A STUDY ON THE LIGHT ATTENUATION CAUSED BY PERIPHYTON IN THE BURGAS BAY, THE BLACK SEA

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Abstract. Quantifying PAR attenuation caused by periphyton is a crucial step in setting good management criteria for seagrass habitats protection. To help forward the process of its implementation in the Bulgarian coastal waters we set out to verify experimentally this relationship within several perennial, shallow, sublittoral seagrass meadows. Four types of functional relationships between the dry weight and the PAR quantity have been tested: exponential rise to a maximum, Michaelis-Menten, natural logarithm, negative exponential function (both constrained and unconstrained form). The exponential rise to a maximum proved to be the most appropriate curve for a description of the data under the current experiment. The coefficients obtained: 73.22 (71.02 ÷ 75.48, 95% confidence interval) and 0.8299 (0.7507 ÷ 0.9300, 95% confidence interval) are in the range reported on other experimental studies. The application of a variable for evaluation of the effect of the species composition, especially within the higher loads interval, would improve the curve precision. Nevertheless, it can be useful to assess the seagrasses habitat suitability and the risk of light stress in the Burgas Bay (the Black Sea).

Key words: seagrass habitat suitability criteria, light stress risk assessment, temperate brackish water bodies

1. INTRODUCTION

A large body of studies on periphyton (epiphyte) load covering the leaves of submerged plants in marine and freshwater environments shows its potential to affect plant's productivity, synthesis and accumulation of reserve substances, as well as its survival. Twilley *et al.,* 1985 summarize that it is due to the attenuation of available PAR (photosynthetic active radiation) (*e.g.* Sand-Jensen, 1977, Phillips *et al.*, 1978 Balthuis and Woelkerling, 1983) and reduction of the inorganic carbon diffusive transport (Send-Jensen, 1977). Epiphytes can also affect nitrogen (Cornelisen and Thomas, 2004) and phosphorous (Jonstone, 1973) assimilation. They can impact the gas exchange which is carried out through the leaf surface of the seagrasses (Brodersen *et al.*, 2015).

The periphyton ability to reduce the available light and nutrient assimilation, and hence seagrass distribution define the epiphyte load as a suitable object in the development of management criteria for nearshore seagrass meadows protection (Nelson, 2009). Many authors relate the disappearance of submerged aquatic plants in regions where euphotic zone reaches the bottom with the abundant epiphytes (Send-Jensen, 1977; Philips *et al.,* 1978; Cambridge *et al.*, 1986; Silberstein *et al.,* 1986).

Light attenuation is a decrease of the quantity of the PAR reaching the leaf surface of the basiphyte. It is caused by two processes:

1. so-called backscattering when light rays that have reached the periphyton surface are reflected into the water;
2. absorption of light when it passes through the periphyton layer (Kirk, 1994).

A lot of experimental works have been done to test the relationship between the periphyton load and the attenuation of the light passing through it in temperate, subtropical and tropical conditions, as well as across all these climatic zones (Nelson, 2017). Following the conclusions of Nelson (2017) for the necessity of regional assessments and taking into account that periphyton species diversity may influence the "attenuation" response we find it useful to test their validity at a local scale. When it comes to the Black Sea conditions, verification is obligatory, because of the specific peculiarities of the local environment: a temperate, naturally nutrient-enriched, mixo-mesohaline and highly-productive basin (Poulos, 2020), with low species diversity.

This study aims to assess the PAR attenuation when passing through the periphyton layer accumulated for different periods as a step towards the definition of safety criteria for seagrasses habitat suitability in the Burgas Bay shallow coastal water bodies.

2. MATERIAL AND METHODS

Thin artificial substrates (polyethylene terephthalate (PET) used as seagrass leaves mimics (Boynton *et al.*, 1999) were left out to be fouled within seagrass meadows in different parts of the Burgas Bay: the Foros Bay, the Chengene Skele Bay, the coastal water area in front of the Ropotamo River mouth (Fig. 1). All the selected seagrass meadows are subjected to freshwater inflow. The substrates were exposed for different time intervals (4, 8, 22, 45 and 63 days) during the summer season (from June until September). The samples from more wave-exposed part of the Foros Bay, Chengene Skele Bay and Ropotamo location were submerged by SCUBA divers at a depth of 2.5-3.0 m, while in the most wave protected Foros Bay part they were located at a 1 m depth.

The strips were anchored in the sandy bottom using bricks. To the upper part of each strip was attached a float made by extruded polystyrene (Fribran®) (Fig. 2).

After being exposed *in-situ* for the necessary time, substrates were carefully taken out of the water, submersed in containers with filtered seawater, sunshade and transported to the place of measurement within 30 minutes. During this operation the most loosely adhered mineral and organic particles were lost, so the conclusions we drown in this paper, reflect the strongly adhered particles and organisms of the periphyton community, which persist on the substrate even after relatively strong physical impacts (*e.g.* waves, currents). The *PAR* attenuation measurement was done according to Boynton *et al.* (1999) and Franckovich and Zieman (2005). A measurement set-up was constructed following the principles of LAMA (Light Attenuation Measurement Apparatus) (Boynton *et al.*, 1999). All the procedures were done in the field under a sunshade to avoid ambient light changes impact on the results. The light attenuation was calculated against a "blank sample": a clear PET strip. Due to the visible heterogeneity of the load along the heavily overgrown strips, each strip was cut into smaller pieces and treated as separate samples.

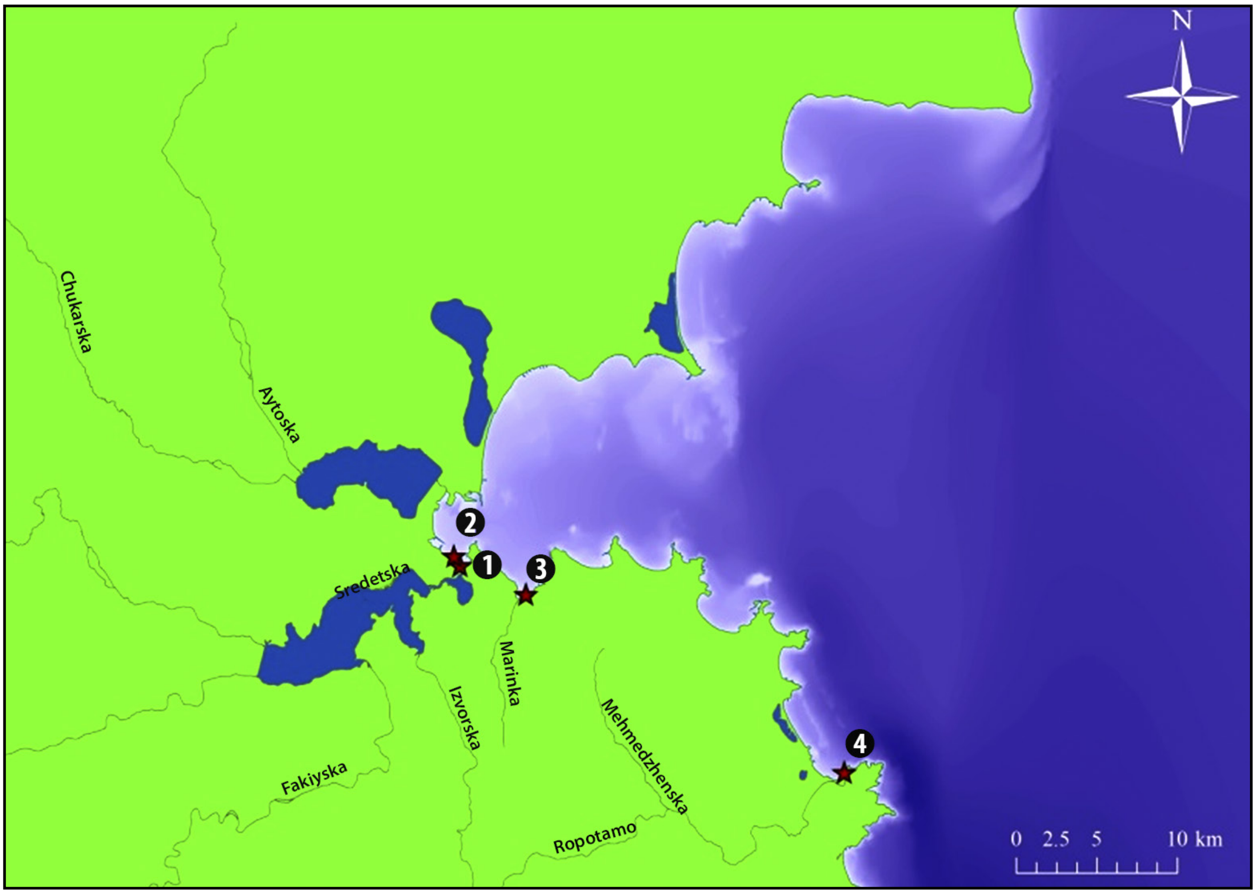

Fig. 1. Study area: locations of artificial substrates immersion are indicated: **1** – Foros Bay – internal part, **2** – Foros Bay – external part, **3** – Chengene Skele, **4** – Ropotamo.

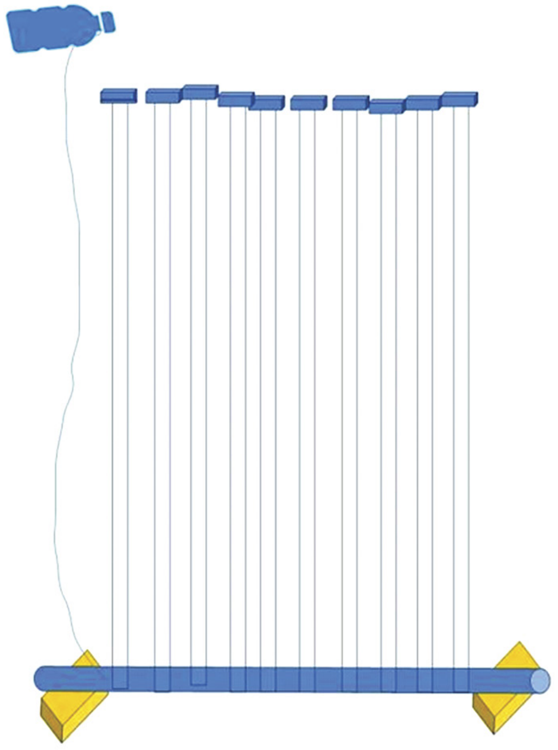

Fig. 2. A schematic representation of the collector array

After the measurements, the samples were fixed in 1-2% formaldehyde in seawater solution. Later in the lab, the dry weight of each sample was determined. The samples were submersed carefully in a container with distilled water to remove the formaldehyde. The load was so strongly attached that there was no visible loss of periphyton during this procedure. Then they were dried at 60°C for 24 hours, cooled in a desiccator and weights were measured on analytical balances. After that, the weight from each sample was removed with a soft toothbrush and a cotton pad soaked in distilled water. Cleaned dry samples were weighted again. The accumulated dry weight was calculated as the difference between the substrate weight with periphyton load and the weight without periphyton load. The area of each sample was measured with the Image J software with a precision of 1 mm after scanning them with an office scanner. A total of 223 samples were used for curve fitting. Statistical calculations were done with the GRAPHPAD software.

3. RESULTS

The results from the field experiments have shown the periphyton potential to decrease the available *PAR*, under certain conditions. The periphyton load accumulated on artificial substrate varied from 0.1 mg/cm^2 (external Foros Bay, 4 days exposure) to 32.4 mg/cm^2 dry weight (external Foros Bay, 45 days exposure). The minimum light attenuation is 0.5% (Chengene skele Bay, 8 days exposure) and the maximum is 88.4% (load equal to 19.4 mg/cm^2 at internal Foros Bay, 60 days exposure).

The relationship between the light attenuation (or light passing through) and the load is of the type "dose-response". The light attenuation data show strong dependency on the periphyton load within the low range of the dry weight. The attenuation reaches a plateau after some threshold value of the load is crossed (Fig. 3).

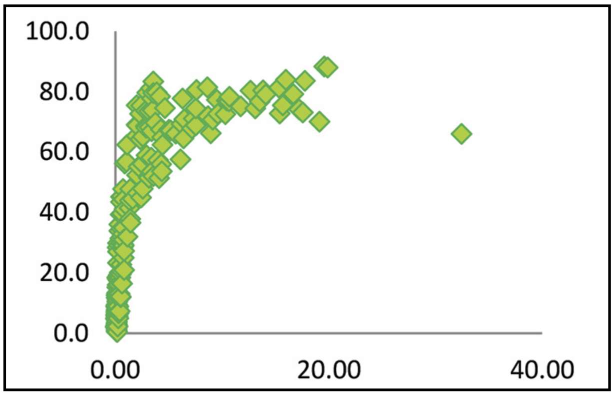

Fig. 3. *PAR* attenuation in % (y-axis), after it has passed through periphyton layer, load in mg/cm^2 (x-axis).

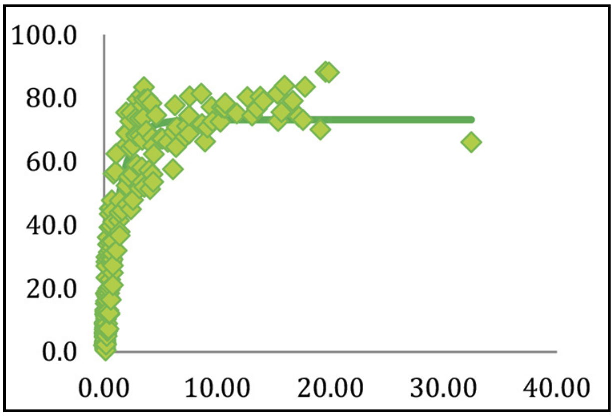

Fig. 4. Exponential rise to a maximum type *PAR* attenuation: $y = A\times(1\text{-}e\hat{}(\text{-}b\times dw))$; y – *PAR* attenuation, %; x – dry weight of periphyton load (dw), mg/cm^2, A and b – coefficients of the equation.

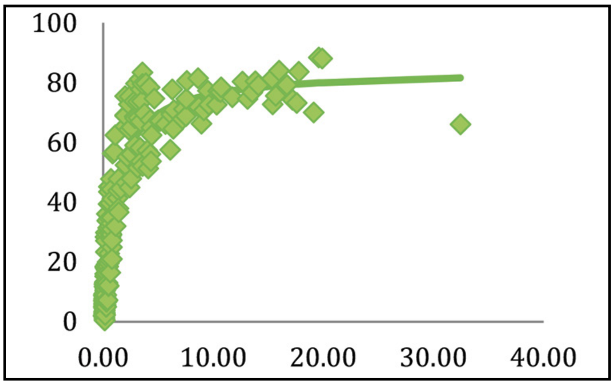

Fig. 5. Michaelis-Menten type *PAR* attenuation: $y = (A\times dw)/(b+dw)$, y – *PAR* attenuation, %, x – dry weight (dw) of the accumulated load, mg/cm^2; A and b – equation coefficients.

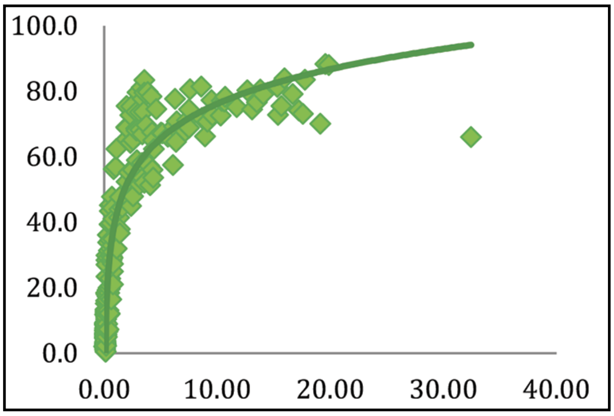

Fig. 6. Logarithmic type *PAR* attenuation: $y = A \times \ln(dw)+b$, *y* – attenuation, %, *x* – dry weight (*dw*) of the periphyton load, mg/cm^2, *A* and *b* – equation coefficients.

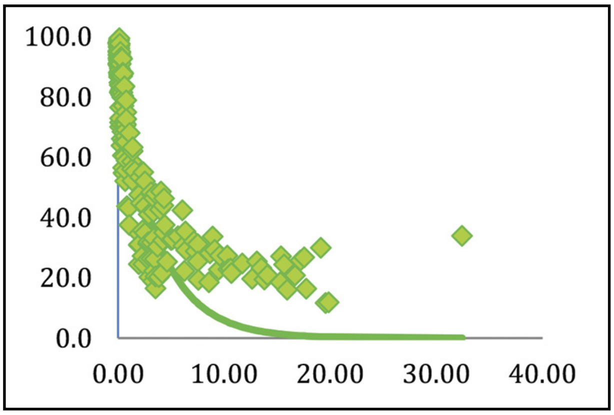

Fig. 7. Negative exponential type attenuation of the *PAR* transferred through the periphyton: $y = A \times \exp(-dw \times b)$, *y* – attenuation, %, *x* – dry weight (*dw*) of the periphyton load, mg/cm^2, *A* and *b* – equation coefficients.

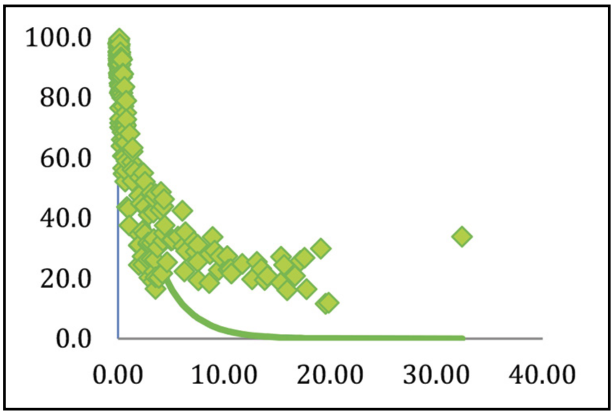

Fig. 8. Negative exponential type attenuation of the *PAR* transferred through the periphyton layer: $y=100 \times \exp(-dw \times b)$, *y* – *PAR* attenuation, %, *x* – dry weight (*dw*) of the accumulated load, mg/cm^2; *b* – equation coefficient.

During different experiments, a variety of functions has been used (both constrained and non-constrained) (review in Neslon, 2017): a second-order polynom, exponential rise to a maximum, linear, logarithmic (natural logarithm), Michaelis-Menten function, negative exponential, negative hyperbolic. In the current study, a relationship has been looked for both between the load and the attenuation, and load and light passed through it.

The form of the relationship on Fig. 3 implies that the change in the attenuation could be best described by exponential rise to maximum (attenuation), Michaelis-Menten function (attenuation), natural logarithm (attenuation) and negative exponential (transferred light).

3.1. Exponential rise to a maximum

The data about the attenuation of light and dry weight of the periphyton accumulated have been used to fit a curve of the type:

$y= A \times (1-e^{\wedge}(-b \times dw))$ (Boynton *et al.*, 1999, Nelson, 2017) (1)

The values of the coefficients of the equation are:

A = 73.22 (71.02 ÷ 75.48, 95% confidence interval) and

b = 0.8299 (0.7507 ÷ 0.9300, 95% confidence interval).

The coefficient A in the equation shows the maximum possible light attenuation when passing through the periphyton layer, *i.e.* the *PAR* attenuation, when the plateau is reached.

The coefficient b determines the slope of the curve in its initial rise range and illustrates the importance of the dry weight accumulated on the substrate (Nelson, 2017). The bigger the value of b the steeper the curve rise is and then less load would attenuate more light. The zero hypothesis (*Ho*) in such a nonlinear form of equation is that coefficients *A* and/or *b* are less than zero. As the value range of both coefficients is bigger than zero the curve could be accepted as a one that describes the data well.

3.2. Michaelis – Menten curve

An equation of the form:

$$y = (A \times dw)/(b+dw) \quad (2)$$

has been used for testing the possibility to describe the *PAR* attenuation (Fig. 5). The values of the coefficients are:

A = 84.24 (81.28 ÷ 87.32, 95% confidence interval),

b = 1.057 (0.9345 ÷ 1.19, 95 % confidence interval).

3.3. Natural logarithm

The equation of the form:

$$y= A \times \ln(dw)+b, \quad (3)$$

was used to test the possibility to describe the data for light attenuation (Fig. 6). The following coefficients have been calculated:

A=15.27 (14.58 ÷ 15.97, 95% confidence interval) and

b = 41.02 (39.75 ÷ 42.28, 95% confidence interval).

When the dry weight is < 0.1 mg/cm^2, the function takes negative values, and when the weight is zero, it is undefined. When an attempt was made to find coefficients giving a positive value if weight is > 0 and < 1, the *A* and *b* had changed so much that the sum of squares of the residuals was much bigger than the minimum. In other words, it is not possible to find a decision which describes correctly enough the results and at the same time avoids the negative values. Because of this reason the natural logarithm function is not acceptable for a description of the experimental results.

3.4. NEGATIVE EXPONENTIAL FUNCTION

A function of the form:

$$y = A\times\exp(\hat{}-b\times dw), \qquad (4)$$

was tested to describe the relationship between dry weight and transferred light (Franchovich and Zieman, 2005).

The exponential attenuation can be tested with both constrained and unconstrained form (Franchovich and Zieman, 2005) (Fig. 7 and Fig. 8). When the function is unconstrained, the coefficients of the equations are as follows:

$A = 91.43$ (88.70 ÷ 94.21, 95 % confidence interval) and

$b = 0.2753$ (0.2386 ÷ 0.3168, 95 % confidence interval).

The maximum of the light transferred is determined by the coefficient *A* and is equal to 91.43 %. The coefficient *b* forecasts steeper slope than observed, in the curve initial decrease region. If the dry weight of the load is above 19.11 mg/cm^2, the curve forecasts a lack of transferred light (< 0.5 %), which is not according to the received results. When the function is constrained, the coefficient *A* (maximum light transfer) is accepted to be equal to 100%. If the above-mentioned constraining is applied the coefficient *b* is 0.3575 (0.3187 ÷ 0.4011). The constrained function does not describe well enough the results, especially in the range of high periphyton dry weight.

4. DISCUSSION

The coefficients A and b obtained in the equation for *the exponential rise to a maximum* are within the range reported by other authors: *A* from 89.7 (temperate zone) to 65.1 (subtropical zone); *b* from 0.6 (temperate zone) to 0.29 (tropical zone) (Nelson, 2017).

Another study has shown very high values of the coefficients (Boynton *et al.,* 1999) which could be due to the experimental conditions. Their experiment has been carried out in a highly productive estuary in a subtropical zone. The high productivity of the environment has impacted the periphyton load rate and probably its quality. This implies that research on the productivity of the environment effect upon coefficients values is necessary.

In a study on *Potamogeton pectinatus* L. (=*Stuckenia pectinata* (L.) Borner) in the Dutch lake Veluwe: a temperate, shallow and eutrophic, heavily modified water body, van Djik, 1993 has received coefficients of the *Michaelis – Menten* curve: $A = 110$ and $b = 1.271$. In the work of Franchovich and Zieman (2005) (cited there as "two parameter hyperbolic attenuation") in the low-productive tropical Florida Keys are reported higher coefficients of the Michaelis – Menten curve: $A = 90.0 \div 108.3$, $b = 3.59 \div 9.0$. It is interesting to note, that coefficients received under the current experiment place the relationship between the two other curves (the graph is not shown) following the productivity gradient assumed by the dominant angiosperm species: from the most eutrophicated lake Veluwe (accommodating only the most tolerant macrophyte), through Burgas Bay (*Zostera noltei*, Hormenann + *Stuckenia pectinata* (L.), Borner), up to Florida Keys (*Thalassia testudinum*, K. D. Koenig). Both temperate bays curves show significant attenuation rate in the lower weight interval and an "early" and relatively "sharp" transition to the plateau, unlike the Florida Keys one. In the latter, the attenuation increases with the increase of the dry weight more gradually reaching the plateau more smoothly, showing lower *PAR* attenuation levels under the same dry weight for a significant part of the dry weight interval (< 27 mg/cm^2).

A comparison of the results received in this study with the results of Franchovich and Zieman (2005) (Florida Keys) in the function of the type *exponential attenuation* shows, that the value of *A* coefficient falls within the range received by both authors 72.77 ÷ 98.6. The *b* coefficient, which reflects the rate of light attenuation by increasing the load, in the present study is higher than the other values for *b* (0.07 ÷ 0.15) in the research of Franckovich and Zieman (2005): *i.e.* the slope of light attenuation in Florida Keys experiments is smoother than the present one.

Following the comparisons of the curve fitting results from different regions (Nelson, 2017) and across curve types a general pattern of coefficients dependence on the productivity of the environment stands out. These results could be interpreted from the point of view of the "response" of periphyton attenuation to the productivity of the environment and the life strategy of the dominant angiosperm species.

In his study Nelson (2017) has found a statistically significant difference between subtropic and temperate zone (exponential rise to a maximum coefficient *A*) and between subtropic and tropic zone (exponential rise to a maximum coefficient *b*), but a general conclusion on climatic zone effect was not drawn, because of the small number of studies in the tropical zone. Nevertheless, the hypothesis that the more productive regions cause steeper initial attenuation increase and higher attenuation maxima does not lack grounds. In low-productive regions (oligotrophic tropical and subtropical zones) in meadows dominated by late-successional species (*sensu* Kilminster *et al.,* 2015) one would expect a lower rate of fouling by epiphytes and stronger control of the environment upon epiphyte abundance (*e.g.* nutrient limitation and/or high level of grazing pressure). Late-successional species have relatively slow leaf exchange rate (review in Borovitzka *et al.*, 2006) and under some conditions (*e.g.* those that would prevent seagrasses from sustaining high enough energetic reserves thus decreasing their tolerance to shadowing) they wouldn't be able to survive in an environment where epiphyte accumulation rate is too fast (high value of the exponential rise to a maximum coefficient *b*). The opposite is valid for colonizing and opportunistic species (Kilminster *et al.*, 2015), at least in temperate zones, which sustain a higher rate of leaf exchange and can survive in an environment with high fouling rate and high value of maximum attenuation. The ability of a basiphyte to support the optimal quantity of epiphytes in the context of the regulation (control) and tolerance appears to be an important part of its adaptive repertoire closely related to both its life strategy for resilience and the local environment. Increasing plant parts renewal rate is a widely exploited adaptation mechanism for reduction of the shadowing stress in the marine environment (Chapaman and Craigie, 1977, cited by Littler and Littler, 1999; Orbita and Mukai, 2009; Fourqurean *et al.,* 2010) and leaf exchange rate is closely related with the life strategy species traits (*e.g.* Raven, 1981, Kautski, 1988, Duarte, 1991, Kilminster *et al.,* 2015). More productive conditions sustain more tolerant higher plants that have a high level of leaf exchange, thus retaining the periphytion shadowing under acceptable levels. Stronger basiphytes control over the epiphytes allows them to survive in an environment with considerable fouling pressure.

On the other hand, the less productive habitats support the dominance of more sensitive, slow-growing seagrass species, having low leaf exchange rate (Duarte, 1991; Kilminster *et al.,* 2015). In such conditions, the low rate of both the periphyton accumulation and light attenuation increase along the periphyton dry weight interval allows for longer living seagrass leaves to photosynthesize under optimal light conditions.

It is necessary to continue the research of the relationship between the fouling pressure and environmental productivity in different geographic zones (Nelson, 2017) and in meadows dominated by a larger number of seagrass species (especially late-successional ones in subtropical and tropical zones) and this hypothesis to be tested again.

4.1. Comparison of the fitted curves

The analysis of the curves used for description and forecast of the relationship of the dry weight and light attenuation by the load (or light transferred through the load) has shown that the curve of *logarithm rise* (exponential logarithm) could not be used because of functional limitations, which could not be overcome and does not correspond to the properties of the data ("a negative" attenuation in some range of load and undefined function when the load is zero).

The comparison of the two *negative exponential attenuation*s of the light transferred (unconstrained and constrained function) gives a relative advantage of the unconstrained function, according to the criterion extra sum of squares *F* test (*DFn*, *DFd*) = 35.87 (1.221). The last has a significantly bigger value than 1, which shows that the more complicated model (the unconstrained one) better describes the data in comparison with the simpler (constrained) model. The comparison is valid if one does not assess the model quality according to the criteria of residuals normality, their clustering and homogeneity of the variation, coefficient of asymmetry and independence of coefficients. Besides the fitted curves (constrained and unconstrained function) do not forecast correctly the quantity of the light transferred in the range of high loads which also lowers their applicability in practice. Due to these shortcomings, both curves are rejected.

The comparison of *the exponential rise to a maximum* and *Michaelis-Menten* curves shows, that the curve of Michaelis-Menten better explains the received results (the difference between *AIC* = -0.9111). As the difference in *AIC* of both models is not so big (61.2 % Michaelis-Menten, 38.8% - exponential rise to a maximum) both curves are acceptable. The collection of additional data could improve the differentiation between the two of them (Motulsky, 2019). The above-mentioned conclusion is valid if the criteria of the normality of residuals, their clustering and homogeneity of the variations, the coefficient of asymmetry and independence of coefficients are not taken into account. If these are taken into account *the exponential rise to a maximum* is the preferred one. The only discrepancy in the relationship is the lack of homogeneity of the variation of the residuals.

A possible reason for the discrepancy is the increasing natural heterogeneity in the spatial distribution of the periphyton load in the range of high values. In the initial stage of the periphyton succession, physical factors are the dominant ones in structuring its biodiversity and spatial distribution (Wahl, 1989). Then the community has low biodiversity, it consists of a thin diatom layer with poor inclusion of Ulvella sp. and *Myrionema magnusii* (Sauvageau) Loiseaux and has a more homogenous spatial structure. This impacts the variability of the light attenuation and at this stage, the residuals are homogenously distributed. This feature is observed when the load is under 0.62 mg/cm^2 and corresponding attenuation up to 17 % (Fig. 9). In the intermediate and final stages of the succession, the biodiversity of the periphyton organisms is high: the encrusting highly calcified species have already appeared (Pneophyllum sp., *Cryptosula pallasiana* (Moll)) together with non-calcified colonial (*Botryllus schlosseri,* (Pallas)) and solitary ascidians. Both groups significantly attenuate light but the former have significantly higher dry weight than the latter. The distribution of these organisms in the space is strongly uneven that is a source of additional heterogeneity. Introducing a variable which better to reflect the peculiarities of the composition can contribute to a reduction in the inhomogeneity of the variation of the residuals and would probably make the curve more precise.

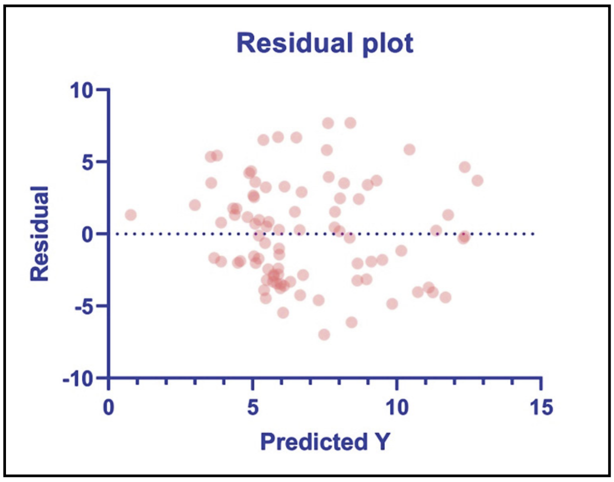

Fig. 9. A residual plot of the dry weight data up to 0.63 mg/cm^2; along the vertical axis are the "residuals" and along the horizontal axis are the predicted values of the dependent variable (the light attenuation)

In summary the most suitable curve for describing the periphyton dry weight - light attenuation relationship for the Burgas Bay based on the current experimental work is the *exponential rise to a maximum* one, according to the following expression (Frankovitch and Zieamn, 2005):

$$LL = Io \times \exp(-Kd \times z) \times [100-(73.22 \times (1-\exp(-0.8299 \times DW))], \quad (5)$$

where *LL* – designates the light (*PAR*) at the leaf surface, *Io*×exp(-*kd*×*z*) is the light attenuation of the water column above the leaf and 100-(73.22*(1-exp(-0.8299×*DW*)) is the light that has passed through the periphyton layer, *DW* – is the periphyton dry weight.

5. CONCLUSION

The comparative analysis of the tested functions shows that the model of the exponential rise to a maximum is the most suitable one to describe the experimental data. Its choice is not unusual as the predominant number of authors have preferred it for data description (review in Nelson, 2017). Introducing a variable which takes into account the species that make up the community would probably improve the mathematical description of the relationship. However, the coefficients received under this study are within the boundaries determined by other authors, which gives us a reason to propose the equation to be used for practical aims when assessing the risk of light stress occurrence and the suitability of a habitat for seagrass presence.

ACKNOWLEDGEMENTS

The authors are thankful for the logistic support to prof. V. Peichev. The dedicated work in the field of A. Machev, N. Nietresta and G. Milkov is highly appreciated.

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