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Use of bioassay CG (Colour Graduation) to determine density of *Skeletonema* sp. at hatchery

Badraeni¹, Dody Dharmawan Trijuno¹, and Irvan Eriswandi²

¹Fisheries Department, Marine Science and Fisheries Faculty, Hasanuddin University, Makassar 90245, Indonesia

²Bachelor Graduate Student, Aquaculture Study Program, Fisheries Department, Marine Science, and Fisheries Faculty, Hasanuddin University, Makassar 90245, Indonesia

Email: badraeni@unhas.ac.id

Abstract. The natural food that is often used in hatcheries is *Skeletonema* sp. Phytoplankton provision of *Skeletonema* sp. on fish larvae or seeds carried out in hatcheries tend to be based on estimates and experiences while in the field. This study aims to obtain indicators that can be used in estimating the range of cell density of *Skeletonema* sp. just by looking at the Bioassay CG (Colour Graduation) kit. This research was conducted at the CV. Puncak Sinunggal, Barru Regency. In this study, researchers use a black CG Bioassay prototype in the form of a box equipped with LED lights, batteries, a colour indicator paper kit, 150 mL glass beaker, and a power button. Samples were observed in the exponential phase or 24 hours after the inoculation was diluted by adding culture media to obtain several different colour gradations. The dilution carried out is mixing between samples with culture media with each ratio, beaker glass (BG) 1 (10: 0); BG 2 (9: 1); BG 3 (8: 2); BG 4 (7: 3); BG 5 (6: 4); BG 6 (5: 5); BG 7 (4: 6); BG 8 (3: 7); BG 9 (2: 8) and BG 10 (1: 9). Each treatment was carried out three replications and assisted by 9 panelists. The results showed 10 different colour *Skeletonema* sp. cell density increases with colour change, where the lowest range is in the S10 colour with density $38-75 \times 10^4$ cells.mL⁻¹ and the highest in S1 colour with a density of $612-740 \times 10^4$ cells.mL⁻¹.

1. Introduction

Hatchery is the first step and the key to success in aquaculture business. The main factor that supports the successful management of larvae is the availability of sufficient and sustainable natural food. Natural feed that is widely used in hatcheries is phytoplankton, *Skeletonema* sp. because it is easy to breed and requires a relatively short time to maintain [1], and has a high protein content which can stimulate larval growth [2].

Giving *Skeletonema* sp. larvae in hatcheries tend to be based on estimates, not on calculations according to larvae needs. This is because the process of calculating natural feed requires a long time, apart from limited supporting facilities such as haemocytometer, dropper pipette, microscope and others and requires special skills to carry out the process of counting natural feed cells. The need for natural food for each phase in fish and shrimp larvae is different, and if the natural feed provided is not in accordance with their needs, it will have an impact on the growth rate and production. Therefore we need the right way to apply natural food according to the needs of larvae which do not require a lot of supporting facilities and a relatively short time.



One of the ways that can be used to determine the density of *Skeletonema* sp. quickly and precisely is the use of Color Gradation (CG) Bioassay. CG Bioassay is an innovation in the form of a standard color paper kit by utilizing different color gradations adjusted to the density of *Skeletonema* sp. This tool is used by comparing the color of the kit paper that has been made with the density of *Skeletonema* sp. the results of calculations using a haemocytometer and a microscope. This paper kit is adapted to the pigments that make up the cells in *Skeletonema* sp. namely chlorophyll a, c, alpha, and beta-carotene, as well as xanthophyll (fucoxantin, diadinoxantin, and diatoxantin) so that the color becomes golden brown where the higher the cell density in the culture container, the more intense the resulting color [3].

Based on the description above, a study is conducted to determine the density of *Skeletonema* sp. using CG (Color Gradation) Bioassay as an alternative that is easy to use and does not require a long time to feed according to cell density *Skeletonema* sp. required.

This study aims to obtain a color gradation (CG) paper kit indicator that can estimate the density range of *Skeletonema* sp. The results of this study are expected to be used as a tool to estimate the density of *Skeletonema* sp. quickly and easily in hatchery natural feeding.

2. Methods

This research was obtained CV. Puncak Sinunggal hatchery, Mallawa Village, Mallussetasi District, Barru Regency, South Sulawesi Province.

2.1. Work Procedures

2.1.1. Testing Container. The testing container that is used is a 150 mL beaker glass made of clear glass, filled with 100 mL of water sample. The number of beaker glass required in one observation with different densities is 10 pieces each.

2.1.2. Testing Organism. The organism that is tested is *Skeletonema* sp., taken from the bulk culture of natural feed which is ready to be given to the rearing tank for shrimp larvae in the CV. Puncak Sinunggal hatchery.

2.1.3. CG (Colour Gradation) Bioassay. CG Bioassay is a color observation testing tool equipped with several components including an LED light, 150 mL beaker glass, a color grading kit paper, a battery and a power button. Each of the components has function, those are, an LED light as an aid in the observation process, beaker glass as a container for observing the color of *Skeletonema* sp., A battery as a source of electric current and a power button to activate the LED light (Figure 2).



Figure 1. CG (Colour Gradation) Bioassay Prototype

2.2. Testing Method

2.2.1. Preparation of Testing Materials. Skeletonema sp culture in tubular fiber tub with a volume of 2 tons. The tub is filled with 800 L of seawater, given UREA fertilizer 6.67 ppm, NPK 8.33 ppm, TSP 5 ppm and Silicate: 5 ppm, equipped witheration, inoculated with Skeletonema sp., Ready to be used as test material to determine density based on grading color.

2.2.2. Testing Steps. Skeletonema sp. which has been cultured are harvested by filtering using a filter bag. The Skeletonema sp. filter results were used as the observation sample. The sample was then diluted by adding culture media water to obtain several different color gradations. The dilution carried out was mixing the samples with culture media with their respective ratios, Baeker glass (BG) 1 (10: 0); BG 2 (9: 1); BG 3 (8: 2); BG 4 (7: 3); BG 5 (6: 4); BG 6 (5: 5); BG 7 (4: 6); BG 8 (3: 7); BG 9 (2: 8) and BG 10 (1: 9). Then an observation was made by matching the sample color with the kit paper in the prototype. Sample dilution and sample color matching were carried out 3 (three) times. Sampling in the exponential phase based on several references shows that the population peak is in the 24 hour maintenance period [3,4], 48 hours [5], 72 hours, 96 hours [6], 120 hours, 144 hours, 168 hours, 192 hours, 216 hours, 240 hours, 264 hours, 288 hours [7].

2.2.2.1. CG Bioassay Working Mechanism. CG (Color Gradation) Bioassay is a tool to determine the amount of natural feed density in a container by comparing the different colors in each density with the help of a predetermined light. The use of this tool is only applied to Skeletonema sp. which will be given to fish or shrimp larvae or seeds.

2.2.2.2. Matching Bioassay CG (Color Gradation). Kit Paper With Density Skeletonema Sp. CG Bioassay paper kit is made of glossy photo paper which is commonly used for printing high quality photos, has a smooth, glossy paper surface and reflects more light, resulting in high-resolution print quality.

The paper size of the CG Bioassay kit was printed using A4 (21cmx29.7cm). The color on the CG Bioassay paper kit is obtained from the Adobe Photoshop CS6 application (Figure 3) by taking the base color in the test sample and then reducing the color level (Hint) from 100-0% which is labeled with letters and numbers to make it easier to distinguish and printed using a printer Epson L365 by doing Head Cleaning first to get maximum print results.

The color indicator for the kit paper is adjusted to the type of algae density that will be calculated. CG Bioassay paper kit uses brown as the main color because the algae to be observed is Skeletonema sp which is a brown algae from the Bacillariophyceae class. Paper kits are made using the Adobe Photoshop CS6 application.

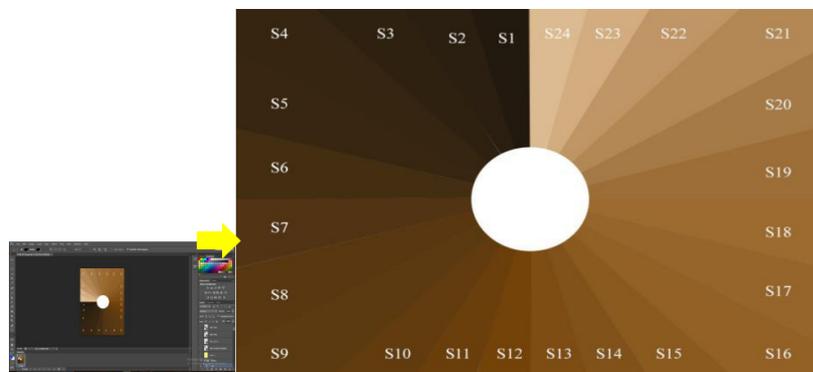


Figure 2. The making of CG Bioassay kit paper (left), CG Bioassay kit paper (right)

The kit paper was placed at the bottom of the CG Bioassay prototype to facilitate observation of matching test samples. The observation process is carried out by placing the test sample on the kit paper, turning on the LED light by pressing the power button, then observing the suitability of the sample color with the kit paper. After the observation is done it can be concluded between color and density. To minimize the occurrence of bias during matching, at least 5 panelists who do not have color sensing problems are assisted.

Color coding using the letters S and numbers 1-10 to make it easier to distinguish each color and density found on the CG Bioassay paper kit. The letter S in each color code is taken from the first letter of the test sample, those are *Skeletonema sp* (S) and numbers 1-10 to make it easier to distinguish each color code on the CG Bioassay paper kit.

2.2.2.3. *Calculate the density of Skeletonema sp. cells.* After matching the paper color of the CG Bioassay kit on the test sample in each beaker glass, then calculating the cell density of the test sample using a haemocytometer, microscope and hand counter. In order to obtain an accurate cell count, we carried out a count of 12 times for each beaker glass.

2.3. Variable Parameters

2.3.1. *The density of Skeletonema sp using a Haemocytometer.* Haemocytometer is used to calculate cell density manually using the formula, Cell Density = number of cells x 10⁴ cells / mL.

2.3.2. *Determination of density using CG (Colour Gradation) Bioassay.* Color Gradation (CG) Bioassay is used to determine cell density by matching the colors contained in the CG Bioassay kit paper with the sample, the kit paper has a color scale, each of which has a different density.

2.4. Data Analysis

Data that is obtained from the observations, matched, and has its sample density calculated is analyzed descriptively with the help of table and figures.

3. Results and Discussion

Observation and *Skeletonema sp.* density calculation results using haemocytometer shows 10 different color composition (Figure 4 and Table 1).

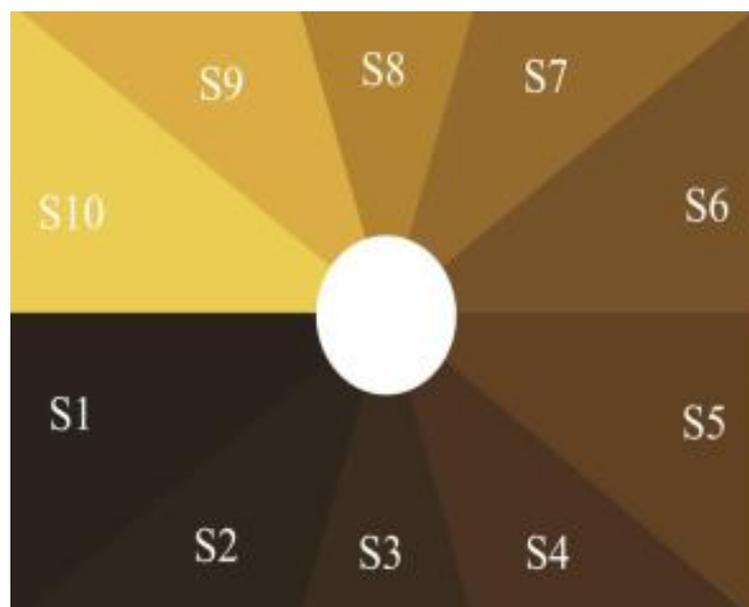
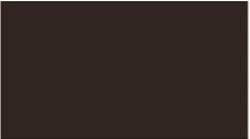


Figure 3. Observation result of CG Bioassay paper kit

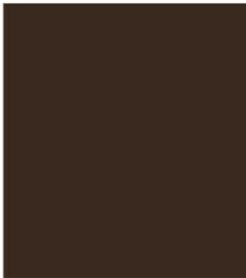
Table 1. Color Composition, Color Code, and Skeletonema sp Density

No	Color	Color Indicator	Density ($\times 10^4$ sel/mL)
1		S1	> 597
2		S2	596–457
3		S3	456–311
4		S4	310–267
5		S5	266–202
6		S6	201–160

7		S7	159-134
8		S8	133-108
9		S9	107-78
10		S10	<78

The color gradation of *Skeletonema* sp. at harvest time in natural feed culture tanks which will be given to shrimp larvae according to the color code on the CG Bioassay paper kit can be seen in Table 2

Table 2. Harverst and CG Bioassay paper kit color result

No	Harvest Color research	Paper kit Color	Color Code	Density (x10 ⁴ sel/mL)
1			S3	457-311
2			S4	311-267



The results of cell density calculations using a haemocytometer and its correlation with the color of *Skeletonema* sp. on the CG Bioassay kit paper, the density range of each color change is obtained as shown in Figure 9 below,

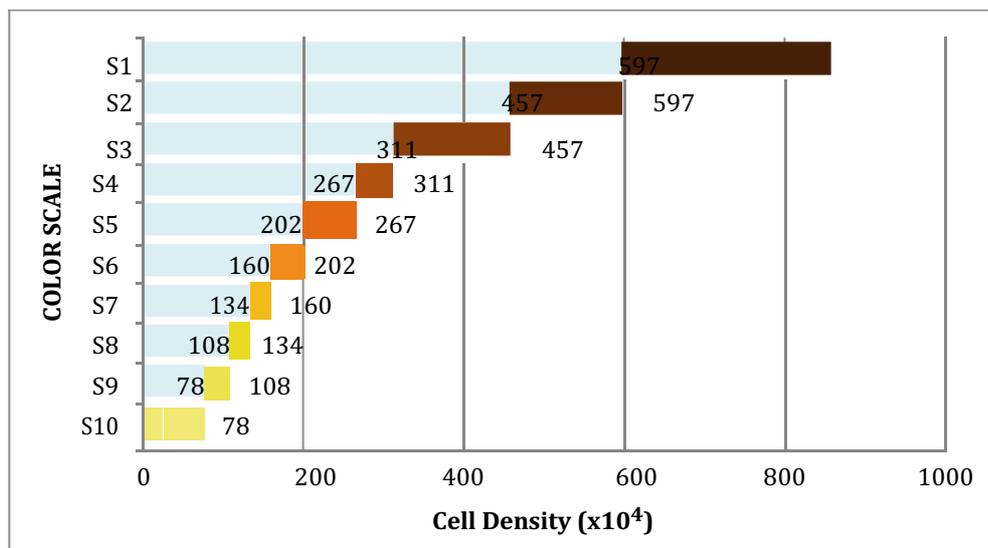


Figure 4. The correlation between cell density and color on the CG Bioassay color grading kit paper

Figure 9 above shows that the CG Bioassay density range scale consists of 10 scales (S1-S10) which show the total density of *Skeletonema* sp. with color indicators. The range of density in *Skeletonema* sp based on the color scale is from high to low density, from S1 to S10. For the S1 color code, which is the darkest color, is the highest density with a density value > 597x10⁴ cells / mL. Furthermore, the density of the color indicator decreased until the S10 scale was the lowest density with a density value < 78x10⁴ cells / mL.

The color change that occurs on each color scale from dark brown to clear golden yellow as the cell density decreases. This color change is due to the lower pigment / dye content due to the low cell population of *Skeletonema* sp. in a container. The waters will be golden brown if it is overgrown by *Skeletonema* sp., The higher the density in the waters, the color that will appear will be a very thick golden brown. This is in line with the research results of Yunus (2008) and Ervandi (2014) that the color change that occurs in natural food in a container is caused by the higher levels of chlorophyll along with the high cell population in the container [8,9].

The colors of the bioassay CG (Color Gradation) kit paper that are usually given to larval rearing tanks in hatcheries are S3, S4, and S5 color codes. One example of natural feeding *Skeletonema* sp. at Hatchery CV. Puncak Sinunggal in the Zoea-Mysis phase is 2 L with a frequency of 4 times / day given to larvae with a density of 1 million tails. The color code that appears is S3 with a density of

457-311x10⁴ cells / ml given each frequency of administration. If given 4 times / day, the number of *Skeletonema* sp. given daily ranged from 3,656-1,688x10⁷ cells / day. Standard use of natural feed dosage *Skeletonema* sp. at each Zoea-Mysis stage in the production of tiger shrimp hatchery based on SNI No. 01-6144-2006 is 20-30x10³ cells / ml / day in each individual. So for giving *Skeletonema* sp. to 1 million tails, it takes 20-30x10⁹ cells / day so that natural feeding of *Skeletonema* sp. in the rearing tank of tiger prawn larvae in the CV hatchery. The peak of Sinunggal in accordance with its needs according to SNI No. 01-6144-2006.

The dark brown to golden yellow color indicator on the CG Bioassay paper kit is based on the color substance or pigment contained in *Skeletonema* sp. In accordance with the statements of Gusriana (2008) and Amanda (2013), *Skeletonema* sp. contains xanthophyll (yellow color) and carotene (golden color) pigments and is included in brown algae (Bacillariophyceae Class) [3,10].

4. Conclusion

The results of the study using the CG (Color Gradation) Bioassay to determine the density of *Skeletonema* sp. concluded that:

1. CG Bioassay is a tool to determine the density of *Skeletonema* sp. cells. use a color indicator.
2. The color indicator scale used in the CG Bioassay consists of 10 color compositions, namely the colors S1, S2, S3, S4, S5, S6, S7, S8, S9, and S10.
3. The color change of the *Skeletonema* sp. culture results changes with the change in cell density, namely the lowest density range is in the S10 color with a density <78x10⁴ cells / mL and the highest is in the S1 color with a density > 597x10⁴ cells / mL.

References

- [1] Rudiyananti S 2011 The Growth of *Skeletonema costatum* on Various Salinity Level's Media *Saintek Perikan. Indones. J. Fish. Sci. Technol.* **6** 70–7
- [2] Sutikno E, Dwi S P and Hermintarti 2010 Pemanfaatan Mikroalga sebagai Bahan Substitusi Tepung Ikan pada Pakan Buatan untuk Ikan dan Udang 1
- [3] Armanda D T 2013 Pertumbuhan kultur mikroalga diatom *Skeletonema costatum* (Greville) Cleve Isolat Jepara pada Medium f/2 dan Medium Conway *Bioma* **2** 49–63
- [4] Fauziah F and Hatta M 2015 Pengaruh pemberian kascing (bekas cacing) dengan dosis yang berbeda dalam kultur *Skeletonema costatum* *Acta Aquat. Aquat. Sci. J.* **2** 11–7
- [5] Mudhakiroh S and Soeprbowati T R Perbandingan Penghitungan Populasi *Skeletonema costatum* (Greville) Cleve Dengan Metode Hemocytometer Dan SRCC
- [6] Kurniawan M H and Agung M U K 2017 Pemanfaatan *Skeletonema* Sp. Dalam Mereduksi Limbah Minyak Solar Di Perairan *J. Perikan. Kelaut.* **8**
- [7] Setyaningsih I, Panggabean L M, Riyanto B and Nugraheny N 2006 Potensi antibakteri diatom laut *Skeletonema costatum* terhadap bakteri *Vibrio* sp *J. Pengolah. Has. Perikan. Indones.* **9**
- [8] Yunus M 2008 *Penentuan Kepadatan Sel Nannochloropsis oculata Menggunakan Indikator Monokrom* (Hasanuddin University)
- [9] Ervandi 2014 *Pengujian Karakteristik Gradasi Warna Dalam Menentukan Kepadatan *Chorella* sp* (Makassar: Universitas Hasanuddin)
- [10] Gusriana 2008 *Budidaya Ikan untuk SMK* (Jakarta: Direktorat Pembinaan Sekolah Menengah Kejuruan Departemen Pendidikan Nasional) p 499