerge *L. Ehrenbergii* и *G. olivaceum var. minustissima.* If they are found their presence indicates a later stage of succession.

➢ *Evaluation of the significance of the experimental area for the qualitative composition of the periphyton.*

The principle component analysis by species for each period of colonization of 4 days clearly differentiates the regions, together with their typical species. Foros - inner part - *C. placentula*, Ropotamo - *N. longissima*, Foros – external part and Marinka are grouped together - *C. scutellum*. This trend of similarity / difference of the regions is maintained for a period of 8 days. Ropotamo is the region with the greatest species richness, with the dominance of *R. musculus+T.tabulata+Navicula sp.1*. The area differs from the others by the presence of *A. brevipers*, *A. Hauckiana*, *B. paxilifera*, *G. marina* etc. In the inner Foros strongly dominate *C. placentula+Licmophora* sp., *F. pygmea*, *Cocconeis sp. 1*, *H. hyalina* are presented. The outer part of Foros and Marinka show similarities in the structure of the diatom community - dominance of *C. scutellum* and its variety. The progress of the colonization process increases the diversity and difference in the structure of diatoms in different regions. In 22 days and especially in 63 days, the areas of the inner and exteranl part of Foros and Ropotamo are distinguished, with their typical species. The analysis (SIMPER, Clarke and Warwick, 2006) of the contribution of species to the difference in the factor "region" shows that there is a significant difference in the qualitative and quantitative composition of diatoms between the selected regions: the minimum difference is between Foros external part and Marinka, 81%), and the maximum - between Foros external part and Ropotamo (88.67%).

Table 7. Results of the analysis for statistically significant indicator species by the indval method (Dufrene and Legendre, 1997): multipattern analyzes, package “Vegan”, “R”, A - specificity of the indicator, B - sensitivity of the indicator.

Indicator species for “region” factor	A	B	Statistic (√indval)	p value (significance level)
Group "Foros internal"				
P. elongata	1	0.75	0.866	0.026 *
H.hyalina	0.9117	0.75	0.827	0.031 *
Group "Ropotamo"				
Nitzschia longissima	0.9967	1	0.998	0.002 **
Group "Marinka+Ropotamo"				
N. parapontica	0.9754	1	0.988	0.036 *
Codes for statistical significance: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1				

Statistically significant indicator species for "region", according to the results of the indval approach (Table 7), are mainly representatives of the high-profile and motile guilds. As a very good indicator for the inner part of the Foros Bay is a certain species that is present in the samples with a longer duration of colonization (> 4 days). *N. longissima* has been identified as a good indicator for the Ropotamo region, which is characteristic of this area, although it is also found in samples from other regions (excellent sensitivity and good specificity). *N. parapontica* is defined as a good indicator for the regions of Ropotamo and Marinka, where it reaches high abundance, although it can be found in samples from other regions. The two approaches to assessing indicator species - SIMPER (Clarke and Warwick, 2006) and indval (Dufrene and Legendre, 1997) indicate not just different species, but species with different life strategies: low-profile and slow-moving, disturbance- and stress-tolerant in the first type of tests, and high-profile and motile species, requiring substrate persistence and good resource availability (Passy, 2007) in the second type of tests. The approaches focus on different aspects of the species adaptation strategy. The indval approach emphasizes the high specialization of species and their strong "preference" for certain condition and good indicators are species that have a narrower ecological niche. SIMPER "allows for" species with wide ecological plasticity, emphasizing abundance as an indicator of species' preference.

V.4.3. Dependence of shading on the amount of accumulated load

In essence the relationship between the accumulated epiphytic load and the shading (transmitted light) is of the "dose-response" type. In various experiments (review in Nelson, 2017) to describe this relationship, different functional dependencies were examined, looking for dependences both between abundance and shading and between abundance and light passing through the periphyton layer. The form of function in this experiment suggests that the change in attenuation could be best described by curves of exponential raise to a maximum (shading), Michaelis-Menten (shading), natural logarithm (shading) and negative exponential (light that has passed through the periphyton layer). These functionas are tested in the study. The comparative analysis has shown that the model of exponential raise to a maximum is the most suitable for describing the results. The choice of this model is not unusual, as the great majority of authors have preferred it in their research (review in Nelson, 2017). Although the qualitative composition of periphyton probably influences the function even without taking it into account the model can be used for practical purposes, as the obtained coefficients are within the limits set by other authors (for temperate climate).

In order to determine "critical" shading thresholds, it must be compared with the minimum light requirement (MLR) for the respective basiphyite (Ochieng, 2008). The calculation of the light reaching the leaf blade is done according to the equation (Francovitch and Zieman, 2005).

For the conditions of the Burgas Bay the equation has the form:

$$LL = Io*\exp(-Kd*z)*[100-(A*(1-\exp(-b*dw))],$$

where A = 73,22; b = 0,8299, according to the equation for exponential raise to a maximum, LL – light (PAR photon flux density) at the leaf surface, Kd - attenuation coefficient of a water column at a depth of z meters, Io – light (PAR photon flux density) at the water surface (0 m), Kepi- attenuation coefficient of the epiphyton layer with the respective dry weight dw (mg/cm^2).

The minimum light requirement (MLR) is defined as the level of light required for a seagrass to maintain its metabolism, below which plants cannot maintain their growth (Ochieng,2008).

Using the compensation light intensity approach (Ic) (Ochieng C, 2008) and taking into account the average level according to different authors for *Z. marina* (6 mol/m²/day = 69 μmol/m²/s, Ochieng, 2008), the following threshold values for the maximum permissible epiphytic load can be derived, depending on the PPFD (PAR photon flux density) reaching the epiphytic layer: from 0.01 mg/cm² at PPFD of 70 μmol/m²/s to 7 mg/cm², at PPFD of 142 μmol/m²/s. When PPFD is above 142 μmol/m²/s and under 69 μmol/m²/s the epiphytes are not important in shadowing the basiphyte: in the first case, the maximum shading is reached, but it is too small to cause a "dangerous" for the plants reduction in light intensity; in the second - the ambient PPFD is limiting and the epiphytes are practically irrelevant (Fig.10)

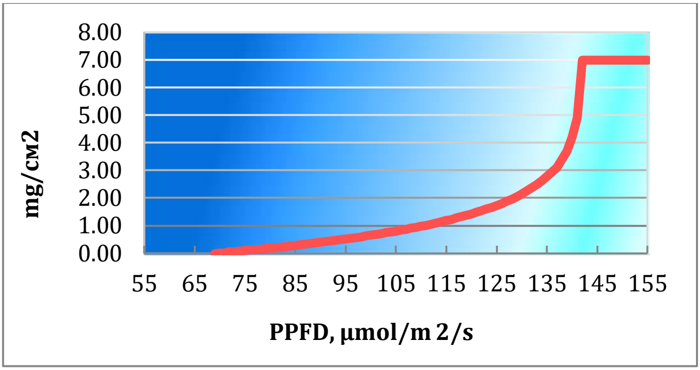


Fig.10 Range of influence of the epiphytic load on the critical levels of available PPFD for Z. marina. The x-axis shows the level of ambient PPFD, and the y-axis the accumulated epiphytic load (mg dry weight/m²).

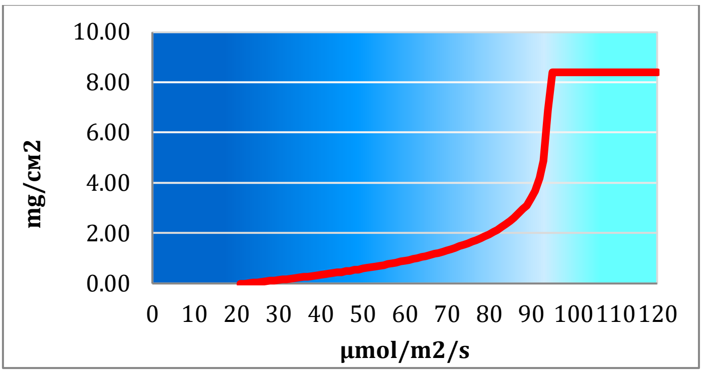


Fig.11 Range of influence of the epiphytic load on the critical levels of available PPFD for Z. marina. The x-axis shows the level of ambient PPFD, and the y-axis the accumulated epiphytic load (mg dry weight/m2).

Taking into account the value derived by Peralta and Vergara (2005) about Ic = 21 µmol/m²/s for *Z. noltei,* the folloing threshold values are received (Fig.11): 0.01 mg/cm² (22 µmol/m2/s) and 8.4 mg/cm² at 64 µmol/m2/s. Fig. 10 and Fig. 11 show that the same epiphytic load has a different importance depending on the available light. The epiphyte attenuation is most important whenavailablePAR is close to the MLR threshold of the species i.e. at the lower limit of it distribution or at increased turbidity. The determination of the critical threshold values for the permissible epiphytic load should be based on the MLR of the basiphyte. In this way, the tresholds that correspond to the actual light stress for the plant can be established.

6. Significance of the wave exposure for the peculiarities of the periphyton.

In order to identify potential indicator species for the individual regions, representative of the combination of watershed load and reduction of its effect, through water exchange with open waters, it it necessary to check the importance of these factors for the quality of the periphyton. The test of the importance of the factors catchment area and integrated fetch for the formation of the qualitative composition of diatoms, using the BEST procedure (PRIMER-E v.6, Clark and Warwick, 2006) showed little importance of both factors if the method is applied to the whole data set. As the importance of the duration of colonization for the qualitative composition of diatoms was shown in order to test the effect of the studied physiographic factors, the procedure was repeated with the matrix, which is supposed to reflect the climax community (Khailov et al., 1992) of 63 days.

Table 8. Results of an examination of the significance of the “structuring” community that colonized the substrate for 63 days, factors (Clarke and Warwik, 2006)

<table>
<tr><td colspan="2">BEST Biota and/or Environment matching</td></tr>
<tr><td>Data: Environmental
Samples: all
Varibales: all</td><td rowspan="2">Parametrs:
Correlation method (rank): Spearman
Method: BIOENV
Max number of variables: 2
Similarity analisys between: samples
Мярка за подобие: Euclidean distance</td></tr>
<tr><td>Diatoms:
Data type: Similarity
63 days</td></tr>
<tr><td>*Varibales:*
1 fetch
2 watershed</td><td>*Results:*
No.Vars Corr. Selections
2 0,943 all
1 0,657 1
1 0,088 2</td></tr>
</table>

The correlation of the matrices with diatoms and physico-geographical factors is the highest if both factors are taken into account (r = 0.943) (Table 8). Even on its own, the wave exposure factor correlates very well with the diatoms data (r = 0.657). Sea waves can affect periphytic organisms directly, causing them to fall off the substrate more easily (e.g. Vermaat, 2005). Intensive hydrodynamics also affect fouling indirectly, by acting on top-down control, maintaining a low level of grazing pressure (Schanz et al., 2002) or by bottom-up control, facilitating rapid dilution, removal and maintenance of low concentrations of nutrients originating from land based sources (Raateoja and Kauppila, 2019), or by supplying nutrients through "renewal" of water (Cattaneo, 1991). The balance between the different direct and indirect effects of water exchange on the periphyton depends on the local characteristics of the environment. In order to assess the direction of impact of the waves on diatoms, it is necessary to repeat the experiment with the inclusion of a larger number of regions and variables - discharged water quantities, loads of nutrients, Corg and BOD5, grazing press, salinity, nutrients, composition of phytoplankton, as a source of plankto-benthic species, modeling of transport and transformation of inflowing loads.

VI. Summary of the results and conslusions

The Bourgas Bay offers a variety of habitats, where the importance of the wave impact and epiphytic load for the limitation of the seagrasses varies depending on the local conditions:

1. Within the Burgas Bay shallow fields of seagrass can be limited by wind waves, depending on the coastal orientation and its waves exposure. Similarly, the importance of wind regime and exposure for limiting the upper boundaries of seagrass fields is changing: in areas open to the south and southeast, seagrass fields are less frequently but more often subjected to physical disturbance by wave action, while in areas found to the north and northeast is the opposite.
2. The upper boundary of the distribution of seagrass is limited by the wind waves in the following areas: the Nessebar Bay, the Sozopol Bay and the Foros Bay. In the bay of Chengene skele, in the area in front of the mouth of the river Marinka, there is no limiting (destructive) effect of the waves on the upper boundary of the vegetation during the summer.
3. Empirical models have been obtained that can be used to predict the presence of marine angiosperms in unexplored coastal areas, having the same wave climate conditions as the area for which they were derived.
4. The obtained results for the rate of leaf exchange and the productivity of Zosetra spp. from our coast are in agreement with those established by other authors (life strategy of seagrass and the season): *Z. noltei* has a faster exchange than *Z. marina*; both the plants have fast leaf exchange rate and low leaf life span in summer.
5. The current assessment of the quality composition and structure of the periphyton during the summer shows that a total of 63 species permanently attached periphyton organisms have been found on the artificial substrates for a

different period of time. The most abundant and diverse are the representatives of the class Bacillariophyceae - 50 species.

6. The duration of the substrate's existence determines the nature of the periphytic/epiphytic community. During the succession, in areas with a large catchment area, protected from the wave action the species and functional diversity of the periphyton, the values of the biodiversity indices (Shannon – Weaver, H) and evenness (Pielou, J) as well as the importance of motile and high profile guilds gradually increase; the tolerance of species to physical disturbances and to low levels of nutrients decrease, and the "efficiency" of the substrate “usage” increases. For a period of colonization up to 63 days, the low-profile guild retains its dominant role in the composition of the periphyton.

7. The enrichment of the diatom component with motile and high-profile species can be used as an indication of increased trophic status only if the community has had sufficient time to mature. Increased trophic status causes quantitative (faster accumulation of periphyton) and qualitative ("shortens" the time from one stage of succession to another) changes.

8. In areas exposed to wave action without constant surface freshwater inflow from the dry land the community has a low number of species and the low-profile guild in the composition of diatoms dominates.

9. The principle-component analysis shows the presence of clearly differentiated areas (inner part of Foros Bay, outer part of Foros Bay, Ropotamo) in terms of the structure of the diatom component of the periphyton. The factor "wave exposure", assessed by the integrated fetch, shows the presence of a statistically significant "structuring" effect on the qualitative composition of diatoms. The wave exposure should be taken into account when studying diatoms.

10. The analysis of the species contribution to the difference in terms of the factors "duration of colonization" and "study area" shows that there are significant differences in both factors. The difference is much more pronounced between the different periods of fouling than between the different regions. It is necessary to take into account the permanency of the substrate in ecological assessments based on the diversity and quantity of periphyton (and epiphyton). Potentially good indicators of 'colonization duration' are: *C. placentula*, *C. scutellum var. scutellum*, *C. scutellum var. intermedia*.

11. The analysis of the similarity in quality and quantity (relative abundance) of diatom flora in the tested areas for a period of average duration of colonization shows that the species *C. scutellum* and its two varieties, and *C. placentula* are good periphyton indicators for the influence of freshwater inflow in coastal waters. The first two forms increase their relative share, with less influence of freshwater inflow (small inflow and / or large exposure), and the third one on the contrary - strongly dominates the periphyton in the conditions of significant freshwater inflow (large inflow combined with small exposure).

12. The obtained results show that when using the qualitative composition (relative number) of the epiphytes as an indicator of the environmental conditions, it is important to determine the average duration of existence of their substrate. The

actual summer period (June, July, August) is not recommended for monitoring of diatoms on the leaves of the Zostera, due to the high rate of leaf exchange and the correspondingly low species diversity of the epiphytes. The end of summer (from the end of August to the end of September) is the most suitable period for assessment by the qualitative composition of diatom epiphytes found on the leaves of Zostera.

13. The function of exponential raise to a maximum is most suitable for estimating the shading caused by accumulated epiphytes. The derived functional dependence (exponential raise to a maximum) shows that the attenuation of light in the geographical scope of the experiment occurs in a pattern similar to that found for other regions of the worlds oceans (temperate climate zone). Similar coefficients have been determined for the Burgas Bay.

14. The following threshold values are derived for the maximum allowable epiphytic load, depending on the light reaching the epiphytic layer: from 0.01 mg/cm^2 at an PPFD equal to 70 μmol/m^2/s to 7 mg/cm^2, at PPFD of 142 μmol/m^2/s for *Z. marina* and 0.01 mg/cm^2 (PPFD 22 μmol/m^2/s) and 8.4 mg/cm^2 at an PPFD of 64 μmol/m^2/s for *Z. noltei*.

The present summarized conclusions are original scientific results obtained by the author in the framework of this study. The use of other authors' results, opinions and interpretations on the discussed issues is duly indicated, through the cited citations, in the respective chapters of the dissertation.

VII. Contributions:

Scientific contribution:

1. The limitation of the upper boundary of marine angiosperms meadows by wind waves in the regions: the Nessebar Bay, the Sozopol Bay, the Foros Bay and the lack of limiting (destructive) effect in the Chengene Skele (estuary of the Marinka River) has been proven. For each studied area the limiting directions of the approach of the wind waves are established.
2. Statistical models of the relationship between the upper boundary of seagrasses and waves have been obtained; they can be applied to unexplored areas with the same or similar wave exposure.
3. For the first time, the species *P. bicaudata* parasitizing on *Z. noltei* was found off the Bulgarian Black Sea coast, which poses the need of further studies of the mechanisms of the parasite's effect on its host.

Scientific and applied contribution:

1. It has been found that the function for exponential raise to a maximum is the most suitable to account for shading caused by the accumulated epiphytes. The coefficients of the equation for the conditions of the Burgas Bay are determined.
2. Threshold values for the maximum allowable epiphytic load are derived, depending on the light (PPFD) reaching the epiphytic layer.
3. The applied approaches and the obtained results allow for more targeted planning and resource allocation on the study of the presence of communities of

marine angiosperms in different regions of the Bulgarian Black Sea coast with contribution to the optimization of the monitoring programs.

VIII. Papers on the topics of disseation:

1. Hineva E. V. and Prodanov B. (2014) Ecological status of macrophytobenthos community along Bulgarian Black Sea coast. Proceeding of the 12 th International Conference on marine science and technologies, Varna, 315-319
2. Hineva E. V. (2017) First record of a parasite Plasmodiophora bicaudata, J. Feldmann, 1941 on Zostera noltei, Hornemann along Bulgarain Black Sea coast. Proceedings of the Institute of fishing resources, vol. 28, 79-86
3. Hineva E. V. Importance of the wind waves for seagrass distribution along the north coast of Nessebar Bay (Black sea) - приета за печат
4. Hineva E. V. (2020) Wind regime and wave fetch as factors for seagrass habitat distribution: a case study from Bulgarian Black Sea coast, Ecologia Balcanika, 12 (1): 123-135
5. Hineva E. V. and Panayotov V. T. (2020) Wind waves and their importance for the ecology of a seagrass field. Fifteenth international conference on marine sciences and technologies, October 28th, 2020
6. Hineva E.V., Panayotov V. T., Stefanova E.S. and Stamatova H.G. A study on the light attenuation caused by periphyton in the Burgas Bay (the Black Sea) – изпратена