Appendix A - A proposed guideline for light data analysis

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One of the most important components for successful eelgrass restoration is ensuring that the restoration site has environmental conditions that allow eelgrass to survive at that location. This type of assessment should begin at least one year before a large-scale restoration is recommended and includes, among other things, test planting of eelgrass and monitoring of light conditions (Moksnes et al. 2016). The 2016 handbook discusses several physical and biological factors that can affect the suitability of the environment and how these can be monitored. Often, light conditions are the limiting factor for eelgrass and determine whether a site is suitable and at what maximum depth planting can occur. Therefore, light conditions should be examined during the site selection process by logging light levels during the summer months. However, there has been a lack of a standardized description of how the collected light data should be analysed and interpreted. Below is a detailed description of all steps in this process. This is followed by an example of analysis using real data.

Table of content

1. Monitoring of light 2

2. Calibration of meters and conversion of light from Lux to PAR 3

3. Exclusion of incorrect data 4

4. Calculation of Kd and Dmax 6

5. Calculation of Mol PAR per m2 per day at the bottom 6

6. Example – light analysis 7

- **6.1 Conversion of light and calibration of light meters** 7
- **6.3 Calculation Kd och Dmax** 11
- **6.4 Calculation of Mol PAR per day at the bottom** 12
- **6.5 Interpretation of results** 14

7. References 14

1. Monitoring of light

The light requirements of eelgrass vary in the literature, but on average, the plants are said to need 20% of surface light to survive (Dennison et al. 1993, Duarte et al. 2007). Research on eelgrass restoration in Sweden has shown similar light requirements (Moksnes et al. 2018), and studies under laboratory conditions indicate that vegetative growth decreases when light levels fall below 5 mol PAR per m² per day, although the plants can survive under light conditions as low as 3 mol PAR per day (Eriander 2017). When selecting sites for restoration, measuring light conditions is therefore an important part of evaluating environmental conditions, and calculating the light attenuation coefficient (Kd) in the water is recommended (Moksnes et al. 2016). However, methods for analyzing light conditions in water vary greatly in the literature, and there has been a lack of detailed guidance on how light meters should be calibrated, whether and how data should be excluded, and how Kd should be calculated. A more standardized method for light analysis is important to ensure that results are comparable between different restoration projects and studies of eelgrass light requirements. Below is a detailed method description and guidance for analyzing light conditions in water.

The light meters used during the development of the method are Lux meters of the brand Onset HOBO, but the methods described for sorting data and calculating light variables are also applicable to other types of light meters that can store data. Many different types of light meters are available on the market, measuring light either as photosynthetically active radiation (PAR) or Lux. This appendix describes the method for measuring light in the unit Lux. In general, data-logging PAR meters are more expensive than Lux meters, and within the ZORRO research program, the cheaper Lux meters of the brand Onset HOBO have primarily been used. The Lux values have then been converted to PAR by calibrating the meters against a PAR meter (see below). Onset HOBO is available in two models: the older UA-002-64, which can only be programmed to take instantaneous Lux readings at selected intervals, and the newer MX2022 model, which can log light values regularly and then calculate an average for a selected time interval. The latter model is recommended, as these meters provide more stable data with fewer outliers (see below).

Light is measured during the eelgrass growing season (May–September) using data-logging light meters. Light can be measured continuously if the meters are cleaned every 1–2 weeks depending on the degree of fouling. Alternatively, light can be measured during a 2-week period at the beginning of the growing season, e.g., in June/July, and again in September. At sites where, for example, runoff from land or resuspension of sediment from the bottom may negatively affect light levels, longer or more frequent measurement periods may be recommended to identify any periods of poor light that could negatively impact eelgrass.

To describe the light conditions in the water, two light meters must be used, placed at different depths at the same location in each potential restoration site. By measuring light at two depths, the attenuation coefficient (Kd) in the water can be calculated. The attenuation coefficient can further be used to calculate the theoretical maximum depth distribution for eelgrass at the site (Dmax), assuming that light conditions are the same at different locations within the site (see below). The deeper meter should be placed about 20 centimetres from the bottom, at the depth where restoration is planned. In this way, the total amount of light (PAR; mol photons/m²/day) reaching the planted eelgrass per day can also be calculated. The shallower meter is placed about 120 centimetres from the bottom so that the depth difference between the meters is 1 meter. It is important that the depth difference between the meters is measured precisely, as small differences have a significant impact when calculating Kd. It is also important that the light meters are placed at a depth of at least 1.5 meters to avoid the risk of the shallower meter being exposed to air. However, one should avoid placing the light meter too far from the planned planting site, e.g., in deeper water outside a shallow area, as deeper areas often have better water quality with less turbidity than shallower ones (Moksnes et al. 2018).

The meters are programmed to record light every 15 minutes to obtain high-resolution data throughout the day while minimizing the risk of the meter's memory becoming full (this applies to light meters of the brand Onset HOBO). To maximize the amount of usable data, the meters should be cleaned at least every other week. Experience from several years of light measurements shows that fouling is generally not a problem during the first two weeks. For more expensive PAR meters, it is now possible to purchase automatic cleaning units.

2. Calibration of meters and conversion of light from Lux to PAR

The Onset HOBO light meters record light in the unit lux, and to convert this value to photosynthetically active radiation (PAR), they are calibrated against a PAR meter. The calibration is most easily performed on land by placing the lux meters together with a PAR meter on a flat surface where all meters are oriented in the same direction and measurements are taken from morning to evening. If possible, a clear day should be chosen to obtain a broader range of PAR values throughout the day. If the PAR meter does not log light values, at least 20 PAR readings should be manually recorded under different light conditions, e.g., from morning to midday, while the lux meter also records a value. When calibrating the MX2022 meters, it is important to use the instantaneous measurement value and not the average value, which is also recorded.

Linear regressions are then performed between PAR and lux for each light meter separately. The equation for the linear relationship between lux and PAR values is derived and can then be used for conversion to PAR values while also calibrating different meters to each other. The analysis often shows significant differences between individual meters, even within the same model, which is why it is important to calibrate all meters individually. The MX2022 meters generally show less variation between units than the older UA-002-64 meters (Figure 1). Comparisons between the two types of meters also show large differences in the relationship between PAR and lux (Figure 1), so if both old (UA-002-64) and new (MX2022) meters are used, calibration becomes especially important. If calibration of the light meters is not possible for some reason, the average conversion factor established for the old and new light meters, respectively, can be used.

Mean formula for UA-002-64 (old meters): $PAR = lux * 0{,}0090 \quad R^2 = 0{,}998$ *Formula 1.*

Mean formula for MX2022 (new meters): $PAR = lux * 0{,}0164 \quad R^2 = 0{,}999$ Formula 2.

The relationship is based on average values from 21 UA-002-64 meters (441 data points) and 22 MX2022 meters (462 data points).

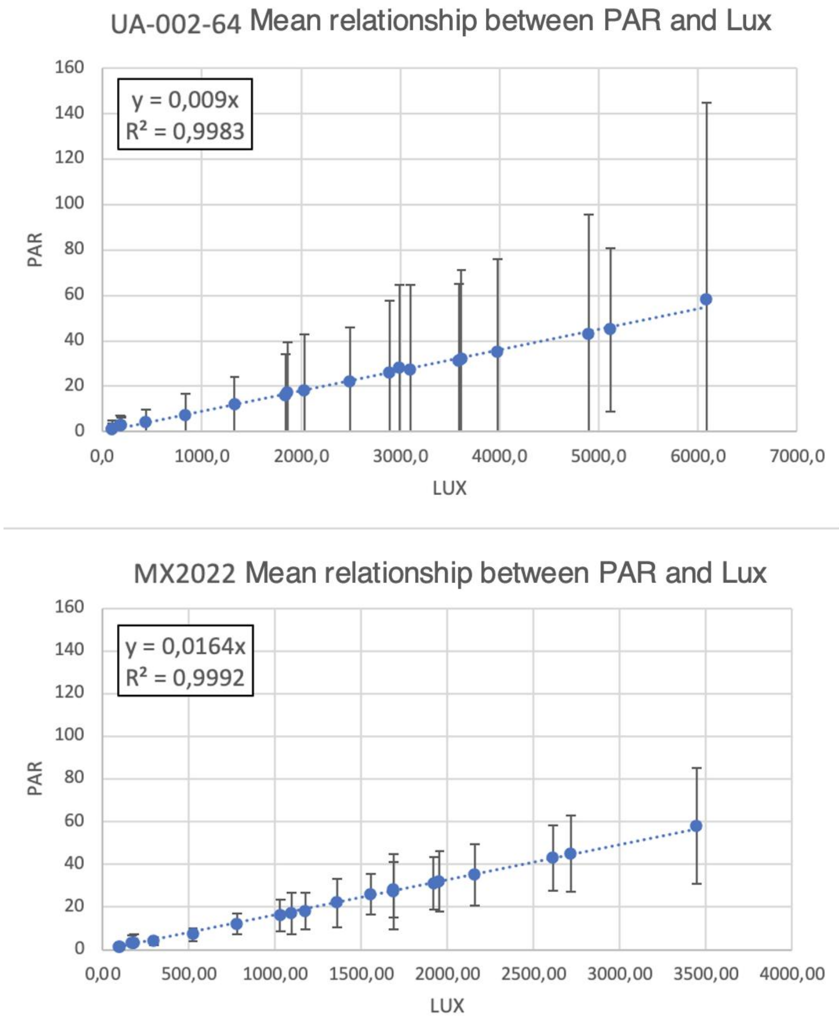


Figure 1. The mean ratio between PAR and Lux (±standard error) for the old (UA-002-64; n=21) and new (MX2022; n=22) Lux meters.

3. Exclusion of incorrect data

After calibration and conversion of the light data to PAR, the data is examined to exclude incorrect values caused by, for example, fouling on the sensors or shading by animals or drifting algae. Furthermore, extremely high values resulting from occasional measurement errors, which can affect the calculation of total daily light (see below), need to be excluded. This section describes how to identify and exclude erroneous data. Section 6 provides examples of how these analyses are conducted.

Identification of fouling and shading

By studying the light at the deep and shallow meters, as well as the ratio between them, it is possible to assess whether the data indicates fouling or is merely a result of changes in light intensity or turbidity in the water. To evaluate this, the average daily light at the surface and bottom is plotted together with the ratio between the average light values (bottom/surface).

We expect that the measured light may vary between days for three different reasons:

1. Changes in solar radiation due to cloud cover and season.
2. Changes in water turbidity.
3. Fouling/shading of the sensors.

The following assumptions can be made, which may facilitate the interpretation of the data:

1. Light variations caused by solar radiation can result in large day-to-day fluctuations, but should not affect the ratio between the meters (deep/shallow).
2. Changes in turbidity primarily affect the ratio, as values from the deep meter are expected to decrease more than those from the shallow meter when turbidity increases (i.e., the ratio decreases with increasing turbidity). Turbidity caused by wind-driven resuspension is expected to lead to relatively short periods of low ratios (day to days), depending on whether it is driven by sea breeze or storms. These periods are expected to affect nearby meters synchronously if they have the same exposure. Turbidity caused by runoff from watercourses is expected to result in slightly longer periods (days to a week), depending on the extent of rainfall/outflow, and may cause more localized effects that vary from site to site. Turbidity caused by algal blooms is expected to have longer-lasting effects over extended periods (days to weeks) and cover larger areas (same trend at nearby sites).
3. Changes due to algal fouling on the sensors are expected to lead to a slow (over days to weeks) decrease in light values on affected meters, more so on the shallow meter than the deep one (as it is less light-limited), which leads to a gradually increasing and persistent ratio. This may, but is not necessarily expected to, be synchronous with nearby sites. Changes due to shading from drifting algal mats on the bottom or fast-growing benthic vegetation are expected to reduce values only on the deep meters and may cause rapid and persistent decreases in the ratio that are not expected to be synchronous with nearby sites.

In summary, turbidity is expected to cause more synchronous, transient effects on the ratio than fouling.

Identifiering of faulty values

After periods with poor data have been excluded, any extreme high PAR values also need to be removed. This is important because the sum of all values over a day is used in the calculation of Kd (see below), meaning that erroneously high values can have a significant impact. These are identified by plotting all recorded values over time, or alternatively by sorting the data by light value. For the newer light meters (MX2022), these extreme values are generally not a problem since the light value represents an average over, for example, every 15 minutes. However, studies of light logged with the older (UA-002-64) meters have shown that extreme high PAR values (>3000 PAR) are sometimes recorded. Measurement points where the ratio exceeds 1 are also excluded (as light can never be higher at the bottom sensor). These are identified by sorting the data by the ratio.

Final check

To verify that the exclusion of poor periods and incorrect measurements has led to improved data quality, linear regressions can be performed between PAR at the surface and PAR at the bottom for each measurement point before and after the data has been ‘cleaned’. If the data quality has improved, the regression should show fewer outliers. In some cases, multiple trend lines may appear, which is not an error but rather indicates a change in the relationship between the meters, suggesting changes in water quality over longer time periods (see example in Section 6 below).

4. Calculation of Kd and Dmax

To calculate the light attenuation coefficient (Kd), all PAR values per day are first summed for both the surface and bottom meters. This is done to stabilize the data and avoid individual high or low ratios between the two meters having too much influence. Kd is then calculated per day using these summed values according to the formula:

$Kd = -\ln(PAR_{\text{deep meter dayX}} / PAR_{\text{shallow meter dayX}})$/depth difference between meters Formula 3.

This provides daily Kd values for the entire measurement period. These daily values can then be used to calculate an average over a selected period, for example, the first two weeks, per month, etc.

Based on the Kd values, the theoretical maximum depth distribution at the site (Dmax; assuming that eelgrass requires 20% of surface light) can be calculated for each day or for a selected period using the following formula:

$Dmax = \ln(0{,}2)/-kd$ Formula 4.

5. Calculation of Mol PAR per m2 per day at the bottom

When calculating the total amount of light in the unit mol photons PAR per square meter per day that reaches the depth where eelgrass grows, or where restoration is planned, the measurements recorded by the meter placed closest to the bottom (20 centimeters above the bottom) are analyzed. Calculations of the total amount of light reaching the bottom provide an ecologically relevant measure of actual light conditions, and since this value is not affected by the surface meter readings, it also serves as an independent measure of light.

Since the value being assessed is the cumulative amount of light reaching the bottom per day, it is important that values are available for all measurement points during a 24-hour period, and that values excluded as extreme or incorrect are replaced. To facilitate this process, all measurements recorded between 21:00 and 05:00 are initially removed, as light is generally zero or near zero during these hours in summer. Missing values are replaced by copying the temporally closest correctly recorded PAR value to the location where a value is missing.

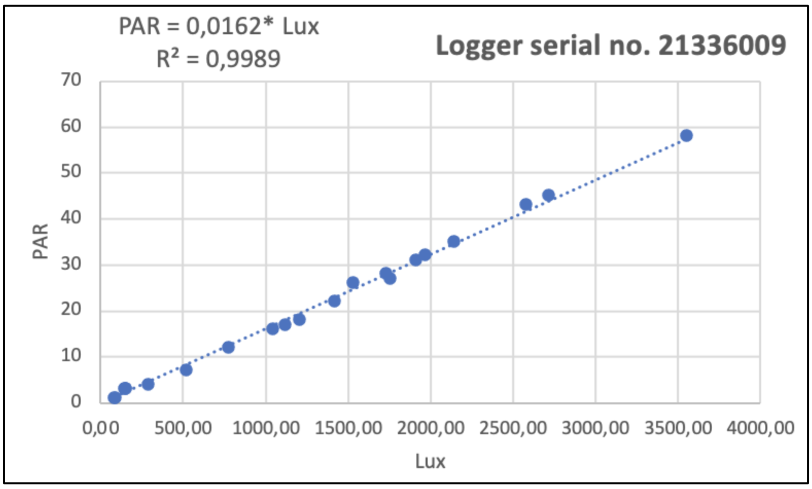
Since PAR is measured per second and values are recorded by the meters every 15 minutes, it is important to calculate the per-second PAR value in order to determine the total amount of light reaching the bottom during a day. Therefore, constant light conditions are assumed between measurement points, and each measurement is multiplied by 15 (if light is measured every 15 minutes) and then by 60, to obtain the PAR value in µmol/m²/second. These PAR values are then summed over the day, and the total is divided by 1,000,000 to obtain the PAR value in the unit mol PAR per m² per day (see Section 6 for example).

6. Example – light analysis

Below is an example of all the steps according to the methods for light analysis described above. The example is based on light data collected at two sites (Triton and Varvsbassängen) in the Port of Malmö during the spring and summer of 2024. Light meters were placed at two different depths (20 centimeters and 120 centimeters) above the bottom at each site and measured light from May 24 to August 26. Light values were logged every 30 seconds, after which an average was calculated for each 15-minute interval and stored in the meter.

6.1 Conversion of light and calibration of light meters

The meters were of the MX2022 model and had been calibrated against a PAR meter in air prior to deployment in order to derive specific formulas for converting from lux to PAR for each individual meter (see example of a regression for one of the meters in Figure 2).



Figur 2. The results from the calibration of a lux meter where synchronous measurements of light in lux and PAR were conducted. Through a linear regression with an intercept at the origin, the equation for converting from lux to PAR is obtained for each specific lux meter.

6.2. Setup and exclusion of incorrect data

Ljusdata laddades ned från mätarna, importerades till Excel och organiserades i ett arbetsblad per lokal. Lux-värdena omvandlas till PAR genom varje mätares unika omvandlingsformel som tagits fram genom kalibrering (se avsnitt 2). För varje lokal placeras data från den grunda och djupa mätaren i kolumner bredvid varandra, där man säkerställer att tidpunkterna för mätningen matchar under hela mätperioden (Figur 3).

#	Date-Time (CEST)	Lux - ytan	PAR - ytan	Date-Time (CEST)	Lux – botten	PAR - botten
147	05-24-2024 05:30:00	451,8	7,3	05-24-2024 05:30:00	340,4	5,7
148	05-24-2024 05:45:00	733,1	11,9	05-24-2024 05:45:00	566,2	9,4
149	05-24-2024 06:00:00	1023,1	16,6	05-24-2024 06:00:00	790,9	13,1
150	05-24-2024 06:15:00	1376,2	22,3	05-24-2024 06:15:00	1066,0	17,7
151	05-24-2024 06:30:00	1886,4	30,6	05-24-2024 06:30:00	1463,5	24,3
152	05-24-2024 06:45:00	1931,3	31,3	05-24-2024 06:45:00	1528,7	25,4
153	05-24-2024 07:00:00	1931,0	31,3	05-24-2024 07:00:00	1544,5	25,6
154	05-24-2024 07:15:00	2339,1	37,9	05-24-2024 07:15:00	1899,6	31,5
155	05-24-2024 07:30:00	3247,6	52,6	05-24-2024 07:30:00	2532,3	42,0
156	05-24-2024 07:45:00	4898,3	79,4	05-24-2024 07:45:00	4074,9	67,6
157	05-24-2024 08:00:00	5457,8	88,4	05-24-2024 08:00:00	4853,7	80,6
158	05-24-2024 08:15:00	6798,8	110,1	05-24-2024 08:15:00	5992,1	99,5
159	05-24-2024 08:30:00	7884,1	127,7	05-24-2024 08:30:00	6683,7	110,9
160	05-24-2024 08:45:00	8524,0	138,1	05-24-2024 08:45:00	7460,9	123,9
161	05-24-2024 09:00:00	9648,4	156,3	05-24-2024 09:00:00	8241,2	136,8
162	05-24-2024 09:15:00	11135,1	180,4	05-24-2024 09:15:00	9940,6	165,0
163	05-24-2024 09:30:00	14667,8	237,6	05-24-2024 09:30:00	12975,1	215,4
164	05-24-2024 09:45:00	8544,9	138,4	05-24-2024 09:45:00	7156,8	118,8
165	05-24-2024 10:00:00	5837,6	94,6	05-24-2024 10:00:00	4740,4	78,7
166	05-24-2024 10:15:00	9129,6	147,9	05-24-2024 10:15:00	7836,4	130,1
167	05-24-2024 10:30:00	7123,5	115,4	05-24-2024 10:30:00	5967,4	99,1
168	05-24-2024 10:45:00	6105,2	98,9	05-24-2024 10:45:00	4915,6	81,6
169	05-24-2024 11:00:00	8976,6	145,4	05-24-2024 11:00:00	7352,5	122,1
170	05-24-2024 11:15:00	8663,2	140,3	05-24-2024 11:15:00	7012,7	116,4
171	05-24-2024 11:30:00	10146,6	164,4	05-24-2024 11:30:00	8160,0	135,5
172	05-24-2024 11:45:00	9641,3	156,2	05-24-2024 11:45:00	7746,8	128,6
173	05-24-2024 12:00:00	11631,9	188,4	05-24-2024 12:00:00	9527,7	158,2
174	05-24-2024 12:15:00	20218,2	327,5	05-24-2024 12:15:00	14503,4	240,8
175	05-24-2024 12:30:00	26134,7	423,4	05-24-2024 12:30:00	18923,5	314,1
176	05-24-2024 12:45:00	29205,2	473,1	05-24-2024 12:45:00	21360,3	354,6
177	05-24-2024 13:00:00	28497,1	461,7	05-24-2024 13:00:00	20443,7	339,4

Figur 3. Shows the initial setup of data in Excel, where data from the shallow (ytan) and deep (botten) lux meters have been placed in columns next to each other, with matching timestamps between meters. PAR has been calculated for each lux value based on the specific conversion formula of the light meter (see section 2).

Identification of fouling and shading

The next step is to identify periods where fouling and shading have caused poor data that needs to be excluded. The analysis begins by calculating daily average PAR values for the shallow and deep sensors, which is most easily done by inserting a pivot table in the Excel sheet. Based on the averages, the ratio between the deep and shallow light sensors was also calculated (PAR bottom / PAR surface). The results were then plotted in a graph with two y-axes so that both PAR values and the ratio can be seen in the same figure (Figure 4).

By placing the results from the two sites side by side, it is possible to check that the sensors have functioned properly, as synchronous changes in light intensity (PAR) should be seen as a result of similar weather, which is the case for the data collected from the two sites in Malmö harbor (Figure 4). During period 1 at the two sites, variations in light between days can be seen, but little change in the ratio, indicating that the variation is due to daily differences in light intensity. On June 20 and 23, two minor dips in the ratio are seen at the site ‘Varvsbassängen’. This indicates increased turbidity, as the values at the deep sensor decrease more than at the shallow one (i.e., the ratio decreases = light conditions deteriorate).

After June 14 (period 2; Figure 4), there is a sharp decrease in light at the deep sensor at the site “Triton”, and a more gradual decrease in light at the shallow sensor, which creates a decrease in the ratio. Since this happens suddenly, it is unclear whether it is due to increased turbidity or fouling (which is usually indicated by a slow trend). However, since it only occurs at this site and the light values at the bottom remain lower than before, it suggests that the sensor has become fouled or shaded by drifting algae. After June 29 (period 3; Figure 4), the ratio increases rapidly at the site “Triton”. This is due to a decreasing amount of light measured by the shallow sensor at the surface. It is clear at this site that the shallow sensor is heavily fouled as the light values approach zero at the end of the measurement period. At the site "Varvsbassängen", there is also a decrease in light recorded by the shallow sensor after June 29 (period 3; Figure 4). The ratio first increases rapidly and then fluctuates around 1 until August, indicating fouling primarily of the shallow sensor.

The reliable measurement period has been assessed to last from May 24 to June 29 at both sites (as it is unclear whether the decrease in light at the deep sensor at the site “Triton” after June 17 is due to increased turbidity or fouling). However, it is important to clarify that this part of the analysis is to some extent subjective, and that values more than two weeks after the start or cleaning of the sensors should be considered more uncertain.

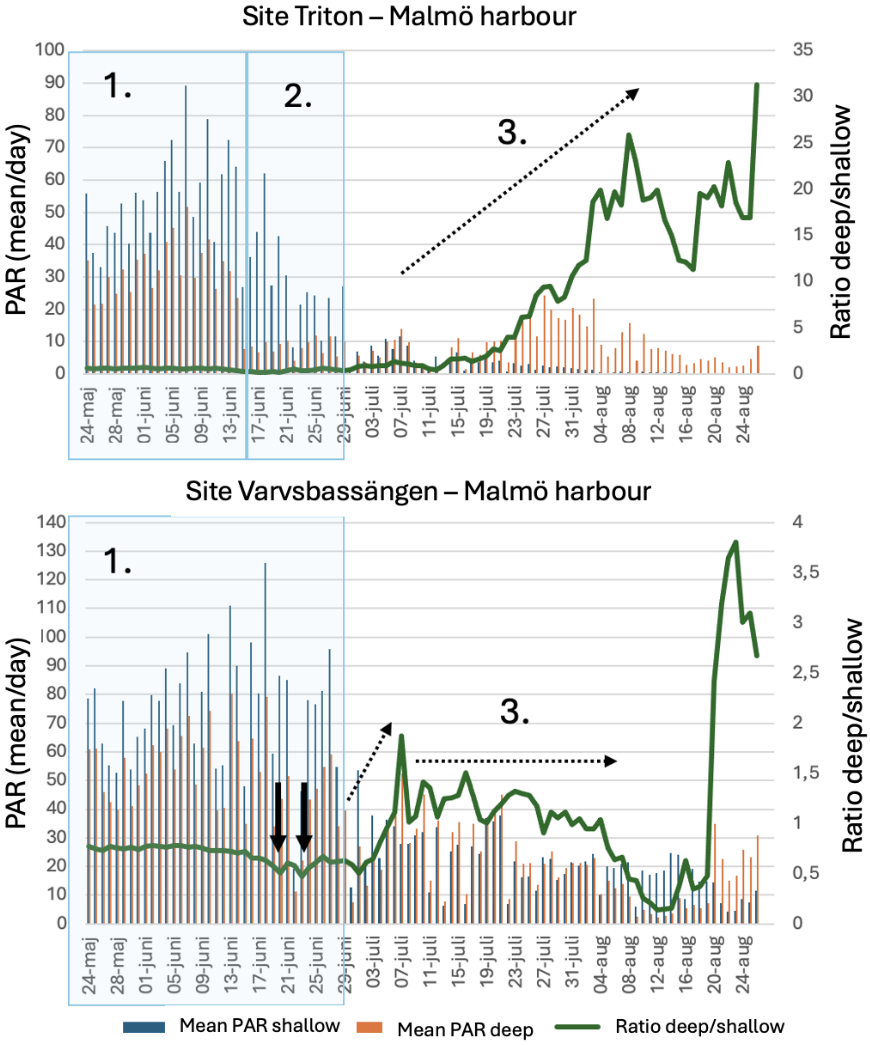


Figure 4. Selection of periods for analysis. The graph shows daily average PAR values at the shallow (surface) and deep (bottom) light sensors, as well as the ratio between them (deep/shallow; right axis). The graph is used to detect incorrect values caused by, for example, fouling, and to exclude these before analysis. The blue-shaded areas represent the periods selected for analysis.

Identification of faulty values and final check

After data from uncertain periods has been excluded, the dataset is re-examined to detect outliers. In this case, no extremely high PAR values were recorded, but data was removed for all time points where the ratio exceeded 1 (as light cannot be higher at the bottom than at the surface). To verify that the data quality had improved, linear regressions between surface and bottom light were compared before and after the data check (Figure 5).

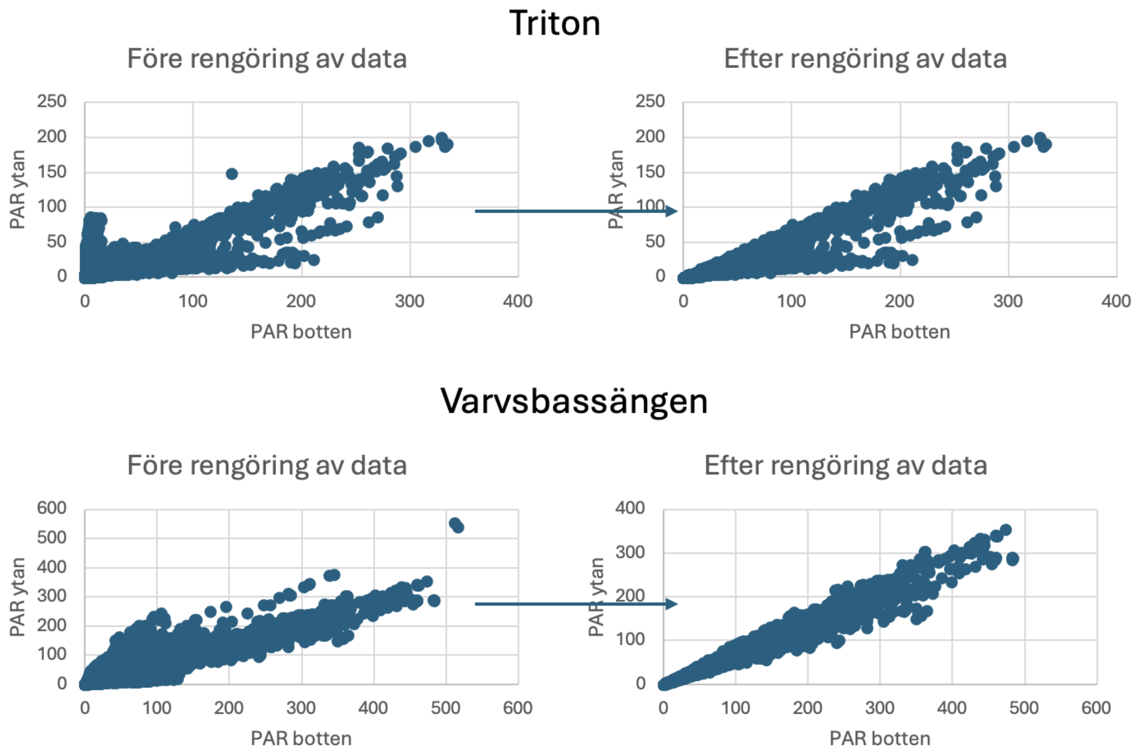


Figure 5. Shows the relationship between light at the shallow and deep light sensors (PAR values recorded every 15 minutes) before and after uncertain measurement periods and outliers were excluded for the site Triton and Varvsbassängen.

6.3 Calculation Kd och Dmax

Using a pivot table for the final dataset in the analysis, the daily sum of PAR was calculated. These values were then used to calculate the daily light attenuation coefficient according to formula 3. The theoretical daily maximum depth for eelgrass distribution (Dmax) was then calculated based on the Kd values using formula 4. Kd and Dmax per day were subsequently plotted in a combined graph, and the results were also compiled in tabular form as averages for the first two weeks and for the entire period (Figure 6; Table 1).

Table 1. Summary of light attenuation (Kd), theoretical maximum depth for eelgrass (Dmax), and the amount of light at the bottom for the first two weeks and for the entire measurement period.

	Mean Kd **First 2 weeks**	**Mean Kd** **Whole period**	**Mean Dmax** **First 2 weeks**	**Mean Dmax** **Whole period**	**Mean Mol PAR/day** **First 2 weeks**	**Mean Mol PAR/day** **Whole period**
Triton	0.49	0.77	3.4	2.6	2.7	1.9
Varvsbassängen	0.27	0.37	6.0	4.8	4.7	4.2

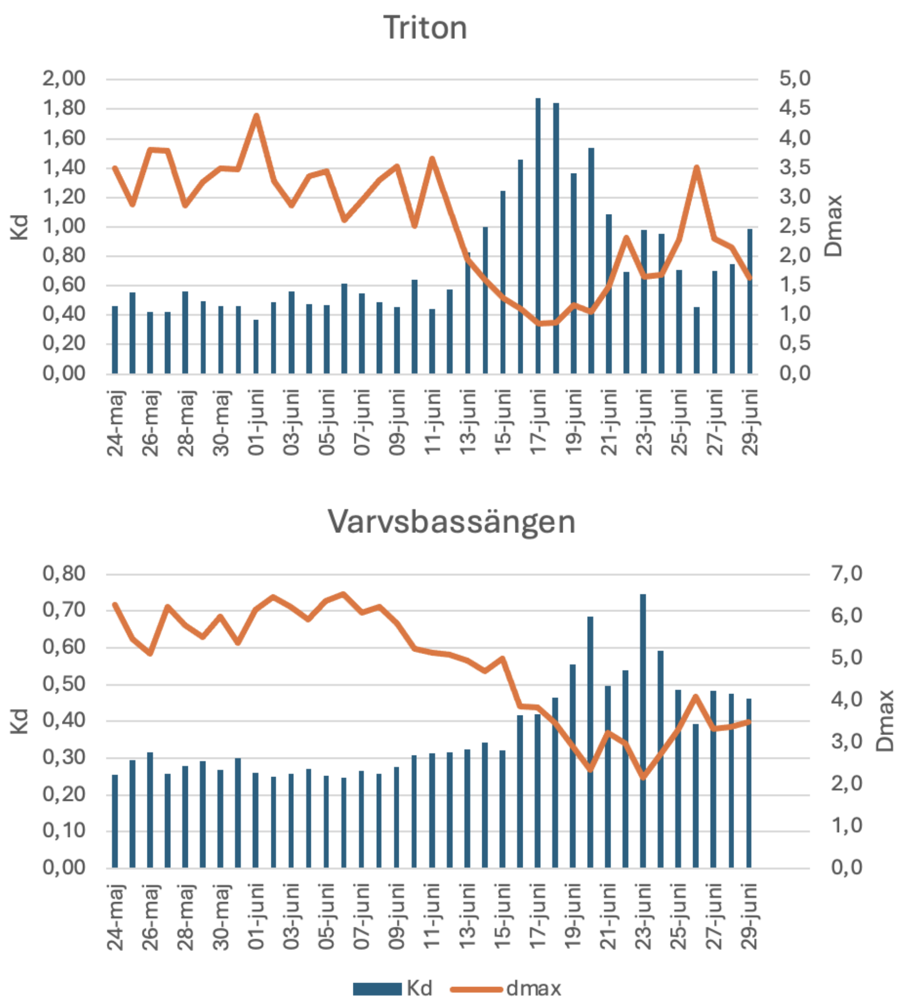


Figure 6. Daily values of Kd and Dmax at the sites Triton and Varvsbassängen in Malmö harbour.

6.4 Calculation of Mol PAR per day at the bottom

To calculate the amount of light in mol PAR/m²/day reaching the bottom, data on bottom PAR, date, and time was exported to a new Excel sheet. Since PAR should be summed, it is important that data is available for all daylight measurement points throughout the day. This means that the start day (when the sensor is deployed in the middle of the day) was excluded. Values between 21:00 and 05:00 were also excluded, as light was essentially zero during these hours. All empty cells where outliers had been excluded were then filled with the nearest correctly recorded value in time (Figure 7). Each measurement point was then multiplied by 15 and then by 60 to convert the PAR value to µmol/m²/second. Using a pivot table, all PAR values were then summed over the day, and the total was divided by 1,000,000 to obtain the PAR value in the unit mol PAR/m²/day. Mol PAR per day was then plotted in a graph, and the results were also compiled in tabular form as averages for the first two weeks and for the entire period (Figure 8; Table 1).

#	Date-Time (CEST)	Date	Time	PAR bottom	
2029	07-27-2022 15:00:00	2022-07-27	15:00:00	283,9	
2030	07-27-2022 15:15:00	2022-07-27	15:15:00	292,4	
2031	07-27-2022 15:30:00	2022-07-27	15:30:00	278,9	
2032	07-27-2022 15:45:00	2022-07-27	15:45:00	267,3	
2033	07-27-2022 16:00:00	2022-07-27	16:00:00	257,5	
2034	07-27-2022 16:15:00	2022-07-27	16:15:00	264,3	
2035	07-27-2022 16:30:00	2022-07-27	16:30:00	260,6	
2036	07-27-2022 16:45:00	2022-07-27	16:45:00	260,6	
2037	07-27-2022 17:00:00	2022-07-27	17:00:00	252,8	
2038	07-27-2022 17:15:00	2022-07-27	17:15:00	246,0	
2039	07-27-2022 17:30:00	2022-07-27	17:30:00	220,1	
2040	07-27-2022 17:45:00	2022-07-27	17:45:00	215,8	
2041	07-27-2022 18:00:00	2022-07-27	18:00:00	205,4	

Figure 7. Example of data setup prior to analysis of mol PAR per day at the bottom sensor, showing how empty cells (for data #2036) are filled with the nearest correctly recorded light value in time.

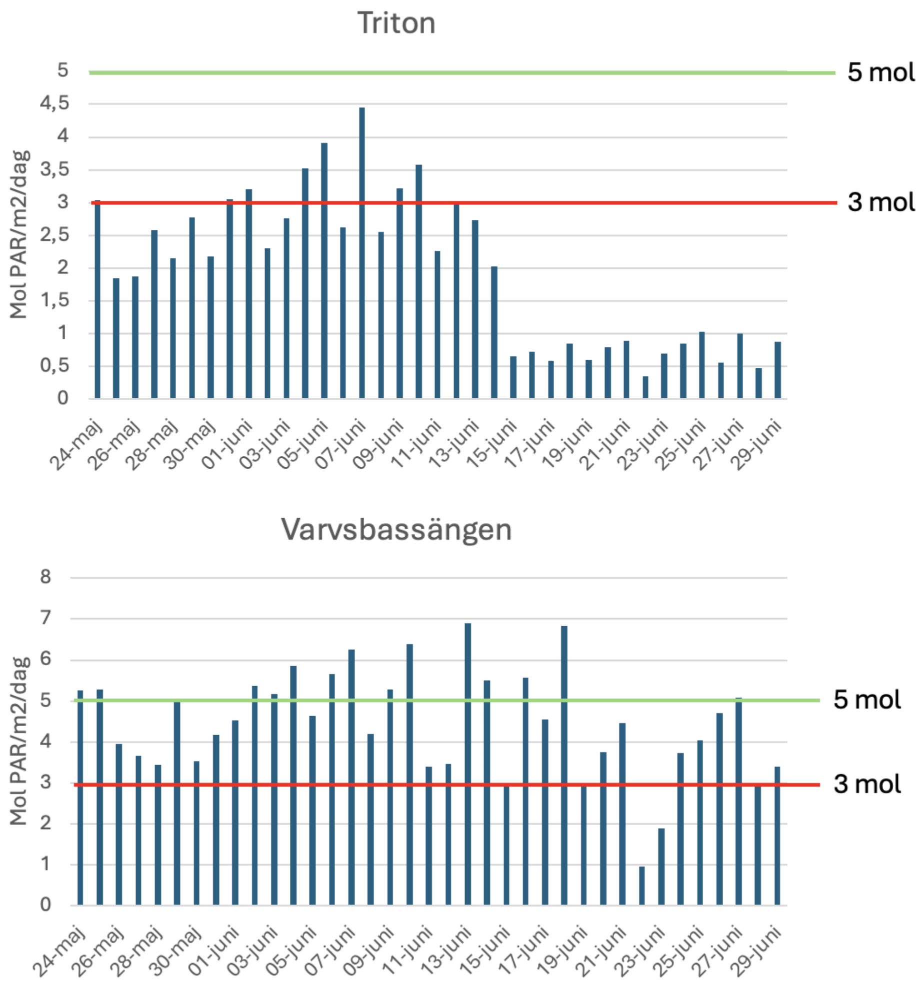


Figure 8. Moles of PAR per day reaching the bottom at the Triton and Varvsbassängen sites. The red line (3 mol) indicates the threshold for eelgrass survival, and the blue line (5 mol) indicates the threshold for unrestricted vegetative growth (Eriander 2017).

6.5 Interpretation of results

The results from Triton and Varvsbassängen indicate variations in water quality, with Varvsbassängen generally having clearer water (lower Kd and higher Dmax) than Triton (Table 1). This is also supported by the actual PAR values measured at the bottom of the two locations. The depth at Varvsbassängen is 4 meters, and the amount of light reaching the bottom exceeds the threshold for eelgrass survival on all but two days during the measurement period (Figure 8). At Triton, the depth was 3.5 meters, and light conditions were below the requirement for eelgrass survival for a large part of the measurement period (however, the low values from June 17 could possibly be due to biofouling on the deep sensor—see previous discussion). According to Dmax, eelgrass could potentially grow down to a maximum depth of 3.4 meters at this location.

7. References

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