

## The Proximate of Natural Foods *Gracilaria lichenoides* and *Ulva fasciata* for Abalone *Haliotis squamata* Culture

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### Abstract

The aim of this research was to analyse the proximate in natural foods *Gracilaria lichenoides* and *Ulva fasciata* for abalone *Haliotis squamata*. The proximate analysis included water, ash, protein, crude fibre, and lipid components. The proximate of *Ulva fasciata* were 82.0% of water, 25.7% of ash, 3.5% of protein, 4.8% of crude fibre, and 1.7% of lipid, while in *Gracilaria lichenoides* were 92.5% of water, 50.3% of ash, 2% of protein, 4.4% of crude fibre, and 1.2% of lipids. Natural food *Ulva fasciata* provides better result of growth of abalone due to the nutrients contained in it particularly the higher value of amino acids and fatty acids compared to natural food *Gracilaria lichenoides*.

**Keywords:** Proximate; Natural food; *Gracilaria lichenoides*; *Ulva fasciata*; Abalone

### Introduction

The utilization of marine resources is turned aside from fishing to aquaculture of late. Marine culture is concerning more to economics fish and oysters, while in Indonesia, one of the high economic commodity which can be developed is abalone (*Haliotis* spp.). The abalone culture is prospective to be developed for some benefits it has technically from the culturing to marketing.

The global production of abalone reached 22.600 metric tones (including poaching of 3700 metric tones) in 2002. Of this, over 8600 metric tones were farmed and the total value of the production was estimated as approximately US\$ 0.8 billion. China is the largest producer in the world with over 300 farms and a total production of approximately 4500 metric tonnes [1]. While South Africa has become the largest abalone producer outside Asia and over-exploitation of wild abalone stocks by poaching and high market prices have been the main drivers for its cultivation [2,3].

The development of abalone culture has stimulated research into abalone digestive physiology, the application of animal feed science principles to abalone, abalone feeding behaviour, on-farm alga product performance of different alga as feed, and the optimal use of both natural and formulated diets under intensive culture condition [4-7]. Some alternatives in culturing systems of abalone's culture development are still needed. For many culture systems have been developed whether the traditional or modern, so the technology transition is more focused to natural food feeding. The study of natural food feeding will be so important because the foods found to be the important factor and have to be noticed in the abalone culture of late.

Alga as abalone natural foods have been studied in some regions to find out the type of alga which influenced to abalone culture [6,7-14].

Types of natural food usually used in abalone culture are *Euclima cottonii*, *Gracilaria arcuata*, *Gracilaria gigas*, *Gracilaria solicornia*, *Gracilaria racillariopsis heteroclada*, and *Gracilaria verrucosa*. The type also used in abalone culture is *Ulva* spp. [15]. Those types have been tested and affected significantly to abalone's growth rate [16,17]. This research is aimed to analyze the proximate in natural food *Gracilaria lichenoides* and *Ulva fasciata* for abalone *Haliotis squamata* culture.

### Methods

#### Time and place

The research was conducted in three months, and it took place in Gondol, Bali and Bogor Agricultural Institute. The abalones were cultured in Gondol-Bali, and the natural foods used for abalone's feeding were analyzed in Bogor Agricultural Institute.

#### Tools and materials

The tools used in this research i.e., porcelain bowls, desiccators, oven, the Bunsen-burner, furnace, flask, Erlenmeyer flask, Kjeldahl flask, distillation apparatus, lipid beaker, Soxhlet tube. While the materials used are catalyst ( $K_2SO_4$  and  $CuSO_4$ ), concentrated  $H_2SO_4$ ,  $H_2O_2$ ,  $H_3BO_3$ ,  $Na_2(SO_4)_3$  (alkali), HCl, filter paper (lipid free), chloroform, aquadest, *Gracilaria lichenoides*, *Ulva fasciata*, and Abalone *Haliotis squamata*.

#### Sampling method

Sample of *Gracilaria lichenoides* and *Ulva fasciata* had cultured in aquarium (Figure 1). Both alga and abalone samples were collected from nature then being cultured in laboratory scale. The abalone used in this research sized 3-4 cm. The step was held to measure the alga's ability to live in culture condition which aimed to keep the foods continuity before fed to abalone. This step was divided in three treatments, i.e., 1 day culture, 3 days culture, and 5 days culture. The cleanliness and freshness were important factors that had to be noticed in this process to keep the quality. The result showed that the foods are still fresh in 5 days culture. The texture, colour, and aroma are not changed and still liked by the abalones.

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Received January 01, 2016; Accepted May 09, 2016; Published May 16, 2016

Citation: Latuihamallo M, Loupatty JW, Manuputty GD (2016) The Proximate of Natural Foods *Gracilaria lichenoides* and *Ulva fasciata* for Abalone *Haliotis squamata* Culture. Fish Aquac J 7: 171. doi:10.4172/2150-3508.1000171

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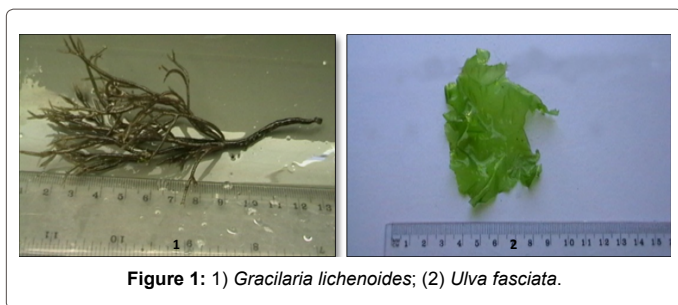


Figure 1: 1) *Gracilaria lichenoides*; 2) *Ulva fasciata*.

## Analysis method

Proximate analysis includes analysis of water, ash, protein, crude fibre, and lipid.

**Water:** Water was estimated based on the difference between weights of sample before and after desiccated. The porcelain bowl had been dried first at temperature of 105°C for about 1 hour and, then desiccated in desiccators for 30 minutes and scale up to constant weight (A). The sample was weighed about 2 grams (B) in the bowl and, then dried in oven at temperature range of 100-105°C for 5 hours or gain the constant weight. The bowl with sample was desiccated in desiccators for 30 minutes then scale up to constant weight (C) [18]. The water was calculated with formula:

$$\text{Water}(\%) = \frac{(A + B) - C}{B} \times 100\%$$

**Ash:** Ash was estimated by weighing the ash remaining as incineration result from organic matter at a temperature of 550°C. The porcelain bowl had been dried first at a temperature of 550°C for about 1 hour, then desiccate in desiccators for 30 minutes and scale up to constant weight (A). Sample weighed about 2 grams (B), put into the porcelain bowl and ignite with the Bunsen-burner until smokeless, then transfer to the furnace at a temperature of 550°C for about 12 hours. The bowl then chilled in desiccators for 30 minutes, and scale up to constant weight [18]. The ash was calculated with formula:

$$\text{Ash}(\%) = \frac{(A + B) - A}{B} \times 100\%$$

**Protein:** Protein was estimated by Micro Kjeldahl Method. 0.75 grams of sample put into Kjeldahl flask, then add 6.25 grams of  $K_2SO_4$  and 0.6625 grams of  $CuSO_4$  as catalyst. 15 ml of concentrated  $H_2SO_4$  and 3 ml of  $H_2O_2$  poured cautiously into the flask and put in acid chamber for 10 minutes.

The next step was destruction process at a temperature of 410°C for 2 hours or acquired the pure solution and, then poured 50-75 ml of aquadest after the temperature was normal.

Erlenmeyer contains of 25 ml solution, 4% of  $H_3BO_3$  with indicator (bromocherosol green 0.1% and mathyl red 0.1% (2:1) as distiller. Kjeldahl flask was affixed to the distillation tool, then add 50 ml of  $Na_2(SO_4)_3$  (alkali). The distillation was conducted and the distillat was collected into the Erlenmeyer until gain 150 ml of distillat volume (the distillat was green). The distillat was titrated excess HCl 0.2 N until the colour changed to grey. Natural form was made liked the sample steps [18]. The sample testing was duplicated. The protein was estimated by formula:

$$\text{Protein}(\%) = \frac{(A - B) \times \text{normality HCl} \times 14,007 \times 6.2}{W(g)} \times 100\%$$

where:

A=ml sample of HCl titration.

B=ml form of HCl titration.

**Lipid:** Lipid beaker dried in oven (105°C) is scaled up to constant weight (A). 2 grams of sample (C) covered with filter paper (lipid free) then put into soxhlet tube. 150 ml of chloroform poured into the lipid beaker. The sample is refluxed for 8 hours. If the solvent is pure-looking, it sign that all lipid have been extracted. Then the solvent in lipid beaker is evaporated to separate the solvent and the lipid. The lipid beaker then dried in oven at a temperature of 105°C for 30 minutes, and scale up to constant weight (B) [19]. The lipid is calculated with formula:

$$\text{Lipid}(\%) = \frac{B - A}{C} \times 100\%$$

**Crude fibre:** The crude fibre was estimated by calculating the remains (by difference):

$$\text{Crude fibre}(\%) = 100\% - [\text{water} + \text{protein} + \text{lipid} + \text{ash}]$$

## Results

### The Proximate of abalone's natural foods

The proximate analysis result in Table 1 shows some nutrition components from abalone food, i.e., Water, Ash, Protein, Crude Fibre, and Lipid. The table shows that water and ash have high percentage in both *Ulva fasciata* and *Gracilaria lichenoides*, 82.0% and 92.5%; 25.7% and 50.3%, respectively. Both the crude fibre and protein are 4.8% and 4.4%, 3.5%, and 2.0%, respectively, and the lowest value are in lipid component which 1.7% for *Ulva fasciata* and 1.2% for *Gracilaria lichenoides*.

### Amino acid profile of abalone's natural foods

The result of amino acid profile shows that the *Ulva fasciata*'s have higher compared to the *Gracilaria lichenoides* (Table 2). Some amino acids in *Ulva fasciata* that have high percentages are i.e., aspartic acid, alanine, and leucine, which valued 2.2%, 1.6%, 1.2%, respectively, while the amino acids that have low percentages are i.e., cysteine, histidine, methionine and tryptophan, which valued 0.3%. In *Gracilaria lichenoides*, aspartic acid and glutamic acid are very high (1.1%), then followed by proline and leucine (0.9% and 0.7%), while the lowest is cysteine (0.2%).

### Fatty acid profile of abalone's natural foods

Fatty acids analysis is varied to saturated fatty acids and unsaturated fatty acids. Both *Ulva fasciata* and *Gracilaria lichenoides* contain of 8 saturated fatty acids and 18 unsaturated fatty acids with different amount.

Table 3 shows that C16 is the highest percentage of saturated fatty acids in *Ulva fasciata* i.e., 51.3%, the followed by C22 and C18, 3.0%

No.	The Composition	Percentage (%)	
		<i>Ulva fasciata</i>	<i>Gracilaria lichenoides</i>
1.	Water	82	92.5
2.	Ash	25.7	50.3
3.	Protein	3.5	2
4.	Crude Fibre	4.8	4.4
5.	Lipid	1.7	1.2

Table 1: Chemical composition of Abalone's natural foods.

No.	Amino Acid	Percentage (%)	
		<i>Ulva fasciata</i>	<i>Gracilaria lichenoides</i>
1.	Alanine	1.6	0.6
2.	Arginine	1.3	0.6
3.	Aspartic Acid	2.2	1.0
4.	Cysteine	0.3	0.2
5.	Glutamic Acid	2.2	1.0
6.	Glycine	1.2	0.6
7.	Histidine*	0.3	0.1
8.	Isoleucine*	0.8	0.5
9.	Leucine*	1.2	0.7
10.	Lysine*	0.9	0.4
11.	Methionine*	0.3	0.2
12.	Phenylalanine*	1.0	0.5
13.	Proline	0.9	0.9
14.	Serine	1.1	0.6
15.	Threonine*	0.9	0.5
16.	Tryptophsn*	0.3	0.1
17.	Tyrosine*	0.6	0.3
18.	Valine*	1.1	0.5

\*=Essential amino acids.

Table 2: Amino acid Profile of Abalone's natural foods.

No.	Fatty Acid	Percentage (%)	
		<i>Ulva fasciata</i>	<i>Gracilaria lichenoides</i>
1	2	3	4
<b>Saturated Fatty Acid:</b>			
1.	C 14	1.2	1.7
2.	C 15	0.6	0.4
3.	C16	51.3	85.8
4.	C17	0.4	-
5.	C18	2.8	2.1
6.	C20	0.4	-
7.	C22	3.0	-
8.	C24	-	0.4
<b>Unsaturated Fatty Acid</b>			
1	2	3	4
9.	C16:1n7	6.5	0.8
10.	C17:1n7	0.9	-
11.	C18:1n9	5.5	2.6
12.	C18:1n7	10.7	1.6
13.	C18:2n6	9.1	2.5
14.	C18:3n3	3.5	-
15.	C18:4n3	1.8	-
16.	C20:1n11	0.3	-
17.	C20:1n9	-	-
18.	C20:1n7	-	-
19.	C20:2n6	-	-
20.	C20:4n6	0.6	2.1
21.	C20:3n3	-	-
22.	C20:5n3	0.3	-
23.	C22:1n9	-	-
24.	C22:1n7	-	-
25.	C22:4n6	0.3	-
26.	C22:5n3	0.9	-

Table 3: Fatty acid profile of Abalone's natural foods.

and 2.8%, respectively, while the lowest are C17 and C20 (0.4%). In *Gracilaria lichenoides*, C16 is the highest percentage of saturated fatty acids i.e., 85.8% (higher compared to C16 in *Ulva fasciata*). While C15 and C18 in *Ulva fasciata* are higher than in *Gracilaria lichenoides*.

The unsaturated fatty acids in *Ulva fasciata* shows that C18:1n7 has the highest percentage i.e., 10.7%, then followed by C18:2n6 and C16:1n7, 9.1% and 6.5%, respectively, while the lowest are C20:1n11, C20:5n3 and C22:4n6 (0.3%). In *Gracilaria lichenoides*, C18:1n9 is the highest percentage of unsaturated fatty acids i.e., 2.6%, then followed by C18:2n6 and C20:4n6, 2.5% and 2.1%, respectively. While the lowest percentage is C16:1n7 i.e., 0.8%. The percentages of unsaturated fatty acid between both foods also have differences which C18:1n7 in *Ulva fasciata* has higher percentage (10.7%) compared to C18:1n7 in *Gracilaria lichenoides* (1.6%). Also for C18:2n6, C16:1n7 and C18:1n9 which are 9.1%, 6.5% and 5.5%, respectively. While C20:4n6 in *Gracilaria lichenoides* is higher than C20:4n6 in *Ulva fasciata*.

## Discussion

### The proximate of abalone's natural foods

Quantitatively, the nutrition contained in both *Ulva fasciata* and *Gracilaria lichenoides* are insignificantly different particularly for the protein and lipid components, though are clearly different in ash component (Table 1). With 50.3% of ash contained in *Gracilaria lichenoides*, the algae become a better resource of mineral to abalone compared with *Ulva fasciata* which has 25.7%.

Table 1 shows that protein component of 3.5% in *Ulva fasciata* is higher than the component in *Gracilaria lichenoides* which is about 2.0%. Also, in lipid component, *Ulva fasciata* has higher percentage (1.7%) compared to the component in *Gracilaria lichenoides* which is about 1.2%. The differences are relatively caused by the water contained in both natural foods are high enough i.e., 82.0% in *Ulva fasciata* and 92.5% in *Gracilaria lichenoides*.

Based on the results, both foods can be assumed to be good as protein and lipid resources for abalone growth, quantitatively, but *Ulva fasciata* is better qualitatively. It caused by the protein and lipid component in *Ulva fasciata* is better than those components in *Gracilaria lichenoides*.

### Amino acid profile of abalone's natural foods

Essential amino acids are the amino acid which are deeply needed for growth of abalone and must invariably exist in abalone's foods because they can not be synthesized by abalone itself. The essential amino acids in *Ulva fasciata* are proved to be higher than the essential amino acids in *Gracilaria lichenoides* (Figure 2).

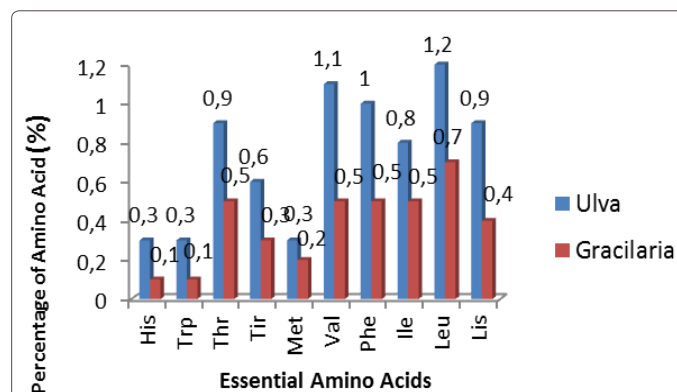


Figure 2: Essential amino acids contained in *Ulva fasciata* and *Gracilaria lichenoides*.

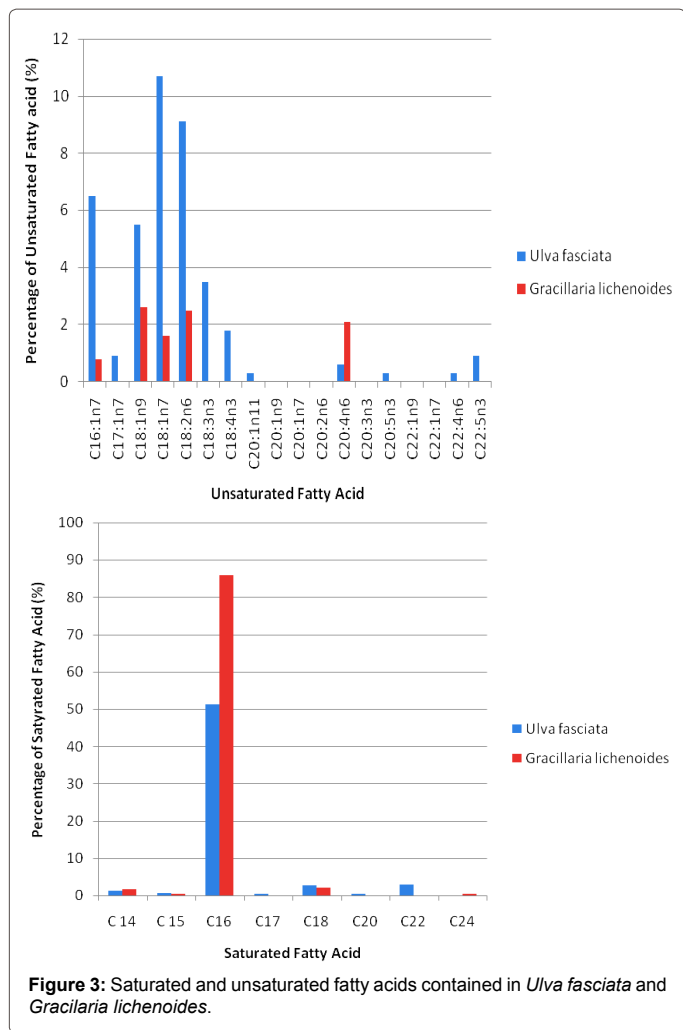


Figure 3: Saturated and unsaturated fatty acids contained in *Ulva fasciata* and *Gracilaria lichenoides*.

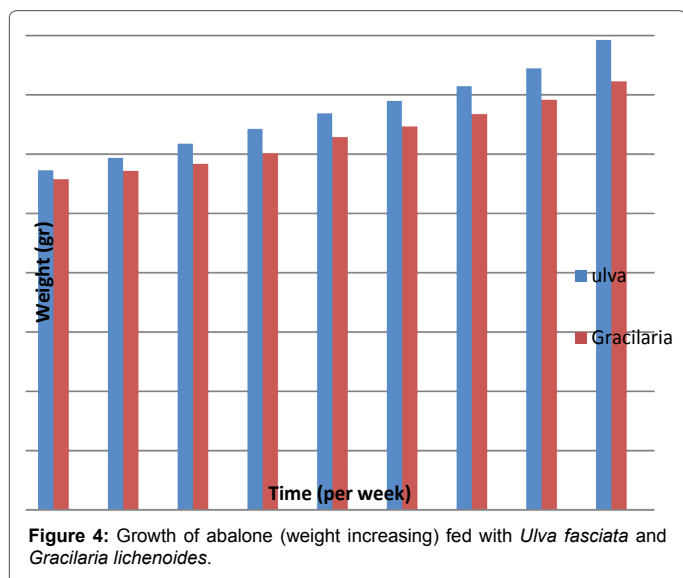


Figure 4: Growth of abalone (weight increasing) fed with *Ulva fasciata* and *Gracilaria lichenoides*.

### Fatty acid profile of abalone’s natural foods

The analysis shows that unsaturated fatty acids in *Ulva fasciata* are greater than in *Gracilaria lichenoides*, either quantitatively or

qualitatively. It is proved by the high percentage of unsaturated fatty acids particularly the potential fatty acids as Omega 3 resources liked linolenic acid (C18:3n3) and eicosa pentaenoic acid (EPA/C18:5n3) (Figure 3). Those fatty acids, particularly the linolenic, are the essential fatty acid, which influenced the growth of abalone.

### Growth of abalone

Natural foods *Ulva fasciata* and *Gracilaria lichenoides* are given to abalone as sample, and found that abalone fed with *Ulva fasciata* has higher growth compared to abalone fed with *Gracilaria lichenoides*. Figure 4 shows the graphic of weight increasing which used as growth indicator of abalone fed with *Ulva fasciata* and *Gracilaria lichenoides*. This result supported by higher analysis result of amino acids and fatty acids contained in *Ulva fasciata* which it provide better growth result to abalone.

As for most other cultured species, it has been shown that different abalone diets produce significantly different growth rates [11-14,20-23]. Abalones are generalist, opportunistic herbivores that readily accept a wide range of diets. In the wild, abalones prefer specific seaweeds, and a number of abalone species have been reported to favour red algae [8,9,24-27].

Natural foods usually used in abalone culture are *Eucheuma cottonii*, *Gracilaria arcuata*, *Gracilaria gigas*, *Gracilaria solicornia*, *Gracilaria racillariopsis heteroclada*, and *Gracilaria verrucosa*. Beside those types, *Ulva* sp. can also be as natural food for abalone [15]. This type has been tested and influenced the growth rate of abalone [16,17] and Sarita dan Effendy suggested that *Gracilaria verrucosa* is the most frequent used as natural food compared to others because contained of mineral and amino acid which giving the best growth rate for abalone [15,28]. Laboratory analysis result shows that the nutrition composition in *E. cottonii* contained 5.68% of protein and 6.22% of crude fibre, and *G. arcuata* contained 6.11% of protein and 14.80% of crude fibre. Cajipe suggested that *G. verrucosa* is composed of 13.85%-30.59% of protein based on the water it contained and also contained essential amino acids (Arginine, Histidine, Lysine, Methionine, Valine, and Phenylalanine) and non-essential amino acid (Alanine, Aspartic Acid, Cystine, Glutamic Acid, Glycine, and Serine) [28]. It means that *G. verrucosa* has the highest percentage of protein than others. Thus, it is a reason *G. verrucosa* giving faster growth of abalone gonad. Besides the protein component, the other reasons, which enables *G. verrucosa* provided the highest rate are the downy texture and finer size compared to other types, so the abalones are preferably consuming that type.

Naidoo tested the effects on growth various diets, including mixed diets consisting of kelp with combinations of both green alga *Ulva* spp. and the red alga *Gracilaria* spp [14]. These diets were also compared with animal-based Abfeed™. It was shown that dry kelp in any form (blades, stipes, and pellets) produced poor growth compared to various fresh alga treatment, including fresh kelp. The result showed that fresh kelp fortified with protein enrich *Gracilaria* and *Ulva* performs best. This growth trials support other studies [26,27] that have shown that mixed diets produce better growth rates than single-species diet.

### Conclusion

By the result, it could be concluded that natural food *Ulva fasciata* provides better result of growth of abalone due to the nutrients contained in it particularly the higher value of amino acids and fatty acids compared to natural food *Gracilaria lichenoides*.

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**Citation:** Latuihamallo M, Loupaty JW, Manuputty GD (2016) The Proximate of Natural Foods *Gracilaria lichenoides* and *Ulva fasciata* for Abalone *Haliotis squamata* Culture. Fish Aquac J 7: 171. doi:[10.4172/2150-3508.1000171](https://doi.org/10.4172/2150-3508.1000171)

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