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Age Variation in Extractive Nitrogenous Constituents of the Cultured Ascidian, *Halocynthia roretzi* Muscle*

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양식산 우렁쉥이, *Halocynthia roretzi* 함질소 엑스성분의 연령차

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ABSTRACT

The muscle extracts of the ascidian, *Halocynthia roretzi* cultured for two and three years old on the southern coast near Chungmu and the eastern coast near Pohang of Korea, were analyzed for extractive nitrogen (EN), free amino acids (FAA), combined amino acids (CAA), nucleotides and related compounds (NRC), quaternary ammonium bases and guanidino compounds using specimens collected in February 1989 and in April 1989, and compared for those contents with each other.

As for the amount of EN, no remarkable difference was found between two- and three-year-old samples collected at St. 1 in the spring and winter seasons, while at St. 2 in the spring season the two-year-old sample was distinctly lower than the three-year-old one.

Taurine, proline, glutamic acid, glycine and alanine were the major FAA in every sample. The amount of taurine, the most prominent FAA, was higher in three-year-old sample than in two-year-old one regardless of sampling station and season. Most of the other major FAA showed a similar tendency to EN at both sampling stations in both seasons.

Adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-monophosphate (IMP), inosine (Ino) and hypoxanthine (Hyp) were detected in all the samples and ATP, ADP and AMP were the major ingredients. The amounts of total NRC were in parallel with those of EN and total FAA.

As for the contents of betaines, two- and three-year-old samples collected in the winter season exhibited a great discrepancy each other, the former being clearly lower than the latter, but no remarkable difference was observed between two samples of two groups in the spring season.

In proximate composition of the muscles, the two-year-old sample was considerably higher in moisture content and lower in protein and glycogen contents than the three-year-

* Studies on the Extractive Components of Ascidians in Korea-IV

old one at St. 2 in the spring season.

The large discrepancies observed between two- and three-year-old samples from St. 2 seems to be attributable to the difference in size of samples rather than to the difference in age.

요 약

양식산 우렁쉥이, *Halocynthia roretzi*의 함질소 엑스성분에 대한 연령차를 검토하기 위하여 양식기간이 서로다른 2년생과 3년생 시료를 1989년 2월과 4월에 남해안의 충무근해와 1989년 4월에 동해안의 포항근해에서 각각 채취하여 근육의 엑스분질소 (EN), 유리아미노산 (FAA), 결합아미노산 (CAA), 핵산관련물질 (NRC), 4급암모늄염기, 구아니시노화합물 등을 분석. 비교하였다.

EN 함량에서 보면 남해안의 겨울과 봄철시료는 연령차가 적었으나, 동해안의 봄철시료에서는 큰차를 보여 지역에 따라 서로다른 결과였다.

FAA는 2년생과 3년생에서 모두 taurine, proline, glutamic acid, glycine, alanine이 주성분으로, 남해안의 겨울과 봄철 시료에서는 연령차가 적었으나 동해안에서는 봄철에 뚜렷한 차이를 보였다. FAA중 함량이 가장 높았던 taurine은 지역이나 계절에 관계없이 연령차가 현저하여 모든 시료에서 2년생 보다 3년생이 높았다.

NRC에서는 2년생과 3년생 모두 ATP, ADP, AMP, IMP, Ino 및 Hyp이 검출되었고, 그 중 ATP, ADP, AMP가 대부분을 차지하였다. NRC의 연령차는 EN, FAA에서와 같은 경향으로서 지역에 따른 차이가 현저하였다.

Betaine함량은 계절변동이 심하여 겨울 시료에서는 연령차가 컸으나 봄철에는 양 해안에서 모두 큰 차이가 없어서, EN, FAA, NRC에서와는 서로다는 결과를 보였다.

일반성분 조성은 동해안에서 2년생이 3년생보다 수분함량은 높은 반면, 단백질과 glycogen 함량은 훨씬 낮았다.

이와같이 동해안의 2년생과 3년생 시료에서 각 성분의 함량간에 특히 큰차이를 나타낸 것은 양식기간의 상이에 따른 연령차 라기 보다는 지역에 따른 성장차에 기인한 것으로 생각된다.

INTRODUCTION

In the previous papers of this series I and my colleague reported the composition and seasonal variation of extractive nitrogenous constituents in some tissues of the cultured ascidian, *Halocynthia roretzi* (Park *et al.* 1990), the composition of extractive nitrogenous constituents of two edible ascidians, *Styela clava* and *S. plicata* (Park *et al.* 1991a), and the regional variation of extractive nitrogenous constituents in the ascidian, *H. roretzi* muscle (Park *et al.* 1991b).

H. roretzi, the most important species of edible ascidian in Korea, is usually marketed after being cultured for more than one and a half years. Although ascidians of different ages have not received different evaluations as to acceptability, it should be necessary from the food chemical and biochemical viewpoints to examine the effects of age on extractive components as well as proximate composition of the muscle, because the age of aquatic organisms is one of the factors affecting those components (Konosu and Yamaguchi 1982, 1985 ; Suyama and Konosu 1987). For these reasons, the author attempted the present study, in which cultured ascidians of two and three years old* were collected at the southern coast near Chungmu in February(winter) and April(spring) and at the eastern coast near Pohang in April(spring), and the muscles were analyzed for proximate composition and for extractive nitrogen(EN), free and combined amino acids(FAA and CAA), nucleotides and related compounds(NRC), betaines, trimethylamine oxide (TMAO), trimethylamine(TMA), creatine and creatinine as those described in the previous paper (Park *et al.* 1990).

MATERIALS AND METHODS

Samples : The biological data of ascidian samples used are summarized in Table 1. Both of two- and three-year-old samples were collected twice in February 1989 and in April 1989 at a culture farm in front of Youngwoon-ri (St. 1) near Chungmu, and once in April 1989 at a culture farm in front of Shinchang-ri (St. 2) near Pohang (Fig. 1).

The two- and three-year-old samples were settled on the ropes in December 1987 and in December 1986, respectively and cultured thereafter at the respective farms. All samples were collected at the depth of 5m. For each collection the muscles of 10 or 30 individuals were pooled and subjected to analyses.

Analytical Methods : The methods for proximate analysis, preparation of extracts and determinations of nitrogenous components were the same as those described in the previous paper (Park *et al.* 1990).

Table 1. Samples of *H. roretzi*

Sample*1	Sampling Station*2	Sampling date	*n*	Weight (g)*3	Width (cm)*3	Height (cm)*3
A-2	St.1	Feb.23, 1989	10	124.8± 21.0	5.3± 0.7	8.4± 0.4
A-3	St.1	Feb.23, 1989	30	200.1± 36.6	6.2± 0.6	9.7± 1.0
B-2	St.1	Apr.20, 1989	10	161.0± 18.8	5.8± 0.4	9.5± 0.7
B-3	St.1	Apr.20, 1989	30	197.7± 33.9	6.2± 0.6	9.5± 0.8
C-2	St.2	Apr.22, 1989	10	11.6± 1.4	2.5± 0.2	3.5± 0.3
C-3	St.2	Apr.22, 1989	10	163.9± 14.1	5.8± 0.4	8.9± 1.0

*1 The figures, 2 and 3, indicate the ages of samples expressed according to the commercial practise.

*2 See Fig. 1.

*3 Average± standard deviation.

* According to the commercial practice in Korea, the ascidian *H. roretzi* cultured over two and three winters are designated as "two- and three-year-old samples", respectively.

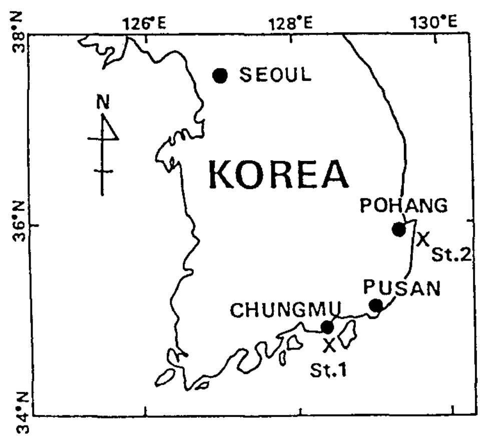

Fig. 1. Map showing two sampling stations.
St. 1, Youngwoon-ri of the southern coast ;
St. 2, Shinchang-ri of the eastern coast.

RESULTS AND DISCUSSION

1. Proximate Composition

When compared the proximate composition of the muscle between two- and three-year-old samples collected at St. 1, little difference was observed between A-2 and A-3 in the winter season, and between B-2 and B-3 in the spring season (Table 2). On the other hand, the proximate composition of the muscle between two samples, C-2 and C-3, collected at St. 2 in the spring season varied considerably ; the former was clearly higher in moisture content (92.5% versus 84.3%), and remarkably lower in protein (1.9% versus 8.2%) and glycogen (1.9% versus 4.0%) contents. These differences between C-2 and C-3 may be ascribed to the extremely small size of the former sample.

Table 2. Age variation in proximate composition of the muscle of *H. roretzi* (%)

Sample*	Moisture	Protein	Lipid	Ash	Glycogen
A-2	83.4	7.9	1.5	2.7	4.0
A-3	86.0	6.4	0.9	2.6	3.6
B-2	79.9	8.1	1.7	2.4	5.9
B-3	82.2	6.5	1.6	2.1	6.3
C-2	92.5	1.9	1.2	2.3	1.9
C-3	84.3	8.2	1.1	2.5	4.0

* See Table 1.

2. Extractive Nitrogen

As shown in Table 3 and Fig. 2, the amount of EN in the muscle of two- and three-year-old samples collected at St. 1, in the winter season did not differ much (244mg*1 for A-2 versus 255mg for A-3). As for samples collected in the spring season, almost no difference in EN was observed between B-2 and B-3, but a significantly large difference was seen between C-2 (224mg) and C-3 (286mg). This discrepancy may be due to the large difference in size between C-2 and C-3 rather than the difference in locality as was the case for the proximate composition.

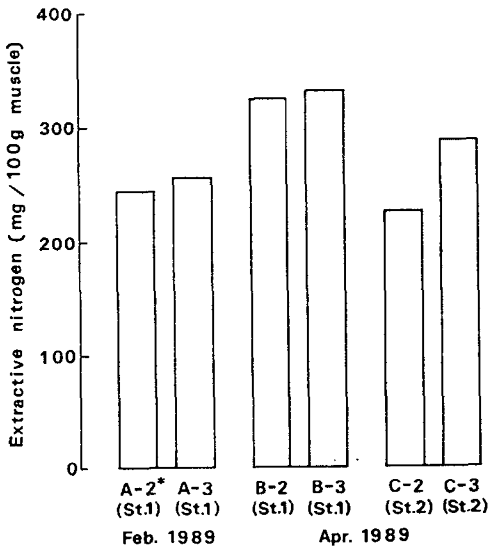

Fig. 2. Age-dependent variation in extractive nitrogen of the muscle of *H. roretzi*.
    * See Table 1 and Fig. 1.

3. Free and Combined Amino Acids

No significant difference in the total amount of FAA was observed between A-2 and A-3 and between B-2 and B-3. However, the level of the total FAA was clearly different between C-2 and C-3 (Table 3).

The content of taurine, which was the most abundant FAA, was lower in two-year-old samples than in three-year-old ones irrespective of season and locality ; it was 436mg in A-2 versus 585mg in A-3, 692mg in B-2 versus 739mg in B-3, and 605mg in C-2 versus 665mg in C-3 (Fig. 3). Almost the same tendency was ovserved in alanine.

As for the content of proline, somewhat different tendency was seeen. At St. 1, A-2 (371mg) and B-2 (420mg) of two-year-old samples were slightly higher than A-3 (243mg) and B-3 (348

*1 Unless otherwise stated, the amounts of extractive components are expressed in terms of mg per 100 g of muscle.

Table 3. Age variation in nitrogenous constituents of the muscle extract of *H. roretzi*[*1]

(mg/100g)

Sample[*2]	A-2	A-3	B-2	B-3	C-2	C-3
Extractive nitrogen	244	255	324	329	224	286
Free and combined amino acids						
Phosphoserine	2	3	3	4	2	3
Taurine	436	585	692	739	605	665
Aspartic acid	38(24)	20	72(3)	44	7	35(1)
Hydroxyproline	14(1)	10(8)	19	15	4(9)	11
Threonine	31	28	33(4)	41(1)	11(2)	26
Serine	13(2)	18	16(2)	22	9	16(2)
Asparagine	-	28	17	26	9	11
Glutamic acid	99(23)	83(7)	106	134	46(13)	105
Glutamine	10	14	-	26	16	-
Proline	371(11)	243	420(17)	348	181(12)	363
Glycine	85	52(18)	103(22)	88(10)	38(12)	56(15)
Alanine	36(1)	37(1)	58(4)	76	34(1)	42
Valine	20(1)	20(1)	11(2)	16(1)	6(2)	12
Cystine	1	1	1	1(1)	1(1)	1
Methionine	9	11	3(3)	5(1)	4(1)	11
Isoleucine	12(1)	11	7	10	4(3)	7
Leucine	17(1)	15(1)	9(1)	14	6(1)	11
Tyrosine	29	28	22	27	5(2)	22
Phenylalanine	13(2)	15(1)	11(1)	14	5(1)	12
β-Alanine	6(1)	6	2(1)	3	4	3
Ornithine	2	2	1	2	2	1
Tryptophan	11	7	17	10	3	13
Lysine	4(2)	4(2)	4(1)	7(1)	6(2)	4(2)
Histidine	32	20(1)	45(1)	43	8(1)	37
Carnosine	—	—	2	—	7	3
Arginine	1(1)	2(1)	2(1)	3(1)	2(1)	1(1)
Nucleotides and related compounds						
ATP	14	12	15	8	6	6
ADP	23	24	55	30	21	29
AMP	25	33	83	108	39	69
IMP	1	1	1	2	2	1
Ino	2	1	7	9	4	4
Hyp	+	+	1	1	2	1
Others						
Glycinebetaine	188	291	340	343	351	338
Halocynine	25	50	25	45	—	19
Homarine	43	80	66	69	94	87
Trigonelline	24	22	18	11	14	8
TMAO	27	34	24	23	26	30
TMA	9	6	6	6	8	7
Creatine	7	11	8	6	27	6
Creatinine	2	2	2	2	1	1
Ammonia	2	2	2	2	2	2

*1 Small amount of α-aminoadipic acid, γ-amino-*n*-butyric acid, β-aminoisobutyric acid, γ-amino-*n*-butyric acid, cystathionine ethanolamine, and τ-methylhistidine were detected in some samples, but they are not given in the table.
The amount of combined amino acids are given in parentheses.
Abbreviations and marks used : TMAO, trimethylamine oxide ; TMA, trimethylamine +, trace ; —, not detected.

*2 See Table 1.

mg) of three-year-old ones, respectively, while at St. 2, C-2 (181mg) of two-year-old sample was clearly lower than C-3 (363mg) of three-year-old one (Table 3 and Fig. 3).

The other major FAA in the samples were almost at the same level between A-2 and A-3, and between B-2 and B-3. But C-2 was lower than C-3 in almost all of FAA. Especially the difference in glutamic acid, aspartic acid and histidine between C-2 and C-3 was evident.

The contents of CAA in the muscle extracts are given in parentheses in Table 3. As compared with FAA, their concentrations were very low, being less than 10mg except a few amino acids. The total CAA levels of two-year-old samples (A-2, B-2 and C-2) were higher than those of three-year-old ones (A-3, B-3 and C-3), respectively.

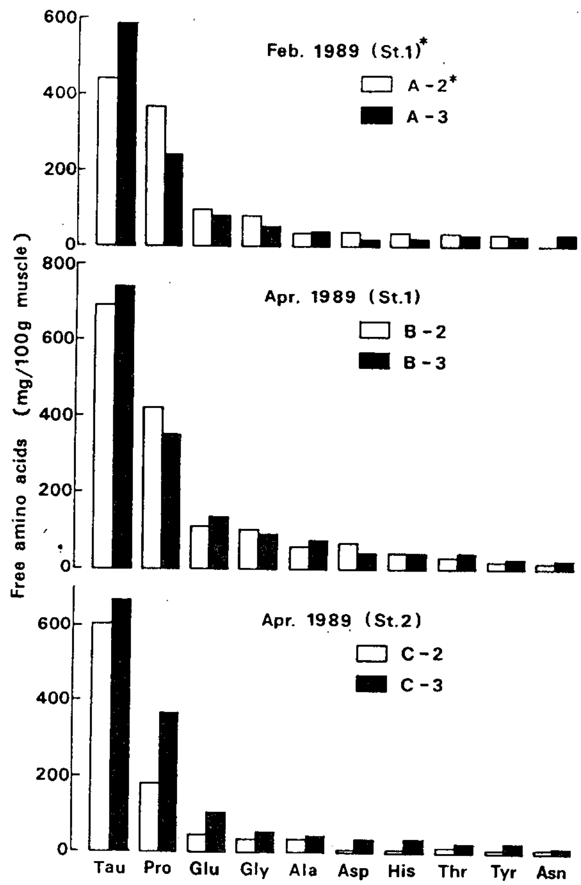

Fig. 3. Age-dependent variation in free amino acids of the muscle of *H. roretzi.*
* See Table 1 and Fig. 1.

4. Nucleotides and Related Compounds

Fig. 4 compares NRC between two- and three-year-old samples. Adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate(ADP), adenosine 5'-monophosphate(AMP), inosine 5'-monophosphate(IMP), inosine(Ino) and hypoxanthine(Hyp) were detected more or less in all the samples.

The sum of NRC expressed in terms of μmol per 1 g of muscle was almost the same between two- and three-year-old samples collected at St.1 (1.67 μmol in A-2 versus 1.89 μmol in A-3 and 4.34 μmol in B-2 versus 4.44 μmol in B-3). However, it was considerably different between the samples of different ages at St. 2(2.10 μmol in C-2 versus 3.05 μmol in C-3). Those differences in the sum of NRC among the samples were in parallel with those in EN and the total FAA described above.

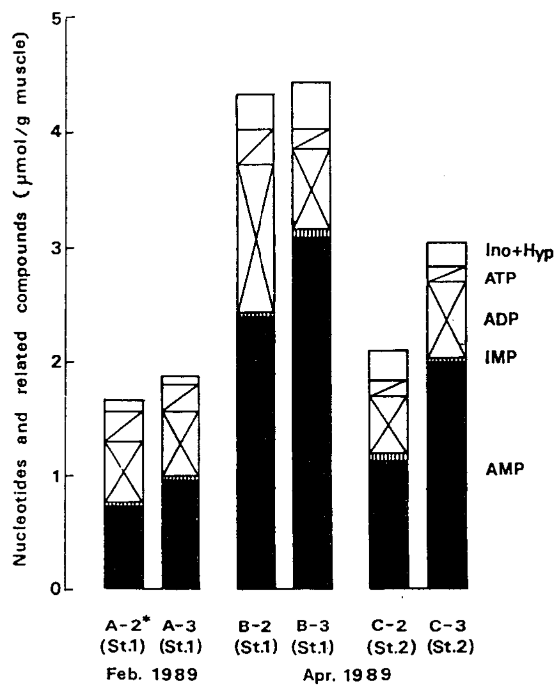

Fig. 4. Age-dependent variation in nucleotides and related compounds of the muscle of *H. roretzi.*
* See Table 1 and Fig. 1.

5. Betaines

Glycinebetaine (GB), homarine (Hom) and trigonelline (Tri) were detected in all muscular samples. Halocynine (Hal) was also found in them except for C-2 collected at St.2 in the winter season (Table 3 and Fig.5).

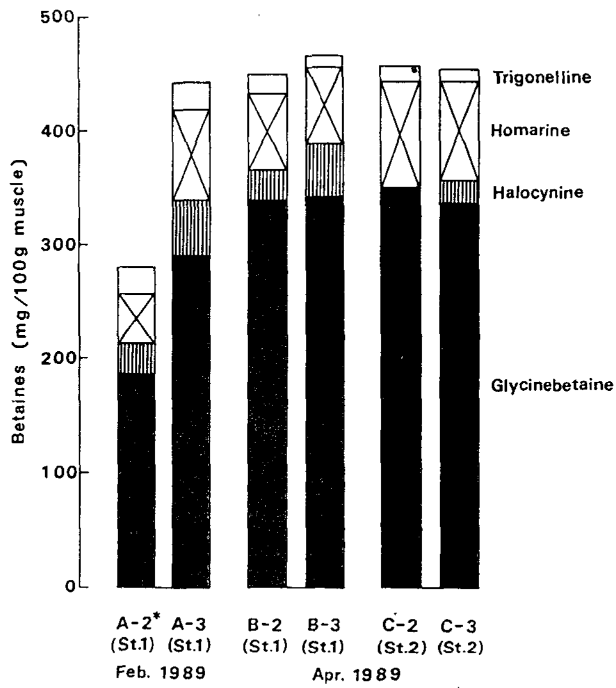

Fig. 5. Age-dependent variation in betaines of the muscle of *H. roretzi.*
* See Table 1 and Fig. 1.

Unlike EN, FAA and NRC described above, the amounts of those betaines differed much between two- and three-year-old samples collected at St.1 in the winter season (280 mg in A-2 versus 443 mg in A-3), but the other two samples of two groups collected in the spring season at St.1 and St.2, respectively, did not differ much from each other (449 mg in B-2 versus 468 mg in B-3 and 459 mg in C-2 versus 452 mg in C-3).

The samples A-2 and A-3 exhibited great discrepancies not only in GB (188 mg versus 291 mg), but in Hom (43 mg versus 80 mg) and Hal (25 mg versus 50 mg) contents. The higher value of Hal for three-year-old sample than for two-year-old one was also seen in the other two groups.

Thus, the age variation in betaines was clearly observed in GB and Hom of the winter samples and in Hal of all the samples.

6. Trimethylamine oxide and trimethylamine

The level of TMAO was very low, ranging from 23 to 34 mg, in two- and three-year-old samples at both sampling stations (Table 3). There was almost no difference in their amounts between B-2 and B-3, and between C-2 and C-3 in the spring season, but a little difference was observed between A-2 and A-3 as was the case for betaines in the winter samples.

The content of TMA was less than 10 mg, and little variation by age was found in all the samples.

7. Creatine and Creatinine

The two- and three-year-old samples in the winter (A-2 and A-3) and spring seasons (B-2 and B-3) from St.1 showed essentially no difference in creatine content (Table 3).

However, the samples from St.2 revealed a great disparity each other, C-2 being more than 4 times as high as C-3. The creatinine content was extremely low (less than 2 mg) and did not differ between the two samples irrespective of season and locality.

8. Comparison of Nitrogen Distribution in the Muscle Extract among Samples.

In order to know the difference in nitrogen distribution in the muscle extract between two- and three-year-old samples, the nitrogen contents were calculated for each group of the analyzed compounds and are summarized in Fig. 6 in terms of mg N per 100 g of muscle.

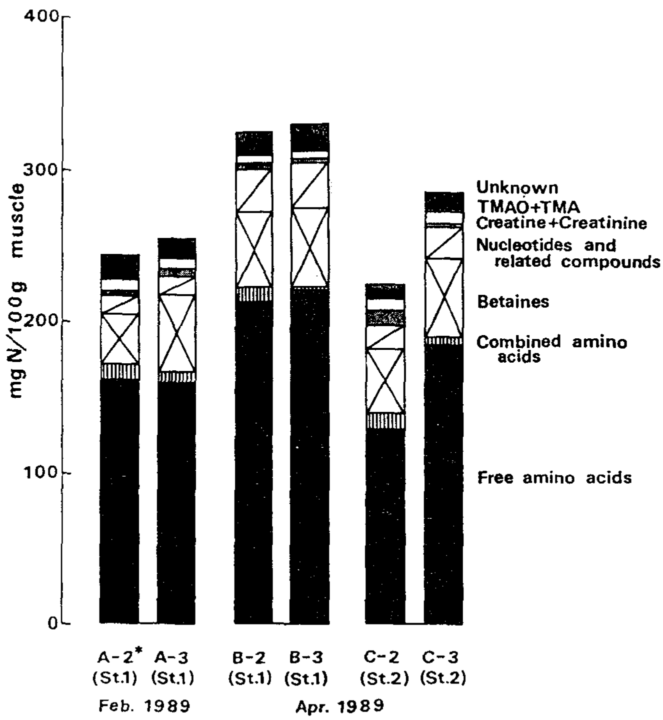

Fig. 6. Age-dependent variation in nitrogen distribution in the muscle extracts of *H. roretzi.*
* See Table 1 and Fig. 1.

The differences in some nitrogenous constituents between the two samples pointed out in the preceding sections, are seen again in this figure. They are CAA and betaines in the winter samples at St.1; and FAA, CAA, NRC, betaines and creatine in the spring samples at St. 2. The figure also exhibits that the variation by age in nitrogenous extractives is not so great as far as the two samples of the two groups from St. 1 are compared with each other. However, the two samples from St. 2 in the spring season are considerably different in FAA-, betaine- and NRC-N, resulting in a marked difference in EN. As described before, these variations may be attributed to the difference in size of samples rather than the difference in age, because the size of C-2 is very small as compared with those of A-2 and B-2 (Table 1). The effects of the size or growth of ascidian on nitrogenous extractives will be investigated again in the next paper.

The recoveries of EN by analyzed components were 92~97% in all the samples, indicating that the nitrogen distribution in the muscle extracts has been determined for the most part.

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