ISSN : 1410-5217
Acrredited : 83/Dikti/Kep/2009

Original Paper

EFFECT OF USING GUILLARD AND WALNE TECHNICAL CULTURE MEDIA ON GROWTH AND FATTY ACID PROFILES OF MICROALGAE *Skeletonema* sp. IN MASS CULTURE

Vivi Endar* , Sarjito, Johannes Hutabarat and Budi Prayitno

Faculty of Fisheries and Marine Science, Diponegoro University
Jl Prof Soedharto, Semarang 50275, Central Java – Indonesia

Received : Accepted :

ABSTRACT

Live food, especially microalgae Skelotenoma sp. is a key success factor in shrimp aquaculture. To that end, the provision of Skeletonema sp. mass with a high nutrient content is needed. Nutritional quality of microalgae depends on the culture media used. The purpose of this study was to investigate effect of the use of different technical culture medium (Walne and Guillard) on the growth, protein content and fatty acid profile in microalgae culture Skelotenoma sp. Skeletonema sp. obtained from the Laboratory of Natural Feed BBPBAP Jepara. Culture method used was a mass with two different media (modified Walne and technical Guillard), with 12 replications. Data analysis were analyzed by using T test, while the protein content analysis was performed by Kjedahl method.The fatty acids were determined by using in situ transesterification. The results showed that the growth of Skeletonema sp. was markedly different between media Walne and technical Guillard. Guillard medium revealed lag phase after 44 hours (observation to 6) with a cell density of 48.00 x 10^4 cells/ml, then entered the exponential phase at 48. (Observation to 7) with a cell density of 70.25 x 10^4 cells / ml, while the stationary phase occurred in after hours to 52 (observation to 8) with a cell density of 86.75 x 10^4 cells / ml and death phase began at the 56 (observations to 9) with a cell density of 54.58 x 10^4 cells / ml. Growth of Skeletonema sp. cultured with culture medium technical Walne showed a similar pattern in the lag phase to 44 hours of observation (observation to 6 with the cell density is 117.17 x 10^4sel/ml, exponential phase and stationary phase were detected from hour to 48 (observation to 7) with a cell density is 160.83 x 10^4 cells / ml. Later phases of death from an hour to 52 (observation to 8) with a cell density of 122.25 x 10^4 cells / ml. then long culvation or Skeletonema sp stationary phase in Guillard media over a period of 4 hours than Walne medium. Total fatty acids of Skeletonema sp. cultured in Guillard medium resulted in higher yields.

Keywords : *Skeletonema* sp, Technical culture media Guillard and Walne, Growth, Protein, Total Fatty Acids

*Correspondence: Phone: +62-24-7474698; Fax: +62247474698 ; E-mail: anshienvie@yahoo.com

INTRODUCTION

Shrimp aquaculture is one of the biggest marine industries that contributes to the economic development in Indonesia. It is has been generally agreed that one of the most important factors to be considered for successful shrimp culture is live foods, especially with their nutrient composistions.

Skeletonema sp. is a diatom that serves commonly as live food for fish and shrimp larvae (Cuzon, 2004). This is because the nutrient content and the size of *Skeletonema* in accordance with the mouth opening, especially shrimp larvae vanname naupli stadia up to mysis. *Skeletonema* sp-shaped chain with cell sizes ranging from 4-15 μm (Maximiano et.al, 2002) with protein content ranged from ranged from 21.63 to 32.05% (BPAP, 2010). Due to the fact that the need of shrimp larvae for *Skeletonema* sp. as a live food needs, therefore, to meet the needs of natural food culture on a mass scale *Skeletonema* sp is essential to to perform research on supporting shrimp seed business.

Skeletonema sp. is needed in a large number,

ISSN : 1410-5217
Acrredited : 83/Dikti/Kep/2009

when a culture of high-density mass will be performed in a shorter time. In addition to getting a high abundance of cells, in mass culture is also expected to obtain maximum nutrition (Amsler, 2008). Various mass scale studies related to growth *Skletonema* mass scale were carried out by (Rousch, 2003; Susanto *et al.,* 2006; BPAP, 2010, Pratiwi, 2009) and the laboratory scales (BBPAP, 2010; Chilmawati, 2009; Suminto and Hirayama,1995).

In order to obtain growth and good nutrition, appropriate media are required. Culture media commonly used for culturing diatoms is Guillard (Anderson, 2005; Amsler, 2008; BBPAP, 2007) and a modified Walne (Rousch, 2003; Susanto *et al.,* 2006; BBPAP, 2010). Modifications of Walne media (2 doses and adding silicate) could increase the growth of the number of cells of *Skeletonema* sp. 3-4 fold (Susanto et al, 2006) and stimulate the growth of 4-5 times the peak growth at day 6 (BBPAP, 2010). In this study, we examined in more depth about the use of the technical differences in the culture medium (modified Walne and Guillard) on growth, protein content and fatty acid profile of *Skeletonema* culture.

The purpose of this study was to investigate the effect of the use of technical culture media Walne and Guillard on the growth, protein content and fatty acid profile of *Skeletonema* sp.

MATERIALS AND METHODS

Skeletonema sp. used was obtained from the Laboratory of Natural Feed BBPBAP, Jepara, North Java, Indonesia. Media test used was is fertilizer Walne (Anderson, 2005) with a modification of the addition of silicate and use two doses (BBPAP, 2007) and technical Guillard (Anderson, 2005) which was a source of nutrients for *Skeletonema* sp. Then all the tools are sterilized and sterilized fiber tub washed and then dried (Cahyaningsih, 2006). Media was previously diluted with distilled water prior to use in order to facilitate the culturing. The composition of the culture medium Walne and Guillard is shown in the following **Tabel 1.**

Table 1. Nutritional composition of Walne and Guillard media

Composition	Walne (gr)	Guillard (gr)
Nutrien component:		
$NaH_2PO4.2H_2O$	20	10
NaNO3	100	84,2
Na_2EDTA	5	10
Na_2SiO_3	40	50
$MnCl_2.H_2O$	0,36	0,36
$FeCl_3$	1,3	2,9
H_3BO_3	10	-
Akuades	1000 ml	1000 ml
Trace metal element :		
ZnCl2	21	-
CoCl2.6H2O	2	2
$(NH_4)_8.Mo_7O_{24}.4H_2O$	0,9	1,26
CuSO4.7H2O	20	1,96
$FeCl_3.6H_2O$	3,15	3,15
Akuades	100 ml	
Vitamin:		
Vitamin B12	0,1	0,01
Thiamin	20	0,2
Biotin	0,1	0,01

Seawater prepared for culture media used in the study, had a salinity of 28 ppt (Abdulgani *et al*., 2008 and Gusrina, 2009). The environmental conditions of the culture during the study is indicated in the **Table 2.**

ISSN : 1410-5217
Acrredited : 83/Dikti/Kep/2009

Table 2. Ecological parameters of the media used in the study

Parameter	Guillard	Walne	Reference
Ph	8,31	8,17	7,2-8,5*
DO	2,96	2,96	2,00 – 4,00**
Temperature	30,20	30,53	25-31***
Salinity	28,75	28,50	17-30****

* Anderson, (2005); Amsler, (2008)
** Anderson, (2005)
*** Cahyaningsih, (2006);Amsler, (2008)
**** Cahyaningsih (2006); Kinoyo (2010)

RESULTS AND DISCUSSION

One goal is to get the algae culture cells in the highest abundance and optimal nutrition (Fogg, 1965), this is because natural food is an absolute feed given to fish and shrimp larvae. The effect of two different media on the growth of *Skeletonema* sp.is presented in the following **Fig. 1.**

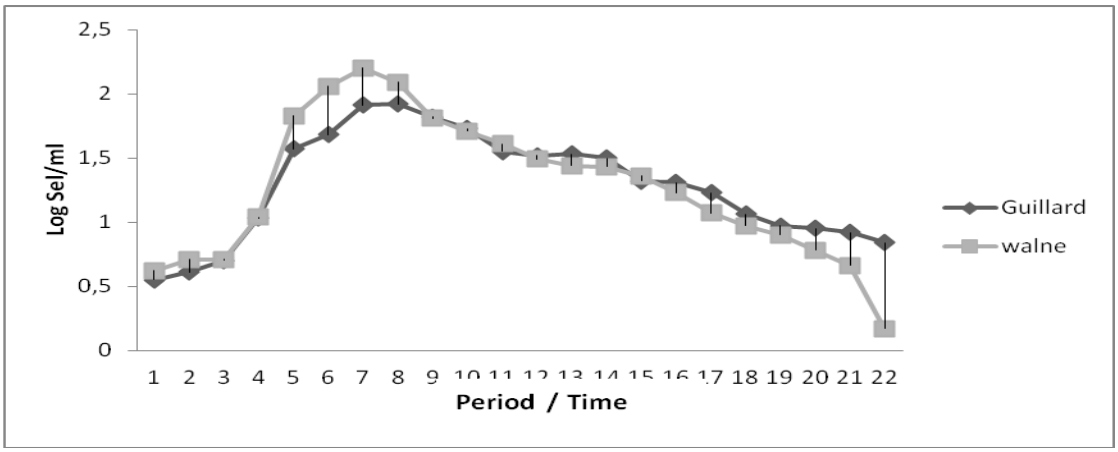

Fig.1. The growth of *Skeletonema* sp. in the Guillard and Walne culture media

Based on the **Fig 1**, it can be seen that *Skeletonema* sp. cultured with medium technical Guillard showed lag phase after 44 hours (observation to 6) with a cell density of 48.00 x 10^4sel/ml, then entered the exponential phase at 48. (observation to 7) with a cell density of 70.25 x 10^4 cells / ml while the stationary phase occurred in after hours to 52 (observation to 8) with a cell density of 86.75 x 10^4 cells / ml and death phase began at the 56 (observations to 9) with a cell density of 54.58 x 10^4 cells / ml.

Growth of *Skeletonema* sp cultured with culture medium technical Walne showed a similar pattern in the lag phase to 44 hours of observation (observation to 6 with the cell density is 117.17 x 10^4 cells/ml, exponential phase and stationary phase were detected from hour to 48 (observation to 7) with a cell density is 160.83 x 104 cells / ml. Later phases of death from an hour to 52 (observation to 8) with a cell density of 122.25 x 10^4 cells / ml. Then long cultivation or Skeletonema sp stationary phase in Guillard media over a period of 4 hours as to as technical Walne culture medium.

The use of technical media Walne 2 doses with the addition of silicate modification provided a more rapid growth. Rousch Research, (2003) explains that the use of two doses of technical Walne with the addition of silicate modification is better used on a laboratory scale culture.

Based on these results, *Skeletonema* sp. lag phase to exponential yield showed difference, this is because of differences in density, cell culture media with body fluids *Skeletonema* sp. The statement is in line with the statement Fogg, (1965) that the concentration of the liquid culture media affect diatom cells for growth. So that the difference in concentrations of the liquid cell culture medium in *Skeletonema* sp influential in restoring the enzyme and substrate concentrations for growth to the next level and the influx of nutrients within cells through the process of diffusion as a result of

ISSN : 1410-5217
Acrredited : 83/Dikti/Kep/2009

the concentration difference between the culture medium with body fluids.

The time difference in the growth phase kultifasi *Skeletonema* sp. with different technical culture media was highly significant, because it is in the culture medium *Skeletonema* Walne more rapid cell division process that cells added per time will be greater than the accretion time it self.

For water quality during the research are in stable condition. Salinity for growth at 28 ppt and the optimal temperature is 28-31^0 C. For the light intensity during the study were in the normal range of 1300 - 2500 lux. Brodie and Lewis, (2007), explained that the appropriate light intensity can help in improving the nutritional quality of the cultured diatoms. Furthermore, Laura and Paolo, (2006) states that the environment plays an important role in the growth and quality mendeterminasi algae cells. Diatoms can absorb nutrients from all walks of water, because it can absorb directly through the cell membrane.

Silicate is an important element in the formation of cell walls and shells for the diatoms (Herawati, 2008). Formation of the diatom cell wall serves as resistance to environmental. According to Siregar et al (2008) demonstrated diatomae characteristics specific to the cell wall sculpture consisting of silica, has a high resistance to environmental stresses. The more optimal utilization of silicate in diatoms, the better for the formation of the cell wall and it just requires a little diatomae silicates. As stated by Liao (1983), silicate is an important nutrient and is only used for culturing diatomae outside the framework for the formation and chlorophyll a would effectively utilized only in small amounts.

The nutrient composition affect the nutritional differences *Skeletonema* sp. especially in the formation of the protein (amino acids) and fats (fatty acids) in the culture medium Walne and technical Guillard is nitrogen and Fe. Nitrate as nitrogen source in the culture medium in transport directly to the cells in the presence of Cl rangasang ATPase, before having assimilated the amino acids combine to form a macromolecule or protein is what will convert fat into fatty acids by enzymatic reaction. This statement is in line with Agustini and Kabinawa, (2002) that nitrogen is makronutrient that can affect growth through metabolism particular protein biosynthesis, resulting in enzymatic reaction resulting protein can convert the fat into fatty acids.

In addition to differences in the composition of nitrogen Fe where the technical Guillard media more than the media technical Walne effect on protein and fat content of *Chaetoceros* and *Skeletonema* sp. Amsler, (2008) stated that FeCl3 (iron) have the ability to reduce nitrate to nitrite and then to reduce nitrite to ammonium. Ammonium is a nitrogen source. Nitrogen is the nutrient most needed for the growth of microalgae (Kaplan, *et al.*, 1986), it as an important element in the formation of chlorophyll a and protein.

The presence of boron in the technical Walne medium had no effect on cultured diatoms, boron more work to color pigmentation for phytoplankton. The statement in line according to the statement Round, (1973) that boron deficiency can cause algae cells lose pigment. So technically Walne media would be more appropriate and suitable for use in culture and *Chlorella* sp and *Spirullina* sp.

The analysis of the fatty acid profile of *Skeletonema* sp. can be seen in the figure below:

ISSN : 1410-5217
Acrredited : 83/Dikti/Kep/2009

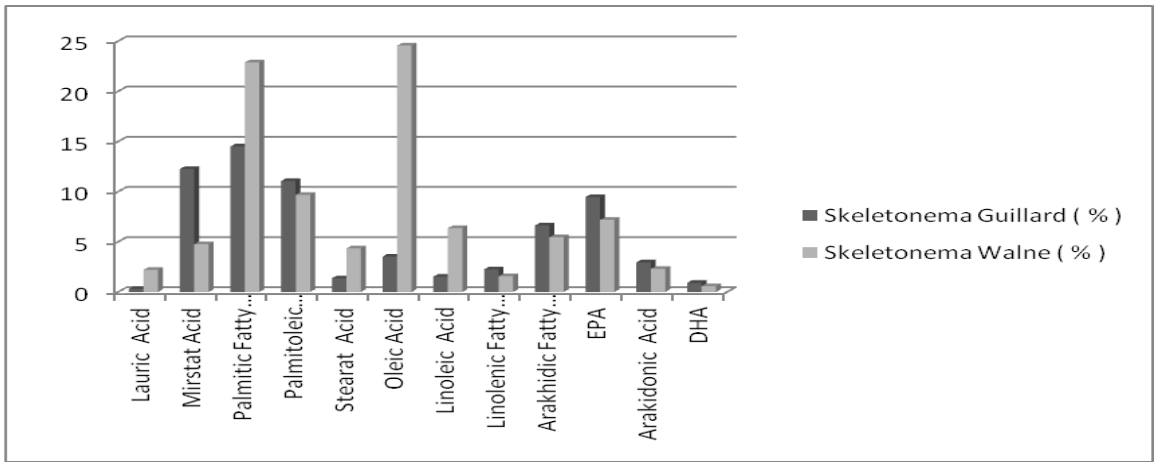

Fig. 2. Fatty Acids *Skeletonema* sp grown in technical culture media Walne and Guillard

From **Fig. 2**, it can be seen that 10 of 12 fatty acid composition in *Skeletonema* sp., were highest obtained on Guillard medium, so this medium is the best for obtaining high content of fatty acids. The following table of total fatty acid content of *Skeletonema* sp technically grown different in culture media:

Table 3. Fatty Acid Total Analysis of *Skeletonema* sp.

Sample	Profile of Methyyl Ester of Fatty Acids		Content (%)
Skeletonema Guillard medium	Lauric acid	C 12 : 0	2,2004 ± 0,005
	Mirstat acid	C 14 : 0	4,7639 ± 0,019
	Palmitic acid	C 16 : 0	22,8913 ± 0,039
	Palmitoleic acid	C 16 : 1	9,6696 ± 0,027
	Stearat acid	C 18 : 0	4,3479 ± 0,034
	Oleic acid	C 18 : 1	24,557 ± 0,045
	Linoleic acid	C 18 : 2	6,3725 ± 0,041
	Linolenic acid	C 18 : 3	1,575 ± 0,015
	Arachidic acid	C 20 : 0	5,4549 ± 0,015
	EPA	C 20 : 5	9,4669 ± 0,009
	Arachidonic acid	C 20 : 6	2,9478 ± 0,016
	DHA	C 22 : 6	0,9059 ± 0,007
Skeletonema Walne medium	Lauric acid	C 12 : 0	0,3157 ± 0,012
	Mirstat acid	C 14 : 0	12,2512 ± 0,037
	Palmitic acid	C 16 : 0	14,5087 ± 0,003
	Palmitoleic acid	C 16 : 1	11,0635 ± 0,039
	Stearat acid	C 18 : 0	1,3654 ± 0,019
	Oleic acid	C 18 : 1	3,5278 ± 0,043
	Linoleic acid	C 18 : 2	1,5302 ± 0,019
	Linolenic acid	C 18 : 3	1,5750 ± 0,011
	Arakhidic acid	C 20 : 0	5,4549 ± 0,016
	EPA	C 20 : 5	7,1858 ± 0,017
	Arachidonic acid	C 20 : 6	2,3067 ± 0,017
	DHA	C 22 : 6	0,5718 ± 0,019

ISSN : 1410-5217
Acrredited : 83/Dikti/Kep/2009

The dominated fatty acids were palmitic and oleic fatty acids. Palmitate is a substrate of SAFA and oleic fatty acid is the substrate of PUFA. Pratiwi, (2009) reported that Safa is the predominant fatty acid composition in all stages of growth. This is because the composition of fatty acids as characteristic of diatom Safa. According to reports Meyer, (2004) and essential fatty acids found in the analysis of total fatty acid composition is the MUFA is oleic fatty acid which is a substrate in the formation of long chains Pufa.

Fatty acids can be divided into 3 namely SAFA, MUFA and PUFA. Safa is composed of saturated fatty acid palmitic acid, fatty acids lauric and fatty acids mirstat. MUFA is monounsaturated fatty acids are fatty acids palmitoleat (usually will appear or come out at the end of the exponential phase or early stationary), while saturated fatty acids Pufa is not double consisting of fatty acids linoleic, linolenic fatty acids, EPA, DHA and arachidonic. Based on research conducted Rousch, (2003) that there are four fatty acids influence the growth of fish and shrimp larvae are fatty acids linoleic, linolenic fatty acids, EPA and DHA. In the opinion of Bufford et al (2004), the most important fatty acids in the growth of larval fish and shrimp are EPA and DHA.

CONCLUSIONS

The growth of *Skeletonema* sp. was markedly different between media Walne and technical Guillard. As for the culture medium technical Walne stationary phase start and end at the same time that observation to 7th (48 hours), while for the media Guillard early stationary observation to 7th (48 hours) and the stationary end to the observation of the 8th (52 hours). Judging from the content of fatty acids provide results that Guillard culture media better than the media technical Walne.

ACKNOWLEDGEMENTS

The author would like to thank Prof. Dr. Ocky Karna Radjasa who has provided input and suggestions during the research and preparation of this paper. Mr. Yunus, Mr. Sakur, Fhibia Jati, Ratna Widiastuti and Christine Yolanda Purba for technical assistance during the research. The research was partially funded by a ressearch grant from DIPA UNDIP, No. 0596/023-04.2-16/13/2012 December 9th 2012

REFERENCES

Arakidonat Acid From *Porphyridium creuntum* microalgae Agustini, N.W.S and Kabinawa, I. N. K. 2002. The Influence of Nitrate Concentration as a Nitrogen Resources AND Culture Media Towards The Forming. Biotechnology and Research Center LIPI. Bogor

Anderson R. 2005. Algal Culturing Tehniques. Aquaculture 154. 239-269 pp

Abdul gani, N., Zuhdi, A. and Sukresi. 2008. Potential *Skeletonema costatum* and Spirulina Microalgae as biodiesel feedstock. Journal Pen Aquatika 5. 15-21

Amsler. D. 2008. Alga Chemical Technology. Department of Biology University of Alabama at Birmingham. Aquaculture 185.105-116 pp

Araujo,S and V. Garcia. 2005. Growth and Biochemical Composition of the Diatom Chaetoceros cf. wighamii brightwell Under Different Temperature, Salinity and Carbon Dioxide Levels. 1. Protein, Carbohydrates and Lipids. Aquaculture, 246:405-412 pp

BBPBAP. 2007. Application Medium 'Walne' 2 Dosage and Preparation Techniques Media On Culture Chaetoceros sp Laboratotium Scale and Semi Bulk. Litkayasa Report. BBPBAP

_____, 2010. Pattern of Growth, Protein Content and Production Biomass Skeletonema sp At Various Levels of Gram. Litkayasa report. BBPBAP

Cahyaningsih, S., 2006. Natural Forage Production Technical Instructions. Department of Marine and Fisheries Directorate General of Aquaculture,

ISSN : 1410-5217
Acrredited : 83/Dikti/Kep/2009

Brackish Water Aquaculture Center Situbondo

Cuzon, Gerard et. al. 2004. Nutrition of Litopenaeus Vanamei reared in tanks or in ponds. Aquaculture : 235.119-123 pp

Directorate General of Aquaculture, 2008. Indonesia Aquaculture Statistics 2007. Department of Marine and Fisheries. Jakarta

Fabregas, Conception Herrero, Buenaventura Cabezas, Julio Abalde. 2008. Mass Culture and Biochemical Variability of The Marine Microalgae Tetraselmis suecica Fylin With High Nutrition Concentration. Aquaculture 115. : 127-139 pp

Gusrina. 2009. Fish Farming. Volume 3. Depdiknas

Herawati, V.E. 2008. Suitability Analysis of Aquatic Segara Anakan Cilacap As Shellfish Cultivation Land Totok (*Polymesoda Erosa*) Seen From Aspects of Primary Productivity Using Remote Sensing [Thesis]. Graduate Program Universitas Diponegoro. Semarang.

Kaplan, D., A. E. Richmond, Z. Dubinsky and S. Aaronson. 1986. Alga Nutrition. In : A. Richmond (Eds). CRC Handbook of Microalgal Mass Culture. CRC Press, Inc. Florida. p. 147-198

Koniyo, Yuniarti. 2010. Biology and Culture Method of Plankton For Natural Feeding Animals Water Larva. Faculty of Sciences. UNG. 3 (2): 1-6

Laura and Paolo. 2006. Effect of Nutrient and Light Limitation on the Biochemical of Phytoplankton. Aquaculture 68 : 145-156

Lavens, P and Sorgeloos. 1996. Manual on the production and use of live food for Aquaculture. FAO Rome, 47 pp

Liao IC, HM. Su, JH. Lin. 1983. Larvae Food for Penaid Prawns. In CRC and Book of Maricultur Vol. I. Crustacea Aquaculture (JP. McKey and JR. Moore,eds). pp 43 – 96

Malcolm R. Brown. 2002. Nutritional Value and Use of Microalgae in Aquaculture. Simposium Internasional de Nutrition Acuicola. Mexico

Meyer.A. 2004. Novel Fatty Acid Elongase and Their Use For The Lipid Reconstruction Of Docohexanoic Byosyntesis. Journal Lipid Research 5: 18999-1909

Pratiwi, Dahrul Syah, Linawati Hardjito, Liliana Goreti. 2009. Fatty Acid Syntesis by Indonesian Marine Diatome. Hayati Journal of Biosciencis. P 151-156

Rousch JM.Scott SE, Sommerfeld MR. 2003. Changes in Fatty Acids Profile of Intolerant Thermo and Tolerant Marine Diatoms During Temperature Stress. Journal Exp. Marine Biology 295: 145-156 pp

Suminto dan K. Hirayama. 1995. Effect on Bacterial Coexsistence on The Growth of Marine Diatom *Chaetoceros gracillis*. Fisheries Science 62 (1), 40-43

Susanto, A., E. Sutanti dan A. Basyar, 2007. Provision of plankton biomass for shrimp hatcheries and aquaculture. Reports test results. Center for Development of Brackish Water Aquaculture, Jepara

Yap Cy, Chen F. 2001. Polyunsaturated Fatty Acids Biological Significance Biosynthesis and Production by Microalgae Like Organism In Chen, Jiang Y. Algae and Their Biotechnology Potential. Netherland. Kluwer Acad Publ