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Annual variation in the composition of major nutrients of the common starfish (*Asterias Rubens*)

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Highlights:

- Nutrient composition of starfish changes during the year.
- Starfish caught from February to May had highest crude protein and lowest ash.
- Fat content did not show a clear seasonal variation.
- Small starfish had the poorest nutrient composition.
- The geographical locations did not significantly affect the nutrient composition.

ABSTRACT

To study the annual variation in the composition of nutrients relevant to pig and poultry feeding, monthly samples of starfish (*Asterias Rubens*) were taken in Denmark. The effect of

different locations and starfish sizes was also assessed. Crude protein (CP) and phosphorus were high from February to May and lower thereafter. The AA profile remained constant and followed the same pattern as CP. An opposite pattern of CP was observed for both ash and calcium. Fat followed no clear annual pattern. Starfish within the smallest size group had the lowest fat and CP levels and the highest ash and calcium levels. The most profound differences between locations were observed in May. The seasonal pattern in chemical composition seemed related to spawning and gonadal developments. Large and medium sized starfish caught between February and May will be most suitable for pig and poultry feed.

Abbreviations: AA, amino acids; Ca, calcium; CP, crude protein; DM, dry matter; P, phosphorus.

Keywords: Feed, marine protein, minerals, nutrients, seastar, starfish meal

1. Introduction

Recently, efforts have been made to use the common starfish *Asterias Rubens* as a feed ingredient in pig and poultry diets (Afrose et al., 2016; Sørensen and Nørgaard, 2016). Starfish have a crude protein level from around 270-450 and up to 700 g/kg in the dry matter (DM) and a high quality amino acid (AA) profile (Levin et al., 1960; Nørgaard et al., 2015; Sørensen and Nørgaard, 2016). The calcium (Ca) level of above 100 g/kg, however, disturbs the balance between Ca and phosphorus (P) in pig diets. Furthermore, the nutrient composition of starfish caught in different months has been found to be variable. Both factors can make the use of starfish in feed formulation challenging. Knowledge on changes in nutrient composition of starfish will allow identification of the optimal fishing period as well

as the circumstances that make starfish meals most suitable for use in animal nutrition and will enable production of a uniform feedstuff.

Different aspects can affect the chemical composition of starfish. Firstly, environmental factors such as temperature, feed source and feed availability can alter availability and requirement of nutrients and utilisation of energy (Ferguson, 1975). Secondly, starfish nutrient composition is believed to be dependent on starfish size (Levin et al., 1960). Lastly, nutrient composition can change between seasons which is partly related to the reproductive cycle. During the reproductive cycle, the gonads vary largely in size and nutrient composition due to deposition and release of nutrients, mainly protein and fat (Oudejans and Sluis, 1979; Rubilar et al., 2008). In *Asterias rubens*, gametogenesis and gonadal developments start around September-October (Oudejans and Sluis, 1979). Ovaries are fully matured and ready for spawning between April-May. Pre-vitelogenesis, a sexual rest period, follows from June to September. The pyloric caeca has a nutrient storage function and will therefore also influence nutrient levels (Jangoux and van Impe, 1977). No research has been published on the annual changes in nutrient composition of the intact starfish body. Nonetheless, annual changes in nutrient composition of starfish have been studied on different body compartments, i.e. the gonads, the pyloric sphincter and the somatic body (Oudejans and Sluis, 1979; McClintock et al., 1990; Rubilar et al., 2008).

The objective of this study was to describe the variation in the composition of major nutrients in starfish. Differences in nutrient composition attributed to month, starfish size and harvesting locations were measured and compared in order to find the optimum catching circumstances of starfish for their use in animal feed.

2. Material and methods

2.1. Sampling and characterisation

Starfish (*Asterias rubens*) were caught monthly between February 2016 and January 2017 and sent for posterior nutrient analysis. Sampling was performed in Limfjorden in the North of Denmark. Starfish were collected by dredging at three different positions: position 1: at a depth of 5.5 m and 56° 46,807; 008° 54,512; position 2: at a depth of 6 m and 56° 47,763; 008° 55,485; position 3: at a depth of 5 m and 56° 48,327; 008° 53,552. Because these locations were part of blue mussel production sites, as they should be according to EU legislation (European Commission, 2017), the water was under continuous quality analysis and declared free of toxins.

Samples were stored at 5 °C during transport and subsequently at -18 °C until nutrient analyses were performed. In April, May and October 2016, additional sampling was performed in Lillebælt, which is in the central part of Denmark. Starfish were collected by dredging by fishermen. In Limfjorden, additional sampling was performed in April (N=312), June (N=206) and December (N=202) 2016 where size and weight of the sampled starfish were measured immediately after fishing. Starfish were allotted to one of three size groups; small (≤ 7.5 cm), medium (7.5-15 cm) and large (> 15 cm). The nutrient composition of starfish was analysed separately for each size class during these three months.

2.2. Chemical analyses

Chemical analyses were performed on intact starfish, i.e. a mixture of the solid phase and the liquid phase leaked during the hours from fishing until freezing. All analyses, with the exception of AA analyses, were performed in duplicates. To assess DM, samples were freeze dried for two to three days at -80 °C until a constant weight was reached (Christ Gamma 2-16 LSC, Germany). Crude protein (CP, $N \times 6.25$) level was analysed using the Kjeldahl method (AOAC, 2000). Crude fat was analysed by hydrolysing the sample with hydrochloric acid before extraction with petroleum ether (European Commission, 2009). To get the ash level,

the samples were oven dried at 525 °C for 6 h. The minerals P and Ca were analysed after ashing at 450 °C and treatment with hydrochloric acid/nitric acid. Phosphorus was measured using spectrophotometry after reaction with vanadomolybdate (Stuffins, 1967). Calcium was measured using atomic absorption spectrophotometry (model SP9, PyeUnicam Ltd, Cambridge, UK). For other mineral than Ca and P, the samples were destroyed using Ultrawave Microwave Acid Digestion System (Milestone Inc, Shelton, USA) at 230 °C and 1500 watt for 35 minutes, acidified with 5 ml of nitric acid (67-69%). The samples were diluted 1:10 with 2% nitric acid before analysis by ICP-MS (inductively coupled plasma mass spectrometry) on an X series^{II} ICP-MS equipped with a conventional Mainhard nebulizer and a Peltier cooled quartz impact bead spray chamber operated at 3 °C (Thermo Electron Cooperation, Bremen, Germany) set with a CETAC auto sampler model ASX-520. Data were collected using the PlasmaLab version 2.6.2.337 (Thermo Electron).

Samples were hydrolysed at 110 °C for 23 h before AA analysis. Sulphur containing AA, cysteine and methionine were oxidised with performic acid before hydrolysis. Amino acids were separated using ion exchange chromatography and quantified using photometric detection after reaction with ninhydrin (European Commission, 1998). Tryptophan samples were hydrolysed with a barium hydroxide solution under alkaline conditions and heated at 110 °C for 20 h. Tryptophan was evaluated with high performance liquid chromatography (HPLC) with fluorescence detection using an internal standard (European Commission, 2000).

3. Results

Figure 1 shows the monthly variation in nutrient composition of starfish. The CP level varied annually between 289 g/kg DM in September and 452 g/kg DM in February (Figure 1a). Crude protein started to increase from October and onwards. High levels of CP were

found from February to May. In this period, starfish contained on average 418 g CP/kg DM. In June, the CP level decreased again. The monthly variation in total AA level followed the same monthly pattern of variation as the crude protein level (Table 1). The AA profiles in g/100 g CP remained constant throughout the year. The largest range within a year was observed for glycine, serine and threonine. Throughout the year, the levels of glutamic acid and glycine were higher than all other AA. In all months, glutamic acid made up 10-11% of CP. The CP contained 15-17% glycine. Cysteine, histidine, methionine and tryptophan were present at the lowest levels, each making up <2% of CP.

Fat levels varied almost 40% between 53.0 and 92.4 g/kg DM (Figure 1b). In contrast to CP and AA, the variation in fat levels did not follow a clear pattern, i.e. fat levels did not have a series of consecutive months in which levels were clearly higher or lower than in the other months. Fat levels strongly fluctuated between the months June to October. Notably, the direction of changes in fat and protein level is the same from month to month.

The lowest level of ash was 357 g/kg DM and the highest level of ash was 578 g/kg DM (Figure 1c). Ash levels were lowest from February to May and highest from June to January. Calcium levels fluctuated between 102 and 167 g/kg DM (Figure 1d) and followed a similar pattern as that of ash. The total variation in P level was 2.93 to 6.45 g/kg DM (Figure 1e). Low levels of P corresponded to high levels of Ca, while high levels of P corresponded to low levels of Ca.

Trace elements and minerals, other than Ca and P, did not show clear annual patterns (Table 2). Annually Na varied between 19 g/kg DM in February and 32 g/kg DM in October. The Mg content differed between 9 g/kg DM in February and 14 g/kg DM in July. The amount of K in starfish varied annually between 7 g/kg DM in May and 13 g/kg DM in September. Potassium values were high between February and May and decreased thereafter to remain low. In May, starfish contained the lowest amount of Mn with 40 mg/kg DM, and

the highest amount of Mn was in January with 127 mg/kg DM. Both in September and January, a large peak in Mn was observed. Starfish annually contained between 446 to 2110 mg/kg DM Fe with large deviations in September and January, similar to those observed for Mn. The lowest value of Cu was found in July with 5.00 mg/kg DM. January had the highest amount of Cu with 7.35 mg/kg DM. Starfish contained 133 mg/kg DM Zn in February and 177 mg/kg DM Zn in January. Selenium varied annually between 1.20 mg/kg DM in September and 1.95 mg/kg in March.

Sizes of starfish ranged from 3.5-21.5 cm (Figure 2a). Medium sized starfish (7.5-15 cm) made up the majority of the total population with 76% in April, 62% in June and 70% in December. In June, the small sized starfish contributed to a larger portion of the population than in the other months, with close to 30% in June compared with roughly 14% in April and December. Large sized starfish formed the smallest fraction of the total population in April and June. The weight of starfish varied between 1-111 g. The average weight of batches of starfish was lowest in June for all size classes (Figure 2b).

Numerically, small sized starfish had reduced CP, P and fat levels and increased ash and Ca levels compared with medium and large sized starfish (Table 3). In June, the differences in CP and P levels between size classes were negligible. The greatest variation between size classes was observed for fat in all months. The difference in nutrient levels between size classes was biggest in December, and the least variation was observed in June.

In April and October, nutrients in starfish were mostly comparable between Limfjorden and Lillebælt (Table 4). In May, the greatest differences were observed in starfish nutrient levels of both locations. Crude protein, fat and P decreased in starfish from Lillebælt, whereas they increased in those caught in the Limfjorden compared with the previous month. Ash and Ca on the other hand, were increased in starfish from Lillebælt and decreased in Limfjorden in May compared with April.

4. Discussion

Starfish meal of *Asterias Rubens* can be a new protein source in feed for pigs and poultry, and *Asterias Rubens*, when harvested in a mollusc production area, was included in the definition of fishmeal and fish oil in the European Union from July 2017 (European Commission., 2017). Starfish are considered a pest by the industry, because they predate on mussels for commercial use. They can be caught and processed into meal by known techniques.

In line with the current study, a study on gonads of the starfish species *Allostichaster capensis* in the southern hemisphere shows that soluble protein levels start to increase at gametogenesis and keep increasing until maturation (Rubilar et al., 2008). Therefore, the annual pattern in CP can likely be linked to the gonadal development and its reproductive stages. The reduction in CP in June can be related to a large release of macronutrients at spawning which is believed to have occurred in May. This finding is supported by Rubilar et al. (2008) who found that CP levels decrease in June and remain low thereafter. Protein bound AA in the gonads and digestive glands of starfish are found to be relatively constant at various months during different years (Ferguson, 1975). This can explain the stable AA profile in the current study. Hence, AA levels can largely be predicted based on the CP level. All essential AA are present at a minimum of 1% of CP level. Lysine, frequently the first limiting AA, is present at a reasonable amount. The ratio of essential AA to lysine is at or above requirement levels of NRC, 2012. Solely histidine and leucine are slightly unbalanced. Cyclical changes are the result of changes in free AA (Ferguson, 1975). The AA that were found to be at the highest or lowest levels correspond to data from Janecki and Rakusa-Suszczewski (2004) on Antarctic Starfish (*Odontaster Validus*). High amounts of free AA, including glycine and glutamic acid, are believed to be important for nitrogen storage in starfish (Ferguson, 1975).

The fat level of close to 100 g/kg DM in some months can be an easily accessible energy source at weaning. However, during the finisher phase, fat can be oversupplied and stored in fat depots. The lack of a clear annual pattern for fat in starfish might be the result of a relation between the gonads and the pyloric caeca. The pyloric caeca accumulates lipids during pre-vitellogenesis, whereas lipids in the gonads start to decrease (Oudejans and Sluis, 1979; Rubilar et al., 2008). On the contrary, at the start of vitellogenesis, lipid levels start to decrease in the pyloric sphincter and increase in the gonads (Oudejans and Sluis, 1979). The stored energy in the pyloric caeca is used for gonadal development (Jangoux and van Impe, 1977). The observed monthly differences in fat levels may depend more on environmental factors than gonadal development.

A possible explanation for the lower ash and Ca levels between February and May is that nutrient deposition for gonad development requires a lot of body resources at the expense of ash deposition. Possibly, P is involved or required for gonadal development and therefore follows a pattern similar to CP. However, the content of other minerals did not display clear annual patterns.

A large gap was observed for all nutrients between February 2016 and January 2017, which is unlikely to occur from January to February during a continuous year. Therefore, the nutrient composition seems to vary significantly between years. Environmental factors can have contributed to the observed difference. These factors might encompass water temperature or the amount and type of feed available.

An aggregation of starfish in Lancashire, England, had a distribution size similar to the current study with a few small sized starfish, and medium sized starfish forming the largest group (Sloan and Aldridge, 1981). The low weights in June are possibly caused by weight loss from the previous release of eggs. This may also account for the low variability of all nutrients between size classes observed in that month; macronutrients such as CP and fat are

removed from the body after spawning, thus Ca and ash make up a larger part of the total DM content. The low macronutrient and high ash level in small sized starfish is possibly linked to a relatively larger surface to weight ratio. The body wall contains large amounts of ash, varying between 510-890 g/kg DM for different species of starfish (McClintock et al, 1990). Moreover, the small size group can include starfish of which organs/gonads are not fully developed.

The different trend of various nutrients between Limfjorden and Lillebælt from April to May may be because starfish in Lillebælt had already spawned before the May sampling. With the removal of gametes from the body, a large amount of protein and fat has left the body. Therefore, in a relative sense, ash and Ca levels are high. It cannot be established whether environmental factors could have caused any differences in the chemical composition of starfish for the different locations, as no additional measurements were carried out.

5. Conclusion

Size, gonadal development and spawning appeared to be predictive of changes in the nutrient composition of the common starfish *Asterias Rubens*. Crude protein, AA, P, Ca and ash had clear annual patterns. Close to reproductive maturity, between February and May, CP and P levels were highest, whereas ash and Ca levels were lowest. Therefore, catching starfish in these months will generate a starfish meal in which CP is high, and in which least difficulty is met in adapting diets to the high Ca level. Fat levels did not show a clear seasonal variation. Fat levels were thought to be interchangeable between the pyloric sphincter and the gonads, resulting in a more constant fat level over the year. Variation in fat level within a year is likely to be more influenced by external factors. Small sized starfish had the most unfavourable nutrient composition with high ash and Ca levels and low CP, fat and P levels. Thus, it would be preferable not to catch the smallest starfish and only use medium and large

sized starfish in the production of starfish meal for pigs and poultry. The geographical locations did not significantly affect the nutrient composition besides in May, where starfish may have spawned before sampling in one location.

Conflict of interest

No potential conflict of interest was reported by the authors.

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Figure captions

Figure 1. Monthly variation in crude protein (a), crude fat (b), ash (c), calcium (d) and phosphorus (e) levels of starfish (*Asterias Rubens*) caught in Limfjorden, Denmark from 2016-2017.

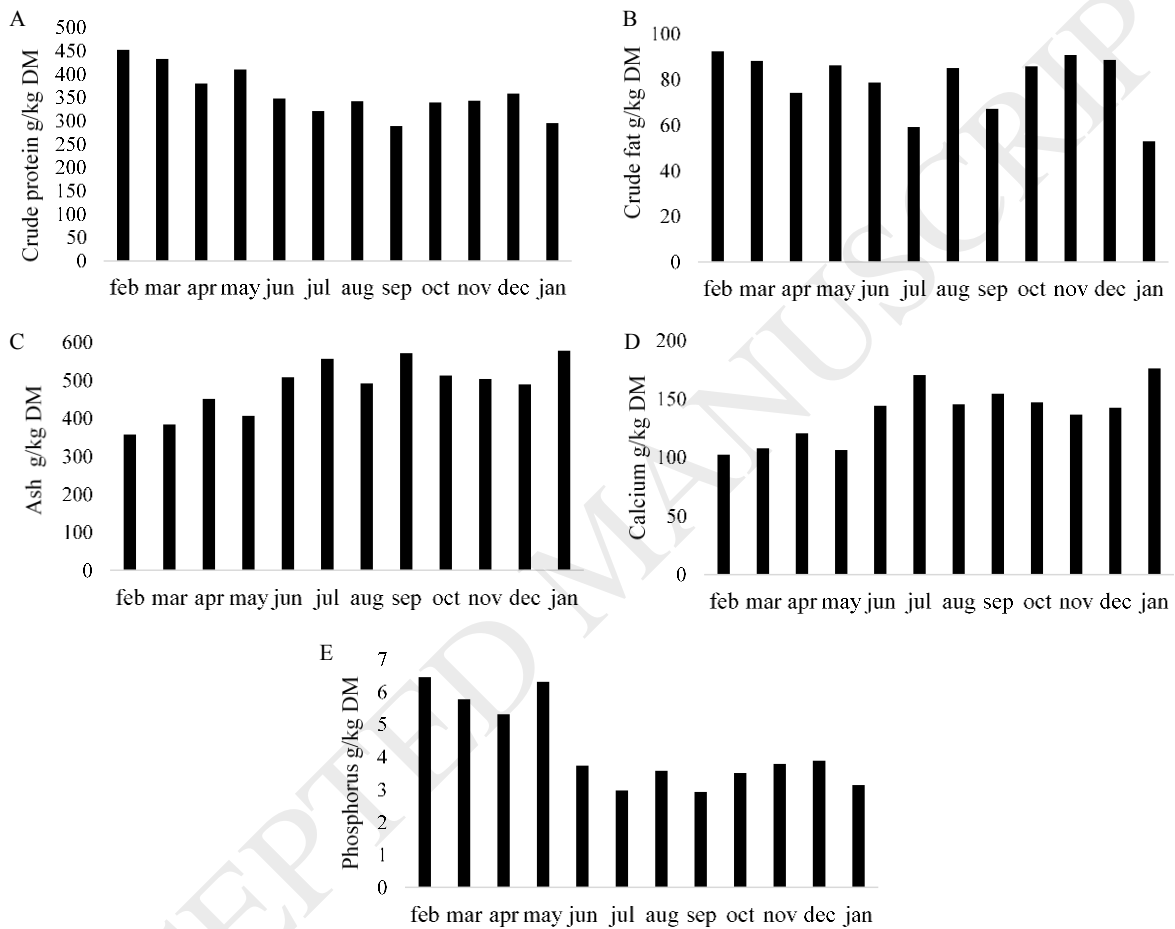


Figure 2. Size (a) and weight (b) distribution of starfish (*Asterias Rubens*) caught in Limfjorden, Denmark, 2016, in the size classes small (0-7.5 cm), medium (7.5-15 cm) and large (>15 cm)

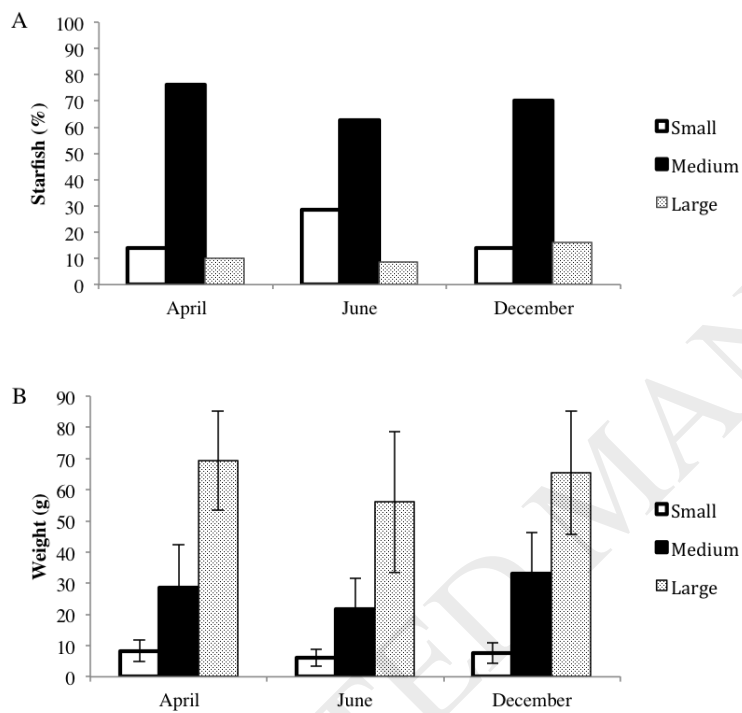


Table 1

Amino acid profile (g/100 g CP) in starfish (*Asterias Rubens*) caught between February 2016 and January 2017 in Limfjorden, Denmark.

Item	Month												Average	Min	Max	Range
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan				
Alanine	4.4	4.6	4.9	5.0	5.0	5.0	4.7	4.8	5.0	5.0	5.0	5.3	4.9	4.4	5.3	0.9
Arginine	5.6	5.7	5.9	5.9	6.0	6.0	5.8	5.8	6.0	6.1	5.9	6.2	5.9	5.6	6.2	0.6
Aspartic acid	8.5	8.5	8.4	8.3	8.4	8.3	8.4	8.3	8.4	8.7	8.5	8.5	8.4	8.3	8.7	0.4
Cysteine	1.4	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.4	1.5	1.4	1.5	1.5	1.4	1.5	0.1
Glutamic acid	10	10	11	11	11	11	10	10	11	11	11	11	11	10	11	0.7
Glycine	14	14	15	15	16	16	15	15	15	15	15	17	15	14	17	3.0
Histidine	1.7	1.7	1.7	1.7	1.6	1.6	1.6	1.6	1.5	1.6	1.6	1.6	1.6	1.5	1.7	0.2
Isoleucine	3.7	3.7	3.7	3.7	3.5	3.5	3.6	3.5	3.5	3.6	3.5	3.5	3.6	3.5	3.7	0.2
Leucine	5.5	5.5	5.4	5.5	5.1	5.0	5.2	5.0	5.0	5.2	5.1	5.1	5.2	5.0	5.5	0.5
Lysine	6.1	6.0	6.0	6.1	5.5	5.2	5.5	5.2	5.2	5.5	5.4	5.4	5.6	5.2	6.1	0.9
Methionine	2.1	2.0	2.0	1.9	1.9	1.8	1.9	1.8	1.8	1.9	1.8	1.8	1.9	1.8	2.1	0.3
Phenylalanine	3.3	3.3	3.3	3.3	3.1	3.0	3.2	3.1	3.0	3.2	3.1	3.1	3.2	3.0	3.3	0.3
Proline	4.0	4.1	4.2	4.1	4.7	4.9	4.6	4.8	4.8	4.8	4.6	4.8	4.5	4.0	4.9	0.8
Serine	4.5	4.6	4.8	4.6	5.0	5.0	4.6	4.8	7.9	7.8	4.9	5.1	5.3	4.5	7.9	3.4
Threonine	4.7	4.5	4.3	4.1	4.9	5.4	4.1	4.9	3.7	4.3	3.4	5.0	4.4	3.4	5.4	2.0
Tryptophan	1.1	1.1	1.0	1.0	1.2	1.0	1.0	1.0	1.0	0.7	0.9	1.0	1.0	0.7	1.2	0.5
Valine	4.6	4.5	4.5	4.4	5.2	4.7	4.3	4.6	4.4	3.6	4.1	4.8	4.5	3.6	5.2	1.7
Sum of 17 AA	85	86	87	87	89	88	85	86	86	85	85	90	87	85	90	5.0

Table 2

Monthly changes in minerals and trace elements from starfish (*Asterias Rubens*) caught between February 2016 and January 2017 in Limfjorden, Denmark.

Item	Month												Average	Min	Max	Range	
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan					
Minerals, g/kg DM																	
Sodium	19	24	25	30	29	30	31	26	32	31	25	25	27	19	32	13	
Magnesium	9	10	11	10	12	14	12	12	12	12	11	13	12	9	14	5	
Potassium	11	12	12	13	9	8	8	7	8	8	8	7	9	7	13	6	
Trace elements, mg/kg DM																	
Manganese	42	40	59	40	54	51	49	111	63	76	80	127	66	40	127	87	
Iron	591	446	903	581	696	496	673	2110	923	1142	979	1714	938	446	2210	1664	
Copper	5.6	6.0	7.0	5.0	5.0	5.0	5.5	5.9	5.7	5.6	5.7	7.3	5.8	5.0	7.3	2.4	
Zinc	133	129	130	139	133	138	154	140	153	150	143	177	143	129	177	47	
Selenium	1.9	2.0	1.6	1.5	1.3	1.2	1.3	1.2	1.2	1.2	1.3	1.2	1.4	1.2	2.0	0.8	

Table 3

Chemical composition (g/kg DM, unless indicated otherwise) of starfish (*Asterias Rubens*) in the size classes small (0-7.5 cm), medium (7.5-15 cm) and large (>15 cm) caught in Limfjorden, Denmark, 2016.

Item	April			June			December		
	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
Dry matter (g/kg)	220	227	230	177	173	143	240	242	250
Fat	32.8	52.9	67.5	30.0	48.1	57.3	38.8	84.8	87.9
Ash	594	549	511	614	589	585	593	485	467
Phosphorus	2.94	3.55	4.07	2.40	2.46	2.34	2.86	3.85	4.12
Calcium	169	146	140	177	173	169	178	142	137
Crude Protein	317	328	349	297	302	297	306	356	366
Alanine	16.7	17.3	17.8	16.3	16.6	16.1	17.3	17.7	17.6
Arginine	19.6	20.4	21.4	18.6	18.7	18.2	19.7	21.1	21.2
Aspartic acid	26.5	27.6	28.8	24.5	25.4	24.7	14.6	10.7	9.68
Cysteine	4.97	5.03	5.18	4.61	4.64	4.50	4.67	5.11	5.16
Glutamic acid	34.4	35.6	37.2	32.0	32.9	32.1	34.3	37.6	38.2
Glycine	50.1	53.1	54.8	47.7	49.5	47.6	53.0	55.1	54.9
Histidine	4.90	5.11	5.39	4.34	4.52	4.42	4.80	5.55	5.74
Isoleucine	11.1	11.7	12.2	9.89	10.0	9.77	10.7	12.2	12.8
Leucine	15.8	16.7	17.6	14.0	14.2	13.9	15.4	17.9	18.6
Lysine	16.5	17.4	19.0	14.3	14.9	14.6	15.9	18.8	19.8
Methionine	5.84	6.23	6.37	5.06	5.40	5.00	5.47	6.28	6.80
Phenylalanine	9.08	9.59	10.3	7.94	8.24	8.12	9.13	11.0	11.6
Proline	15.6	16.4	16.6	15.6	16.9	15.4	15.9	16.2	16.4
Serine	16.3	16.9	17.5	15.4	15.9	15.4	16.4	17.3	17.3
Threonine	12.4	13.1	13.9	11.3	11.6	11.5	14.9	12.2	12.0
Tryptophan	2.85	2.99	3.11	2.57	2.65	2.53	3.33	2.78	2.70
Valine	12.9	13.4	14.4	11.7	12.0	11.9	15.1	12.8	12.5

Sum of 17 AA	277	289	301	310	299	277	256	263	256
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Table 4

Chemical composition (g/kg DM, unless indicated otherwise) of starfish (*Asterias Rubens*) caught in Limfjorden and Lillebælt, Denmark, 2016.

Item	April		May		October	
	Limfjorden	Lillebælt	Limfjorden	Lillebælt	Limfjorden	Lillebælt
Dry matter (g/kg)	216	243	183	211	199	220
Fat	74.2	77.7	86.3	60.9	85.8	74.1
Ash	451	444	406	530	512	527
Phosphorus	5.31	6.59	6.31	4.70	3.51	3.63
Calcium	121	139	106	141	147	165
Crude protein	380	389	410	347	339	315
Alanine	18.7	21.5	20.5	18.2	16.8	17.1
Arginine	22.4	24.6	24.1	21.0	20.3	19.7
Aspartic acid	31.9	33.8	33.9	29.1	28.6	28.4
Cysteine	5.74	6.02	5.97	5.23	4.89	4.66
Glutamic acid	40.1	42.6	43.4	36.8	36.0	34.6
Glycine	55.5	44.4	61.4	42.8	51.0	38.9
Histidine	6.47	6.84	6.98	5.51	5.19	4.98
Isoleucine	14.0	15.0	15.1	12.4	11.8	11.4
Leucine	20.7	22.1	22.6	18.0	17.0	16.6
Lysine	22.8	28.0	25.0	20.9	17.6	18.7
Methionine	7.41	8.21	7.74	6.64	5.95	6.00
Phenylalanine	12.6	13.2	13.4	11.0	10.3	10.1
Proline	15.8	17.0	16.9	16.3	16.2	16.0
Serine	18.1	19.6	18.9	17.3	16.7	16.4
Threonine	16.4	15.2	17.3	15.4	14.7	15.7
Tryptophan	3.73	3.23	4.05	3.23	3.07	3.48
Valine	17.1	15.3	17.9	15.4	14.5	15.9
Sum of 17 AA	330	337	355	295	306	279