Short Note

# Seasonal variation in the nutritional analysis of *Caprella scaura* (Amphipoda: Caprellidae) from the biofouling of fish farm cages in Central Greece

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**Abstract**: The total lipid and protein content of the invasive caprellid amphipod *Caprella scaura*, from the biofouling communities of fish farm cages in the Pagasitikos Gulf were analyzed and compared among seasons. Proteins were the most abundant component (48.5 – 49.3%). Lipid content was relatively lower, with a wider range (6.7 – 34%) and showed a distinct seasonal fluctuation with high values in the winter population and a gradual decrease in spring and summer, with the lowest values in Autumn. Composition of the fatty acids profile was consistent among the seasons, with palmitic (16:0), Oleic (18:1n-9), Eicosapentanoic (20:5n-3)(EPA) and Docosahexanoic acid (22:6n-3 )(DHA) being the most abundant fatty acids. The presence of high levels of EPA and DHA fatty acids makes the species a potential candidate for use of these organisms in aquaculture.

**Keywords:** fatty acids; lipid content; protein content; invasive species; Kjeldahl; Gas chromatography; Integrated Multi Trophic Aquaculture; Pagasitikos Gulf

## 1. Introduction

One of the most crucial challenges which humanity is facing nowadays is the pressing need to feed an ever growing population, but with the limited and finite natural resources available on our planet [1]. Seafood, in general, and especially fish, have always been an important part of the human diet and nowadays they represent an essential component of the global food basket, in terms of nutrition, health, and well-being of all people [2]. Aquaculture is a promising industry which could support this pressing need. In fact, aquaculture is considered one of the fastest growing animal food production sectors, and production will probably continue to grow to meet the rising demand for seafood [3], mainly owning to the generally static or declining capture fisheries [4].

However, aquaculture itself is also facing new challenges in terms of cost and ecological impact. Feeding the fish is often the costliest production input, and can have important

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downstream ecological effects. At present, many marine finfish aquaculture efforts are directed at high trophic level carnivorous fish that are often reliant upon on-growing aquafeeds derived from catches of lower trophic level fish, with increasing associated capture costs and biodiversity/ecosystem impacts [5].

Up to date, the aquaculture sector has focused on searching feedstuff alternatives from the terrestrial environment at a significant degree of success by using protein from plants [6], animals [7,8] and even insects [9,10]. However, the marine aquatic environment is still not fully explored and could possibly provide credible and sustainable solutions for fishmeal and fishoil replacement. In this context, a promising feedstuff alternative could be proved to be the marine amphipods of the Caprellidae Family. These marine organisms have recently attracted the scientific interest of aquaculture researchers, as they are rich in animal protein and at the same time contain significant amounts of the, rarely found in nature, omega-3 fatty acids [4,11-13].

Caprellid amphipods have a number of suitable characteristics which make them a potential resource in the aquafeeds industry and aquaculture: (i) they have a widespread global distribution and can be easily found as the dominant species in biofouling communities attached to sea cages and fixed structures, (ii) they already consist part of the natural diet of several marine fish species, (iii) they exhibit high reproduction performance, fast growth rates and under appropriate conditions can reach high biomass [4,13]. Furthermore, caprellid amphipods could be also utilized as novel aquatic organisms as live feed in aquaculture, particularly for larval or juvenile finfish stages [14,15]. Currently, the aquaculture industry uses *Artemia* spp., rotifers, copepods and mysid shrimps as live feed for juvenile fish production, but these organisms are not nutritionally optimal, since they have low levels of essential fatty acids, so there is a need to enrich them prior to use thus increasing the cost of feeding [16]. Moreover, caprellid amphipods could be, also, utilized within the framework of an Integrated Multi-Trophic Aquaculture (IMTA), serving as extractive species which feed on detritus from the farmed fish, thus reducing the environmental impact of the farming process [13].

The aim of this study was to analyze the nutritional content of the invasive caprellid amphipod *Caprella scaura*, from the natural occurring populations on the fish cages from an aquaculture facility in Pagasitikos gulf, Greece. Our main focus was the fatty acid profile, crude lipid and protein content and if there are any fluctuations between the seasons.

#### 2. Materials and Methods

Samples of the biofouling community, including *C. scaura* populations, were collected following a similar technique described in Lolas, et al. [17]. Plastic meshes were used as an artificial substrate for biofouling to establish and develop and were submerged very close to the nets of the fish cages. The initial deployment commenced in early June 2018 and the meshes were left submerged for 3 months, then collected and replaced with new ones every 3 months, in order for each batch to represent one season (Summer, Autumn, Winter, Spring). During their collection each season, meshes were put in plastic containers filled with sea water and were then transferred to the laboratory. They were, then, rinsed with tap water and sieved through a 250  $\mu$ m mesh in order to collect the majority of the fouling fauna. Because of the distinctive morphology of *C. scaura*, mature male and female individuals were easily sorted out from the rest of the fauna and were stored in glass vials in deep freeze conditions (-80°C).

Lipid fraction was extracted from the wet biomass of *C. scaura* individuals, according to the method described by Folch, et al. [18] and three replicates for each season were analyzed. The crude protein content was determined with Kjeldahl analyses, using a conversion factor of 6.25 [19].Total lipid was extracted using a chloroform:methanol solution (C:M 2:1 V/V) containing 0.05% (W/V) of butylated hydroxytoluene (BHT) as an antioxidant agent. Organic solvents where evaporated with a stream of nitrogen until dried and lipid content was determined gravimetrically. Extracted lipids were stored in C:M (2:1, v/v) with a standard concentration of 20 mg lipid mL<sup>-1</sup>.

Fatty acid methyl esters (FAME) were prepared by acid catalyzed transesterification according to [20]. Extracted lipid sample containing 1 mg of total lipids and 0.1 mg heptadecanoic acid (17:0) (Sigma Chemical Company, St. Louis, MO, USA) as internal standard was evaporated

under a stream of nitrogen. 2 mL of methylating agent was added containing sulfuric acid in methanol (1% H<sub>2</sub>SO<sub>4</sub> in methanol) and 1 mL of toluene. Vials were flashed with nitrogen and dry incubated on a hot block (SC154-240, Environmental Express, SC, USA) at 50°C for 16h. Crude FAMEs were purified by thin layer chromatography (TLC) on  $20 \times 20$  cm glass plate pre-coated with silica gel G (Merc silica gel 60), developed in a isohexane: diethyl ether: acetic acid (90:10:1, v/v) solution and visualized with a 1% (W/V) iodine in CH<sub>3</sub>Cl<sub>3</sub> solution. After scraping silica gel containing FAMEs, they were removed by elution with an isohexane: diethyl ether (1:1, V/V) solution and re-dissolved in isohexane containing 0.05% BHT as an antioxidant agent. Vials containing the samples were flashed with nitrogen prior storage at -80°C.

Separation and quantification of FAMEs was conducted by Gas-Liquid chromatography (GLC) with a Perkin Elmer Clarus 680 coupled with a Col-Elite FAME Wax capillary column (30 m × 0.25 mm id, film thickness 0.25  $\mu$ m) (PN N9316694, Perkin Elmer, Waltham, MA, USA). Hydrogen was the carrier gas with constant flow rate of 1 mL min<sup>-1</sup>. Injector temperature was set at 240°C with a split ratio of 1:10 at a total flow rate of 5 mL min<sup>-1</sup>. The column oven was programmed from 60°C to 190°C for 5 min at a rate of 20°C min<sup>-1</sup> and from 190°C to 240°C at a rate of 5 C min<sup>-1</sup>. The final temperature was maintained for 20 min. Identification of individual FAMES were conducted by comparison to known standards (CL40.13093, FAME MIX 37, Sigma-Aldrich, St. Louis, MO, USA). Peak areas were quantified with reference to the peak area of 17:0 as an internal standard.

#### 3. Results

According to the analyses, *C. scaura* was characterized by high levels of crude protein content (48.5 – 49.3%) with no apparent seasonal fluctuation. On the contrary, lipid content seemed to follow a seasonal pattern, with significant differences among the seasons, with relatively high values in the Winter population (34%), lower values in Spring and Summer (17.5 and 16.3%) and the lowest values in Autumn (6.7%) (Figure 1). The saturated fatty acids (SFA) ranged between 34.4 - 36.5% of the total fatty acids in *C. scaura*, the polyunsaturated fatty acids (PUFA) were between 33.5 - 36.3% and the monounsaturated (MUFA) were 28.0 - 33.7% (Table 1).



**Figure 1.** Seasonal values of the percentage per wet weight (%) of total lipids (A) and crude protein (B) of *Caprella scaura* from fish cages in Pagasitikos gulf, Greece. Values with different letter are significantly different

Fatty Acids	Summer	Autumn	Winter	Spring
SFA				
12:0	0.5	0.2	0.4	0.4
14:0	2.3	2.6	2.5	2.5
15:0	0.8	0.6	0.8	0.7
16:0	22.8	24.2	24.7	25.0
18:0	5.0	4.8	5.1	5.2
20:0	1.8	2.0	2.0	2.1
22:0	1.0	0.5	0.9	0.7
Total	34.4	34.9	36.4	36.5
MUFA				
16:1n-9	0.9	0.6	0.9	0.6
16:1n-7	5.9	3.8	4.9	4.8
18:1n-9	16.5	22.2	21.1	17.1
18:1n-7	3.6	2.6	3.7	2.9
20:1n-9/n-11	1.5	1.7	1.7	1.5
22:1n-9/n-11	0.3	0.3	0.3	0.3
24:1n-9	1.3	0.6	1.0	0.7
Total	30.0	31.6	33.7	28.0
PUFA n-6				
18:2n-6	7.4	10.3	9.1	8.3
18:3n-6	5.0	0.5	3.1	4.1
20:2n-6	0.1	0.2	0.1	0.2
20:3n-6	n.d.	n.d.	n.d.	n.d.
20:4n-6	2.9	4.1	3.4	3.7
22:4n-6	1.8	0.6	1.7	1.0
22:5n-6	1.7	0.9	1.2	1.0
Total	18.8	16.6	18.6	18.2
PUFA n-3				
18:3n-3	0.6	0.9	0.6	0.9
18:4n-3	0.3	0.3	0.3	0.3
20:3n-3	n.d.	n.d.	n.d.	n.d.
20:4n-3	0.3	0.2	0.3	0.2
20:5n-3 (EPA)	8.4	8.3	8.8	8.0
22:5n-3	0.6	0.7	0.8	0.7
22:6n-3 (DHA)	6.8	6.6	6.9	6.6
Total	17.0	16.9	17.7	16.7
Total PUFA	35.7	33.5	36.3	34.9
Ratio (n-6):(n-3)	0.90	1.02	0.95	0.92

**Table 1**. Percentage of fatty acid composition of *Caprella scaura* from fish cages in Pagasitikos gulf, Greece. SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; EPA: Eicosapentanoic acid; DHA: Docosahexanoic acid.

The most predominant fatty acids (FA) in *C. scaura* content were the saturated 16:0 (22.8 - 25.0%) and the monounsaturated 18:1n-9 (16.5 - 22.2%), whereas the very important PUFA Eicosapentanoic acid (EPA) and Docosahexanoic acid (DHA) were found in relatively lower values but still, higher than most of the other FA and they could actually be considered as adequate (EPA 8.0 -8.8% and DHA 6.6 - 6.9%).

## 4. Discussion

The fatty acid (FA) profile of C. scaura was characterized by the presence of four major FAs, 16:0, 18:1n-9, 20:5n-3 (EPA) and 22:6n-3 (DHA). These FAs were also found to be the most dominant in several other studies, either in caprellids [4,11,12,21] but also other groups of amphipods [15,22-24]. Although content levels vary between locations and species, which is something to be expected, the consistent abundance of those four important FAs in amphipods shows that they actually could be a promising natural marine resource in the aquaculture sector.

The small differences in content values of FAs in *C. scaura* among the seasons did not follow a specific pattern. Although temperature is known to affect the composition of the FA profile in marine amphipods [21], this did not seem to be the case for *C. scaura* from Pagastikos gulf. Although some unidentified peaks were detected during the chromatographic analyses, which could potentially indicate that other FAs were also present, the identified FAs were quite consistent among the seasonal samples. On the other hand, total lipid content was evidently affected by water temperature, since it followed a specific seasonal pattern. Marine amphipods, caprellids included, follow a certain nutritional strategy which dictates the storing of energy in lipid reserves to cope with the changes in feed availability during the low winter temperatures [25]. These energy reserves are then being used during the spring and summer reproduction and growth, which are the main processes involved in the metabolism of lipids [26].

Based on the composition of the FA profile of amphipods, a wide range of ratios can be calculated which sometimes are considered as bioindicators of diet preference [22,27]. In the case of *C. scaura* from fish cages in Pagasitikos gulf, the ratio EPA/DHA which is an indicator of a diatom-based diet [22,27], was lower than the ratio 18:1(n-9)/ 18:1(n-7) which is an indicator for a more carnivorous preference [28]. This finding is in agreement with the predation attribute which characterizes the feeding behavior of *C. scaura* [29-32]. This aggressive behavioral trait could also be one of the factors which enhance the invasive success that *C. scaura* by displacing congeneric species from their niche, such as *Caprella equilibra* [17,33-35].

It seems that there is a considerable potential for the use of C. scaura as a key resource in the aquaculture sector. Its nutritional value can be considered, at least, adequate [13,36]. In this context, it could be either harvested from natural occurring populations or from dedicated artificial substrates, integrated within in the IMTA framework or even intensively cultivated on a large scale, in order to make its exploitation more sustainable [4,13,36]. Further studies are required both in local and regional level in regards to the estimation of favorable conditions which could maximize the biomass growth and nutritional output for the harvested / cultivated products and would be very beneficial, in this regard.

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