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THE NUTRIENT AND STEROID CONTENT OF SOME DEEP SEA FISH SPECIES FROM WEST SUMATERA OCEAN

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ABSTRACT

The Purpose of this research is to observe the content of nutrient and steroid of some deep sea fish from west Sumatera ocean. The result of the research shows that 11 kinds of deep sea fish *such as: Dietmoides pauciradiatus, Benthodesmus tenuis, Beryx splendens, Haplosthethus craaaipinus, Hoplothethus* **sp,** *Ophidiidae, Ostracoberyu doryge, Godamus colletinis, Hyteroglype japonica* **contain protein 23,0-24,8 %, fat 1,9-4,1 %, carbohydrate 0-1,75 %, ash 1,7-2,4 %, water 70.1-72,1 %. In amino acid test, it can be identified 17 amino acid (9 essential amino acid and 8 non essential amino acid) Meanwhile in steroid test using Libermann Burchad, it can be identified 8 kinds of deep sea fish containing steroid and the greatest concentratton is in Dietmoides pauciradiatus, Benthodesmus tenuis,Beryx splendens, Haplusthethus craaaipinus.**

I. INTRODUCTION

Based on the total prediction of Indonesian fishery ocean potential which is amount to 6.6 million tons/year, consist of 5 million ton in Indonesian ocean and 2.1 million tons in Zee ocean. The potential prediction comes from some kinds of ocean fish, such as the small pelagic fish 3.5 tons, coral fish 0.048 million tons/year (Anonymous 2000).

According to the potential estimation, production and utilization of pelagic fish in Indonesia in 2001, Malaka Street and Java Sea are in the state of over fishing (BRKP 2001) therefore *it* needs to look for new fishing ground area instead of area in coastal area and pelagic area. Part of ocean environment which is predicted as alternative area is deep sea area.

Deep sea area is located under shining dept area in the open ocean and deeper than continental shelf (>200m). The habitat is the widest part in the world where seldom organisms live in; its water volume is predicted of amount up to 85% of 70% world surface (Nybakken 1992).

The seldom fishes however, are important food source and often looked for by some people in the markets. In Europe, deep sea fish (lung lip) is marketed as cusk eel. In New Zealand called Hung, South America called Cangrio and in Japan called Kingu. This fish is marketed by retail and seldom appears in restaurant, because of the good quality and unique meat texture. Gold king lip, red and black

are marketed internationally, but USA preferably the gold and red king lip (Perkins 1992). In Australia, deep sea tish (Beryx aptendens) was exploited, even occurred over fishing Anonymous (2004), in addition Soselia & Rustam (1993) reported that Cubiceps whiteleggi is one of important economical fish in the future. even thought in Indonesia deep sea fish is not utilized optimally yet.

The result of Baruna Jaya IV expedition leaded by The Agency for Marine and Fisheries. Research Ministry Marine Affairs and Fisheries showed about *529* kinds of deep sea fish. Some of those fishes are presumed to be containing aphrodisiac matter. Based on this information, the current work tried to analyze the content of bio substance of some fish collected from Indian Ocean of *west* Sumatra.

II. MATERIALS AND METHODS

Fish materials used in this work consisted of 11 species of deep sea fish, such as Dietmoides pauciradiatus, Benthodesmus tenuis, Beryx splendens, Haplosthethus craaaipinus, Hoplothethus sp, Hyteroglype japonica. The work is divided by 3 parts, as well; 1) proximate test, 2) Amino acid test and *3)* Steroid test.

1. Proximate Test

Proximate analysis was done including protein, fat, water, ash and carbohydrate.

Water level (Apriantono *et al.* 1989)

Porcelain cup was dried in temperature of 102-105 ^{0}C, for approximately 10-12 hours. Then cup was put in desiccators (± 30 minutes), measured (A grain), the cup was measured by homogenated sample (B gram), the amount 5 gram, placed in the cup was measured (C gram).

Measurement:

$$\% \text{ water content} = \frac{B-C}{B-A} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where: A = Cup (grain)
B = Cup and wet sample (gram)
C = Dried cup (grain)

Ask level

Ask level was analyzed according to Apriantono *et al.* (1989). Porcelain cup was burned in *650* ^{0}C for 1 hour, after the temperature fallen about 200 ^{0}C. The cup was cold in desiccators for 30 minutes and measured (A gram). Then the

cup was measured by homogenate sample (B gram), at 5 gram. The cup and the sample are put in fireplace under temperature increasing gradually until 650 "C. The white ask produced was get out. After that. the fireplace was fallen in 200 °C. The cup was cold for 30 minutes and measured the weight (C **gram).**

$$\% \text{ Ask} = \frac{\text{C-A}}{\text{B-A}} \text{ a } 100\% \quad \text{.....} \quad (2)$$

Where; A = Cup (gram)
B = Cup and wet sample (gram)
C = Dried cup (gram)

Protein level

Protein level was analyzed according to Apriantono *et al.* (1989)

a. Destruction

The sample was measured at 0.3 gram and put in a kjeltec tube. A kjeltec tablet was put in the tube; the tablet was used to catalisator.

The reaction:

$$\text{Organic nitrogen } H_2SO4 \xrightarrow[\text{Catalyst}]{} CO_2+H_2O+(NH_4)_2SO_4 \quad \text{.....} \quad (3)$$

Mercury (Hg); Ag and Selenium (Sc) were usually used to catalyst matter kjeltec tablet consist of K_2SO_4 and Se, after that destruction was done until the color of liquid changes to clear.

b. Distillation

The cup of result of destruction was cold and put to the filter cup, than it was diluted with 200 ml of water, not contain nitrogen, added sotne boiled stones and 100 ml of NaOH in order rhat the solution became base. The filter cup was put above filter fast. The process went on until nitrogen was caught by H_2SO_4, which is in the erlenmeyer or if 2/3 part of the legend in the cup steamed.

HCl was added to a buret, done titration until the color of liquid in erlenmeyer changes to reddish, noted the volume of HCI

$$\% \text{ N} = \frac{14.01 \text{ x (A-B) x C}}{\text{D}} \quad \text{.....} \quad (4)$$

% Protein = % Nitrogen x conversion factor (6.25).

Where A = titration (ml) C = Molarities of standard acid
B = Blanko titration (ml) D = Sample (mg)

Fat level

Fat level was analyzed according to Apriantono *et al.* (1989). The sample was measured at 3 gram (W1), than covered with filter paper in the up part while down part was given free fat cotton then prepared fat cup which was know the weight (W2) and attached with soxhlet tube. The tube was put in the extractor of soxhlet tube, then sprayed with fat dissolved (petroleum benzene), after that extraction was done for 16 hours in 40 ^{0}C. After the extraction had fished, the tube brought out. The fat dissolver, who is in fat cup, was distillated until all of the fat dissolver steamed. and then the fat cup was dried in oven at 105 ^{0}C for 3-5 hours. The cold fat cup was measured in desiccators until constant weight (W3).

$$\% \text{ Fat} = \frac{W3-W2}{W1} \times 100\ \% \quad \text{.......... (5)}$$

Carbohydrate level

Carbohydrate level was analyzed according to Winamo, (1992) Carbohydrate level was reached by calculating the difference of 4 components water level, protein, fat and ask. The measurement is;

% Carbohydrate = 100% - (%Water + %Fat + %Protein +%Ask)

2. Amino and Analyses

Amino acid level was analyzed according to Adijuana (1984). The composition of amino acid was examined by using HPLC. The kind of HPLC used in this research is HPLC water with the principle of amino acid separation based on acid base, moreover it used pico tag amino acids water color. The process used Na-acetate: asetonitril (60:40) as movement phase and trimetil xylena as quite phase. The completing explanations are:

a. Acid hydrolysis

The sample at ± 0.25 gr was measured in closed tube reaction then added 5 ml HCl 6N and blown by N2 gas and closed. After that, the sample was put in oven at 100 ^{0}C for 18-24 hours, the liquid sample was filtered by using fitter paper.

b. Drying

Sample liquid, resulted for hydrolysis process, was taken at 10 1 to reaction tube and added at 30 I drying liquid (methanol: Na-acetate : tri etil acetate = 2:2:1). After that, the matter by vacuum pump having pressure 50 torr (3x)

c. Derivatisation

Derivat **liquid** (metanol: trimetil asetat. penilisati asianat = 7:1:1). The dried sample was added at 30 µl, and then it **was ignored** for about **20** minutes, dried by vacuum pump, 50 torr pressure. After that, the dried sample **was** diluted **by** 200 µl **diluter** liquid (Na-acetate 1 M) and gained **satnple** liquid **which is** ready to be analyzed.

d. Amino Acid analysis by HPLC

l'he condition of HPLC when analysis occurred **is:**

1. **Color** temperature : 38°C
2. Color : pico tag 3.9 x 150
3. **Water speed** : **1.5** ml/minutes
4. Pressure . *3000* **psi**
5. Program : Gradient
6. Movement face : - Asetonitril 60%
   - **Buffer N**
7. **Detector** ; **UV**
8. **The length of** wave : 254 nm

The percentage of amino acid in 100 grams deep sea **can be** measured **by**

$$\% \text{ Amino acid} = \frac{\text{The width of sample area} \times \text{standard concentration} \times 5\text{ml} \times \text{BMA} \times 100}{\text{Standard area} \times \text{weight sample}} \quad \ldots\ldots (6)$$

BMA = the weight of amino acid **molecule**

3. Steroid Hormone Test

Steroid extraction **was** done based on a method, reported by Touchtone and Kasparov (1970) referred **by** Riris (1994). The amount of deep sea at **20** grams. homogenized by **blender was added by 45 ml of** cold acetone, and then saved for 24 hours in cold room at 40 "C, centrifuged in 500 rpm for 10 minutes. **The** obtained deposit **was separated frotn the liquid phase.** The **liquid** phase **was** steamed in **water** heater **at** 40 °C. The obtained **residue** was estated **2 times in etil acetate,** chloroform and **water** (1:1:1) **by** using separation tube so at makes 2 layers, extraction liquid solution down **layer** is chloroform and up **layer is etil** acetate **was steamed in water heater at 4 °C until** dry. The extract **was used** to identify steroid.

Steroid identification was done by Liebennan Burchard **Test-Addition** of some acetate anhydrate acid and 0,5 **ml** chloroform **to a** little extract of deep sea-then stirred it was added a drop of sulfate. The green color showed that the **extract contained steroid** (Cook (1958) **referred** by Riris (1**994).**

III. RESULTS AND DISCUSSION

1. Proximate Test

Result of pmxitnate analysis of deep sea shows that there are differences of nutrient for each deep sea fish. It depends on kind at biota species, age and the conduction at living area (Zaitsev *et al.* 1969 cited by Septarina 1999).

Table 1. The result of proximate analysis

No	Sample code	Analysis Result				
		Water	Ash	Fat	Protein	Carbohidrate
1	A1	70,4	1,9	2,9	23,1	1,7
2	A2	70,9	2,0	2,6	24,5	0
3	A3	72,0	1,9	2,1	23,0	1
4	A4	70,4	2,1	4,1	23,4	0
5	A5	70,1	2,4	2,7	24,8	0
6	A6	71,2	2,1	2,9	23,2	0
7	A7	70,6	2,2	3,6	23,6	0
8	A9	71,9	1,7	2,1	23,1	1,2
9	A10	72,1	2,1	1,9	23,4	0,5
10	A11	72,1	2,2	2,4	23,3	0

Explanations.

A1 : Dietmoides pauciradiatus
A2 : Benthodesmus tenuis
A3 : Beryx splendens
A4 : Hoplosthethus crassipinus
A5 : Hoplothethus sp
Ah : Ophidiidae
A7 : Ostracoberyu dorygenis
A9 : Godamus colleti
A10 : Myctophidae sp
A11 : Hyteroglypne japonica

Protein level

Figure 1 shows that the content of deep sea fish's protein varies from 23.0 to 24.8 %. Based on Stanby & Olcott 1963 cited by Santoso 1998, deep sea fish's protein is in high level at protein and low at fat, it comparing with pelagic fish, the content of deep sea fish's protein is higher than that at pelagic fish,

It shows in Table 2 that Holoplothetus crassipinus contain the highest at protein of 27.4% compared with other researched deep sea fish.

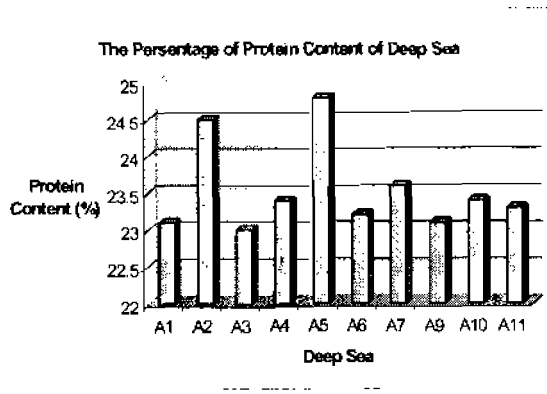

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Figure1. Persentage Histogram **of Deep Sea protein**

Table 2. Composition nutrient some **pelagic fish** in 100 g **RDD**

Name of Fish	**Protein Content(%)**	**Fat** Content (%)	Ash Content (%)	**Water** Content (%)	Carbohidrate Content (%)
Lemuru [a]	20.00	3.00	1.00	76.00	**0**
Teri [a]	**16.00**	1.00	**3.00**	80.00	***0***
	19.90	2.70	**1.20**	76.20	***0***
Ten iiri	**18.50**	2.70	**1.40**	77.40	5.13
Tuna [c]	**22.00**	**1.01**	**1.30**	70.56	0.27
Hiu [c]	**20.98**	**4.51**	**1.39**	72.85	0

Sources : a. **Hardiansyah and D** Briawan (1994)
b. FAO (1972) c. Riana **(2000)**

Fat level

Fat level of some deep **sea** fish **was** in average of 1.9-4.1%, **it is relatively** higher than **that of** both **pelagic** fish and **fresh** water fish. However **this difference in fat level is not cleary significant since fat level of deep** sea fish was **categorized low** one (Stanby & Olcott 1**963).**

The content of deep sea fish`s fat level, was shown in figure 2.

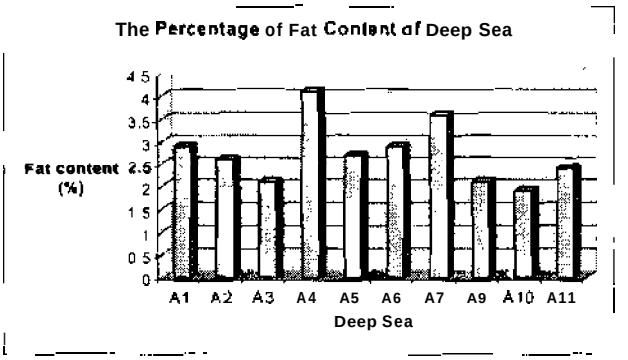

Figure 2. Histogram of **percentage** of **deep sea fish's fat level**

Water level

Deep sea **has** high **salinity with** value 31.2 $^{0}/_{00}$ (Karleskint, 1998). **The** high **rate of inorganic salt** cause hypertonic. **To** maintain **the condition of body** is isotonic **deep** sea organism **enters solution** to their **body for adapting. The condition causes water** level **of** deep sea **fish is less** than **of** fresh water fish and **pelagic** fish.

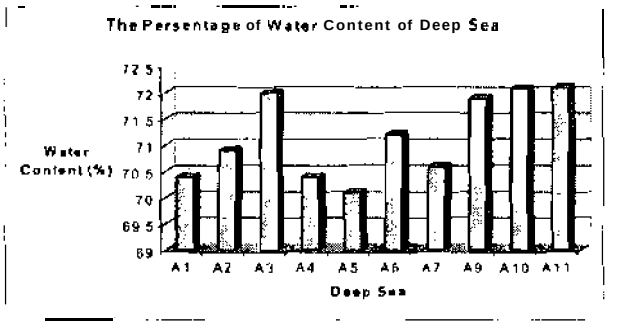

Figure 3. Histogram **of percentage of deep sea fish's** water level

2. Amino Acid Test

Table 3. Amino acid content (%) **of** deep **Sea Fish**

NO	Analisis Result	Kode Sample									
		A1	A2	A3	A4	A5	A6	A7	A9	A10	A11
1	Aspartat Acid	0,308	0,526	0,348	0,308	0,449	0,467	0,501	0,320	0,289	0,649
2	Glutamat Acid	0,530	0,650	0,692	0,530	0,678	0,795	0,745	0,662	0,515	0,815
3	Serin	0,184	0,208	0,220	0,184	0,339	0,310	0,316	0,284	0,232	0,416
4	Glisin	0,130	0,108	0,150	0,130	0,121	0,171	0,166	0,168	0,156	0,309
5	Histidin	0,146	0,170	0,232	0,146	0,126	0,158	0,158	0,160	0,182	0,284
6	Arginin	0,532	0,470	0,572	0,532	0,461	0,647	0,495	0,508	0,525	0,780
7	Threonin	0,360	0,254	0,328	0,360	0,273	0,247	0,304	0,336	0,241	0,503
8	Alanin	0,380	0,456	0,534	0,380	0,376	0,339	0,355	0,462	0,359	0,512
9	Prolin	0,604	0,408	0,500	0,604	0,506	0,478	0,415	0,574	0,431	0,579
10	Tirosin	0,270	0,384	0,680	0,270	0,550	0,495	0,471	0,518	0,568	0,474
11	Valin	0,420	0,388	0,414	0,420	0,261	0,603	0,363	0,412	0,570	0,534
12	Methionin	0,172	0,212	0,194	0,172	0,175	0,191	0,288	0,186	0,302	0,298
13	Sistein	0,250	0,194	0,238	0,250	0,213	0,494	0,249	0,244	0,152	0,192
14	Isoleusin	0,278	0,220	0,278	0,278	0,245	0,201	0,431	0,300	0,235	0,776
15	Leusin	0,680	0,960	1,068	0,680	1,036	1,174	0,856	0,970	0,812	1,109
16	Fenilalanin	0,260	0,968	0,924	0,260	0,730	0,630	0,437	1,032	0,613	0,899
17	Lisin	0,230	0,360	0,248	0,230	0,161	0,250	0,125	0,236	0,217	0,449

note:
A1 : Dietmoides pauciradiatus
A2 : Benthodesmus tenuis
A3 : Beryx splendens
A4 : Hoplosthethus crassipinus
A5 : Hoplothethus sp
A6 : Ophidiidae
A7 : Ostracoberyu dorygenis
A9 : Godamus colleti
A10 : Myctophidae sp
A11 : Hyteroglypne japonica

Marine fish have 17 important amino acids which are required by human body. Nine of those are essential amino acid and the other non essential amino acids. Both Dietinoides pauciradiatus and Hoplothethus crassipinus contain the greatest quantity of atnino acid in leusin and prolin. Benthoides tenuis and Beryx splanden have the highest content of amino acids in leusin and phenilalanin. Mean while, Holoplethus sp, Myctophidae sp and Hyteroglypne japonica have the highest content of amino acid in leusin and phenilalanin. Ophidiidac and Ostracoberyu dorygenis have highest in glutamat and leusin.

From all of deep sea fish analyzed, it seems that leusin is dominant in the quantity among all kinds of ainino acid found in those fish. Leusin is an essential amino acid and include in hatogenic, producing ketone in hearth. Other amino acids included this category is lisin and tripthopan (Lehninger 1994). The function of this amino acid in as important biochemical component, needed by body - to **produced** energy, then to stimulate up part of brain and keep body for reflection http://www.realtime.net/anr/aminoacd.html.).

Arginin having role as aprodisiach was in great quantity among some deep sea fish, moreover it has potential as material of strong medicine Arginin related with Nitrogen oxidize (NO) enzyme, having role in width of blood.

Ostracoberyu dorygenis and family of ophidiidae were dominated by glutamat acid. Glutamat acid gives mild flavors and delicious smelt, the same as cod, but it was sweater since glutamate acid is a amino acid, change to glucose and glycogen by metabolism current called glucogenic (Lehninger,1994).

Other ainino acid included alanin, arginin, asparagin, aspartat acid, sistein, glutamin, glisin, Prolin dominated in Dietmoides pauciradiatus and Hyteroglypne japonica. They are glocogenic ainino acid.

The kinds of amino acids which are combination of ketogenic and glucogenic arc fenilalanin and tirosisn. The domination of fenilalanin was dominate in Benthodesmus tenuis,Godamus colleti Beryx splendens, Myctophidae sp, Hoplothethus sp, Hyteroglypne japonica.

3. Steroid Hormone Test

Table 3. Steroid hormone composition

NO	Fish Name	Test Result (Etil Asetat)	Test Result (Chloroform)
1	Dietmoides pauciradiatus	(-)	(+)(+)
2	Benthodesmus tenuis	(-)	(+)(+)
3	Beryx splendens	(-)	(+)
4	Hoplosthethus crassipinus	(-)	(+)(+)
5	Hoplothethus sp	(-)	(+)
6	Ophidiidae	(-)	(+)
7	Ostracoberyu dorygenis	(-)	(+)
8	Godamus colleti	(-)	(+)

The result of steroid hormone analysis
(+): There is steroid hormone in deep sea fish
(-): There isn't steroid honnone in deep sea fish

Steroid hortnonr is hormone containing steroid nucleolus. Aphrodisiac is matter enlarging libido. Steroid hormone is one of the matters having function as aphrodisiac.

Liebennan Burchard Test is used to analyze cholesterol in which sample was deleted in chloroform and mixed by sulfate acid and acetate glacial. The positive result was shown by the changing of the color from red to violet, blue to green in some minutes. This test was not specific for cholesterol, but for other stcrol such as stignasterol and ergo sterol which will give positive respond (Dence, 1980).

The result on the table shows that deep sea fishes analysis has steroid content. It was seen in Liebennan Burchard Test, extract of isolation using chloroforrn gave positive result, changing extract's color to green. and ergosterol. The color of Fish giving great effect was shown in Dietmoides pauciradiatus, Benthodesmus tenuis and Hoplosthethus crassipinus. The changing extract's color to green shows the existence of sterol-stigma sterol organic indicate the existence of two or more double chain of carbon. While extract isolated by using ethyl acetate did not show the changing of color.

Steroid is white solid crystal. Steroid has some shapes such as little needle, leaf, plate and amorf. It depends on kind of solution used in crystallization, skill and the luck of chemistry expert. Since steroid has 17 or more carbon atoms, so this component tend to undeleted in water (Wilson & Gisvold, 1982). Ethyl acetate mixed water has possibility the steroid which is not restricted, caused the polarity of ethyl acetate is higher than chlorofonn, including non polar.

The color of mix which does not change is predicted by the existence of saturated steroid i.e. chonestanol. It is caused the saturated steroid i.e. chonestanol shows negative result toward Liebernenn Burchad test (Dence,1980).

The peak which easy to be observed is 6 conjugated ketone ring in 1670 cm^{-1}, asetilenik C-H close to *3000* cm^{-1}. From the infra red (IR) analyze, it shows that doubled C=C chain appears as the weak peak which close to 2000 cm^{-1} and vibration C=C is the peak of medium intensity above 1600 cm^{-1}. The confusion between carbonyl peak and siklohexsane peak in 1670 cm^{-1} seldom occur, it is caused the carbonyl having one of the strongest spectrum. The strong chain of 1250 cm^{-1} gives respond to C-O from D ring relation.

From the infra red analyze, it can be see that Dietmoides pauciradiatus, Benthodesmus tenuis, and Hoplosthethus crassipinus show positive result toward IR test. All of the peak are expected showing nearness to the existence of steroid. Meanwhile Beryx splendens, Hoplothethus sp, Ophidiidae, Ostracoberyu doryge Godamus colleti nis, show negative result to the existence of siklohexsane. It shows the positive correlation between Liebermann Rurchad test and Infra red spectrometry test. The strongest existence of steroid is in Dietmoides pauciradiatus, Benthodesmus tenuis and Hoplosthethus crassipinus.

IV. CONCLUSION

Some deep sea fish content proximate, protein between 23,0 and 24,8%. It was higher than pelagic fish. The highest content is in Hoplothethus sp, at 27.4%. In amino acid test, deep sea fish had 17 amino acids, 9 of which essential amino acid and the others were non essential amino acid. Leusin and arginin dominated in the quantity some deep sea fish. Meanwhile steroid hormone test using

Liebermen **Burchard, 8** kinds **of** deep sea fish content steroid and the greatest concentration is in Dietmoides pauciradiatus and Hoplosthethus **crassipinus**

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