Turk. J. Fish.& Aquat. Sci. 19(8), 661-667
http://doi.org/10.4194/1303-2712-v19_8_04

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Response of Fatty Acid Composition of the Green Tiger Shrimp *Penaeus semisulcatus* During the Overwintering Period

Metin Kumlu[1], Enes Kinay[1], Hatice Asuman Yilmaz[1], Asuman Beksari[1], Orhan Tufan Eroldogan[1], Merve Sariipek[2,*]

[1] Çukurova University, Faculty of Fisheries, 01330 Balcalı, Adana, Turkey.
[2] Sinop University, Faculty of Fisheries, 57000 Akliman, Sinop, Turkey.

Article History
Received 24 August 2017
Accepted 08 July 2018
First Online 06 August 2018

Corresponding Author
Tel.: +90 535 578 81 83
E-mail: sariipekm@gmail.com

Keywords
Starvation
Refeeding
Green Tiger Shrimp
Temperature
Fatty acids

Abstract

Response of proximate and fatty acid (FA) composition of the green tiger shrimp *Penaeus semisulcatus* in relation to changing water temperatures during over wintering (for 8-weeks at 11-16°C) and then refeeding periods (at 28°C for a further 2-weeks) was investigated in this study. The shrimps did not appear to need to catabolise their either body proteins or lipids during the overwintering period, as the feeding was not ceased completely. While muscle protein, ash and dry matter compositions of the shrimps did not change by changing temperature, lipid increased from 1% during the overwintering period to 1.2% during the recovery period ($P<0.01$). Saturated FAs (SFA), mono-unsaturated FAs (MUFA), and partially poly-unsaturated FAs (n-6 PUFA) declined, on the contrary, n-3 PUFA significantly rose in the muscle and hepatopacreas during the cold exposure. Shrimps tended to consume especially SFA and, to a lesser degree, MUFA under sub-optimal conditions ($P<0.01$). PUFA and LC-PUFA appeared to be selectively retained or even elevated in the shrimp muscle during the cold season. Over wintering strategy might generate additional benefit for a heathier food supply for human nutrition, as shrimps can be enriched for LC-PUFA, by simply keeping them under cold water temperatures (10-15°C) during overwintering period, with minimal cost of feeding.

Introduction

Unlike in the tropics, farmed crustaceans face great temperature fluctuations in the sub-tropical parts of the World, particularly when farmed in uncovered tanks or ponds. Pond water temperatures might drop down to 7-8°C in January/February in most parts of the North-eastern Mediterranean regions, causing mass shrimp mortalities in open ponds. Therefore, in such regions, tropical shrimps are generally cultured from early spring to late autumn (5-6 months) and harvested before the cold season, achieving only one crop per year (Seidman & Issar, 1988; Kumlu, Kır, Yılmaz, Göçer, & Eroldoğan, 2010a). In order to avoid crop losses, maintain the stock or even proceed with production cycle during the winter months, recirculating aquaculture systems (RAS) installed in considerably cheap greenhousing facilities and utilising some sort of heating systems are off importance for successful shrimp farming in the subtropics. Over wintering facilities can provide fexibility to extend marketing period, enlarge marketable size of shrimps and even provide an opportunity to produce more than one crop per year.

Maintaining good shrimp growth rate during the cold season even in the RAS requires cheap energy sources, like photovoltaic solar panel systems or underground warmwaters if the system is to be cost-effective (Hoang, Lee, Keenan, & Marsden, 2002). In

most cases, shrimp farmers would have no access to reach cheap energy alternatives and therefore are forced to hold their stock at above critical temperatures (12-15°C) in overwintering facilities with minimal or sometimes without any kind of heating until the cold-season is over. In the meantime, some of their crops can be sold fresh to catch premium price in the market. In temperate regions, commercial shrimp farms operating in temperature-controlled RAS prefer to offer their products fresh to the market especially during winter months to fetch attractive premium prices, as such products are often not available on the market during off-season. . As a result, studies on shrimp culture under Mediterranean climatic conditions have been directed towards intensification and indoor RAS culture techniques to better control water parameters particularly temperature to increase yield per water volume and to regularly supply market with fresh products throughout the year (Kumlu & Kır, 2005; Kumlu *et al.*, 2010a; Kumlu, Kumlu, & Turkmen, 2010b; Kumlu, Türkmen, Kumlu, & Eroldoğan, 2011).

The green tiger shrimp *Penaeus semisulcatus* (De Haan) is one of the major shrimp species for aquaculture in the northeastern Mediterranean achieving a marketable size of 20-25 g within the limited-growing season (Seidman *et al*., 1988; Kumlu *et al.*, 2010a). Some studies have proven that this species can easily be overwintered at water temperatures above 12°C in greenhouses (Kir, 2004) without substantial mortality and it could tolerate temperatures as low as 9°C for short periods. Kumlu *et al.* (2005) studied the effects of low water temperatures (14, 18 and 22°C) on moulting, growth and food consumption of *P. semisulcatus* in a 70-day trial and found that this species can be sucessfully overwintered with 87% survival at 14°C and 39 ppt. These authors stated that under such stressful low temperatures, the species reduced food consumption to a minimal level (less then 0.45% of body weight). So far, no study has ever adressed as to how fatty acid composition is affected at chronic low overwintering temperature conditions, when a very limited amount of food is consumed by the shrimps.

A small number of studies have dealth with the effects of temperature and/or food restriction on various tissues lipid contents particularly fatty acid dynamics in marine shrimps and most of the knowledge in this field is derived from studies on other crustaceans (Goss &Bunting, 1983; Schlechtriem, Arts, & Zellmer, 2006; Werbrouck, Van Gansbeke, Vanreusel, & De Troch, 2016a; Werbrouck, Van Gansbeke, Vanreusel, Mensens, & De Troch, 2016b). It has been reported that many poikilothermic organisms adapt to changing water temperatures by modifying the degree of unsaturation of their lipids (Goss *et al.*, 1983; Pruitt, 1990). It has been agreed by many authors that poikilothermic aquatic animals tend to accumulate highly unsaturated fatty acids (HUFA) in their lipids at low temperatures in order to maintain cell membrane fluidity. In a study with the copepod Daphnia pulex, Schlechtriem *et al.* (2006) showed that a long-term exposure to cold temperature caused a significant increase in eicosapentaenoic acid (20:5n-3, EPA), while arachidonic acid (ARA) and EPAwere highly conserved during starvation. Werbrouck *et al.* (2016a) studied several intervals of food deprivation under two temperatures (4 and 15°C) in copepods and found that ambient water temperature affected both the degree of FA depletion and mobilization

In a study with the crab *Carcinus maenas*, Chapelle, (1978) investigated the effects of three acclimation temperatures (7, 14 and 27°C) and found an overall tendency for the degree of unsaturation of fatty acids to increase with a decrease in acclimation temperatures. Similarly, Pollero, Baró, and Irazú (1991) observed a higher content of total phospholipids (PL) in the muscle, hepatopancreas, and gill tissues of *Macrobrachium borellii*, as the water temperature decreased. The higher degree of fatty acid unsaturation generally observed in response to decreased temperature takes place mainly in the polar lipid fraction (Chapelle, Zwingelstein, Meister, & Brichon, 1979; Farkas, 1979). The restructuring changes in lipid composition during thermal adaptation have been explained in terms of maintaining the fuidity of cell membranes, essential for biological activities (Perez-Velazquez *et al.*, 2003).

There is a need for exploring new sustainable and cost-effective production strategies suitable for shrimp farming in sub-tropical regions, without causing any defect on the nutritional contents of the product. While trying to extend shrimp production towards winter months, it is important to know what alterations would occur in the nutritional contents of the shrimps that are hardly feeding and surviving in overwintering ponds. Understanding how lipid metabolism of HUFA fatty acids (especially EPA and DHA) is affected especially during the winter season will help to manufacture more suitable feed formulations for shrimp to be grown in cold-periods, and will also provide valuable information on possible changes in the quality of the product to be destined for human consumption as food.

Therefore, the aim of this study was to evaluate how fatty acid composition of *P. semisulcatus* under overwintering conditions responds to low and/or high water temperatures with relation to restricted food consumption over the 12-weeks of growout, followed by 8-weeks of over wintering period and finally 2-weeks of re-feeding culture period.

Material and Methods

General Culture Practices

This study was carried out at Marine R&D Unit of Cukurova University of Faculty of Fisheries in Adana–Turkey. The juveniles of *P. semisulcatus* (6.7±1.9 g), which had been grown for 12-weeks (pre-overwintering

period) in heated water in four concrete ponds (1x10x1 m) at 28°C and 39 ppt salinity under a greenhouse in a recirculation system were sampled (about 10 individuals from each pond) for fatty acid analyses before being acclimated to 16-17°C for a period of 1 week. The acclimation was simply done by ceasing heating prior to transporting the juveniles to overwintering ponds.

A group of 400 juveniles were stocked into two 3x10x1 m concrete ponds and were fed with 2-mm extruded pellets (45% protein, 12% lipid; Camlı Yem, İzmir - Turkey) by using two feeding tables (0.8x0.8x0.1 m) for each pond. The shrimp were fed ad libitium and the feeds were placed onto feeding trays four times a day (08:00, 12:00, 18:00, 20:00). The feeding trays were checked 2 h post-feeding and about 10-15% remaining food was tried to be maintained on the feeding trays throughout the study.

Water quality parameters as total ammonia, nitrite and nitrate were measured weekly with commercial test kits, pH with a pH-meter (Hanna HI 9812-5, Germany), dissolved oxygen (DO) with an oxygenmeter (YSI Pro 20, USA) and salinity with a refractometer. The results were; total ammonia and nitrite always < 0.2 ppm, nitrate <40 ppm, DO >5.5 ppm, pH 8.05±0.21 and salinity 39±0.03 ppt in all the culturing conditions.

At the end of the 8-weeks overwintering culture, when water temperature dropped down to 11-12°C another sampling was done (10 shrimps from each pond) for fatty acid analyses. Finally, a total of 40 juveniles (8.7±2.6 g) were transferred from the overwintering ponds and stocked into each glass aquarium with 100-L capacity (0.6x0.45x0.45 m) in water at 39 ppt, in two replicates. The animals were acclimated to 28°C here for two days by using 300 watt thermostatically controlled aquarium heaters. Following the acclimation period, the animals then were fed ad libitum four times a day for a further 2-weeks culture (recovery period). At the end of the recovery period, again about 20 shrimps (12.0±4.5 g) were sampled for fatty acid analyses.

During each sampling period, muscle and hepatopancreas of the shrimps were removed and separately placed into plastic tubes (20-mL) before being kept frozen at -20°C'de until the analyses.

Proximate and Fatty Acid Analyses

Before the proximate and fatty acid analyses, the samples were defrosted at 4°C and all the analyses were performed at the labs of Faculty of Fisheries, Cukurova University.

The muscle, hepatopancreas and feed samples were ground to a homogeneous consistency using a centrifugal mill fitted with a 0.25 mm screen. The homogenate from each replicate pond/aquaria was pooled and analysed for proximate composition or fatty acid analyses. Determination of moisture, ash and protein contents in the shrimp tissue samples were conducted as described below. Samples were dried to constant weight at 103°C. Ash content was determined by burning the samples at 450°C for 5 h (AOAC, 1995). Protein (N×6.25) content was assessed using an automated Kjeldahl Kjeltec 2200 (Foss Tecator, Höganäs, Sweden). Lipids were extracted according to Folch, Lees, and Sloane Stanley (1957).

Lipids were extracted according to Folch, Lees, and Sloane Stanley (1957). Following the lipid extraction, fatty acid methyl esters (FAME) were prepared according to Metcalfe and Schmitz (1961) and analysed as described previously by Czesny and Dabrowski (1998) with some modifications. Briefly, the FAME obtained were separated by gas chromatography (Agilent 6890N, Santa Clara, USA), equipped with a flame ionization detector and fitted with a DB 23 capillary column (60 m, 0.25 mm i.d. and 0.25 µm) ejector and detector temperature program was 190°C for 35 min than increasing to 30 °C per min up to 220°C where it was maintained for 5 min. Carrier gas was hydrogen (2 mL min−1 and split ratio was 30:1). The individual fatty acids were identified by comparing their retention times to that of a standard mix of fatty acids (Supelco 37 component FAME mix).

Statistical Analyses

The data were analyzed by one-way analysis of variance (ANOVA) following confirmation of normality and homogeneity of variance. Where significant differences were detected, data were subjected to Student-Newman-Keuls post-hoc test. All calculations were performed by using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA).

Results

Muscle protein contents of the juveniles were not affected by either long-term overwintering period or recoveryperiod (P>0.05, Table 1). Yet, although lipid level did not vary during the 8-week overwintering period (1.0%), the level statistically increased significantly during the two-week recovery period (1.2%, P<0.01). Neither dry matter nor ash contents were affected during the study (P>0.05).

Shrimp tissue and feed fatty acid compositions are summarized in Table 2 and 3. The most abundant fatty acid groups in the muscle of *P. semisulcatus* juveniles were found to be PUFA (38.2%), SFA (32.9%) and MUFA (28.9%). In general, dominant fatty acids in these groups were found as 16:0, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 20:5n-3 and 22:6n-3. During the overwintering, the shrimps tended to consume especially SFA and, to a lesser degree, MUFA under thermal stress and restricted food consumption. Under the long-term cold exposure 18:0 declined by 28.9% in the muscle and 22.2% in the hepatopancreas, while others at a lesser level (P<0.01). Though n-6 PUFA were not affected during this period,

Table 1. Proximate composition (%) of themuscle of *Penaeus semisulcatus* during pre-overwintering (12-weeks), overwintering (for 8-weeks) and recovery (2-weeks) cultureperiods

Proximate Composition (%)	Pre-Overwintering Period	Overwintering Period	Recovery Period
Protein	21.2±0.6	21.1±0.6	20.5±0.2
Lipid	0.9±0.0^{b}	1.0±0.0^{b}	1.2±0.0^{a}
DryMatter	24.3±0.7	24.4±0.3	24.2±0.2
Ash	1.2±1.1	1.3±0.9	1.3±0.8

*Values in each row aremeans ± standard deviation (n=3 readings from the pooled samples). Means marked with different letters are significantly different from each other (P<0.01).

in general, the levels of n-3 PUFA and LC-PUFA were magnified in the tissues of the shrimps during the cold-season. Among the n-3 PUFA, the profoundly influenced fatty acids during thermal stress and recovery period were found to be 18:3n-3 (226.7%), 20:5n-3 (39.4%) and 20:3n-3 (26.3%) (Table 2) in the muscle, and 18:3n-3 (46.7%), 20:5n-3 (218.8%) and 22:6n-3 (51.1%) (Table 3) in the hepatopacreas. The first fatty acid (18:3n-3) rose about three-fold during the overwintering period, while the level decreased by approximately four-folds during the recovery period in the muscle (P<0.01, Table 2). The level of the latter (EPA), however, increased sharply by 218.8% during the overwintering period, whilst declined 21.4% during the short recovery period. The hepatopacreas DHA level did not significantly change during the overwintering period, but the level rose by 63.0% during the full-feeding recovery period.

In general SFA, MUFA, and partially n-6 PUFA declined, on the contrary, the levels of n-3 PUFA significantly rose in the hepatopacreas tissues during the cold exposure stress (Table 3). More specifically, the MUFA (especially 18:1n-9) declined by 49.4%, while LC-PUFA and PUFA increased by 60.8% and 33.5%, respectively, during this period. The most abundant fatty acid groups in the hepatopacreas tissue of *P. semisulcatus* juveniles were found to be PUFA (51.6%), MUFA (32.4%) and SFA (15.8%). In general, dominant fatty acids in these groups were found as 16:0, 18:1n-9, 18:2n-6 and 22:6n-3. Hepatopancreas DHA level also rose dramatically from 31.3% (at pre-overwintering period) to 47.3% at overwintering period but then declined to 17.5% (recovery period) during the study (P<0.01).The most significant change in fatty acid levels occurred in the hepatopancreas tissue was EPA, which rose by more than three-folds during the overwintering period but again significantly declined to 5% once the juveniles had an acces to full-feeding and optimal environmental conditions (P<0.01, Table 3).

Table 2. Fatty acid composition (%) of the muscle of *Penaeus semisulcatus* during pre-overwintering (12-weeks), overwintering (for 8-weeks) and recovery (2-weeks) culture periods

Fatty acids	Commerical Feed	Pre-overwintering Period (A)	Overwintering Period (B)	Recovery Period (C)	Change Between A and B (%)	Change Between B and C (%)	P
16:0	11.4±0.1	17.6±0.1^{a}	16.7±0.0^{b}	14.4±0.17^{c}	-5.1 ↓	-13.77↓	0.00
18:0	3.5±0.0	14.9±0.4^{a}	10.6±0.2^{b}	10.4±0.31^{b}	-28.9 ↓	-1.89 ↓	0.00
SFA	18.7±0.1	32.9±0.5^{a}	27.5±0.2^{b}	25.1±0.50^{c}	-16.4 ↓	-8.73 ↓	0.00
18:1n-7	3.4±0.00	2.9±0.0^{a}	2.6±0.0^{b}	2.6±0.0^{b}	-10.3 ↓	0.0 -	0.00
18:1n-9	32.6±0.2	23.5±0.3^{a}	22.7±0.2^{b}	23.0±0.3^{ab}	-3.4 ↓	+1.32 ↑	0.03
MUFA	45.0±0.3	28.9±0.3^{a}	26.5±0.2^{b}	26.3±0.4^{b}	-8.3 ↓	-0.75 ↑	0.00
18:2n-6	14.9±0.1	7.9±0.0	8.3±0.1	8.5±0.4	+5.1 ↑	+2.41 ↑	0.07
20:4n-6	0.7±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.0 -	0.0 -	0.08
n-6 PUFA[1]	17.2±0.1	10.7±0.1	10.8±0.0	10.8±0.4	+0.9 ↑	0.0 -	0.88
18:3n-3	3.8±0.00	1.5±0.2^{b}	4.9±0.0^{a}	1.0±0.0^{c}	+226.7↑	-79.59 ↓	0.00
20:3n-3	4.1±0.11	3.8±0.1^{b}	4.8±0.0^{a}	4.7±0.1^{a}	+26.3 ↑	-2.08 ↓	0.00
20:5n-3	5.0±0.2	10.4±0.4^{b}	14.5±0.1^{a}	11.6±0.1^{b}	+39.4 ↑	-20.0 ↓	0.00
22:6n-3	4.9±0.2	10.1±0.8^{b}	10.8±0.0^{b}	17.6±0.7^{a}	+6.9 ↑	-62.96↑	0.00
n-3 PUFA[2]	19.0±0.4	27.0±0.9^{b}	35.9±0.1^{a}	35.7±0.8^{a}	+33.0 ↑	-0.56 ↓	0.00
PUFA	36.3±0.4	38.2±0.8^{b}	47.3±0.1^{a}	47.2±0.7^{a}	+23.8 ↑	-0.21 ↓	0.00
LC-PUFA[3]	12.0±0.3	22.2±1.1^{c}	26.4±0.1^{b}	30.2±0.7^{a}	+18.9 ↑	+14.39 ↑	0.00

*Values in each row are means ± standard deviation (n=3 readings from the pooled samples). Means marked with different letters are significantly different from each other (P<0.01).

[1] n-6 PUFA: 18:2n-6, 20:2n-6, 22:2n-6, 18:2n-6, 20:3n-6, 20:4n-6, 22:4n-6.

[2] n-3 PUFA: 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3.

[3] LC-PUFA: 20:4n-6, 22:4n-6; 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3.

Table 3. Fatty acid composition (%) of the hepatopancreas of *Penaeus semisulcatus* during pre-overwintering (12-weeks), overwintering (for 8-weeks) and recovery (2-weeks) culture periods

Fatty acids	Pre-overwintering Period (A)	Overwintering Period (B)	RecoveryPeriod (C)	Change Between A and B (%)	Change Between B and C (%)	P
16:0	11.5±0.2^{b}	10.2±0.2^{c}	12.6±0.1^{a}	-11.3 ↓	+23.5 ↑	0.00
18:0	3.6±0.0^{b}	2.8±0.1^{a}	3.7±0.4^{a}	-22.2 ↓	+32.1 ↑	0.01
SFA	15.8±0.3^{b}	13.8±0.3^{c}	17.7±0.5^{a}	-12.7 ↓	+28.3 ↑	0.00
18:1n-7	2.7±0.0^{b}	1.6±0.1^{c}	2.8±0.0^{a}	-40.7 ↓	+75.0 ↑	0.00
18:1n-9	28.3±0.3^{b}	14.0±0.4^{c}	29.3±0.3^{a}	-50.5 ↓	+109.3↑	0.00
MUFA	32.4±0.3^{b}	16.4±0.6^{c}	34.4±0.4^{a}	-49.4 ↓	+109.8↑	0.00
18:2n-6	8.3±0.1^{b}	5.9±0.2^{c}	12.4±0.1^{a}	-28.9 ↓	+110.2↑	0.00
20:4n-6	0.3±0.1	0.4±0.0	0.4±0.0	+33.3 ↑	0.0 -	0.10
n-6 PUFA[1]	10.8±0.2^{b}	8.3±0.2^{c}	14.5±0.1^{a}	-23.1 ↓	+74.7 ↑	0.00
18:3n-3	3.0±0.0^{a}	1.6±0.1^{c}	2.7±0.0^{b}	-46.7 ↓	+68.8 ↑	0.00
20:3n-3	2.5±0.0^{c}	2.8±0.2^{b}	3.5±0.0^{a}	+12.0 ↑	+25.0↑	0.00
20:5n-3	2.2±0.3^{c}	7.0±0.2^{a}	5.5±0.0^{b}	+218.2↑	-21.4 ↓	0.00
22:6n-3	31.3±0.8^{b}	47.3±1.3^{a}	17.5±0.8^{c}	+51.1 ↑	-63.0 ↓	0.00
n-3 PUFA[2]	39.8±1.0^{b}	59.7±0.8^{a}	30.5±0.7^{c}	+50.0 ↑	-48.9 ↓	0.00
PUFA	51.6±0.8^{b}	68.9±0.9^{a}	46.8±0.5^{c}	+33.5 ↑	-32.1 ↓	0.00
LC-PUFA[3]	34.7±1.0^{b}	55.8±0.9^{a}	24.7±0.7^{c}	+60.8 ↑	-55.7 ↓	0.00

*Values in each row are means ± standard deviation (n=3 readings from the pooled samples). Means marked with different letters are significantly different from each other (P<0.01).

[1] n-6 PUFA: 18:2n-6, 20:2n-6, 22:2n-6, 18:2n-6, 20:3n-6, 20:4n-6, 22:4n-6.

[2] n-3 PUFA: 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3.

[3] LC-PUFA: 20:4n-6, 22:4n-6; 20:4n-3, 20:5n-3, 22:5n-3, 22:6n

Discussion

Crustaceans, which are known to have well adaptations to short or even long-term starvation under instable habitat conditions, are able to catabolise their body lipids to obtain their energy requirement when they cannot consume enough food from the environment (Wen, Chen, Ku, & Zhou, 2006). Barclay, Dall, and Smith, (1983) reported that P. esculentus consumed proteins and lipids as major source of energy when subjected to 14-days of starvation. In a previous study that carried out by Çiçek and Kumlu (2013) with *P. semisulcatus*, the authors found that 1 and 3 weeks starved-groups consumed 17% and 40% of their muscle lipids, respectively and that re-fed shrimps for only 2-weeks were able to recover 10% of their lipids. In our current study, despite a long-term (8-weeks) partial-starvation under overwintering conditions, the shrimps did not need to consume any of their muscle protein or lipid reserves, most likely, due to unceased feeding activities during the overwintering cold period, ranged between 11 and 16°C. A significant elevation in the muscle lipid content during the re-feeding period when depot lipids built up in periods with high energy influx could be explained as the preparation of the shrimps for periods of possible energy shortage.

In general, dominant fatty acid groups were reported as 16:0, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 24:0 and 22:6n-3 in muscle tissue of *P. semisulcatus* by Çiçek *et al.* (2013). In addition to these, the current study revealed that 20:3n-3 as well as 20:5n-3 fatty acids were also present at high levels in the muscle tissue of this shrimp species. Similar to our results, Perez-Velazguez *et al.* (2003) were also resported 16:0, 18:00, 18:1, 18:2n-6, 20:5-n3 and 22:6n-3 as dominant fatty acids in *L. vannamei*. Our data showed a clear overall tendency towards an increase in unsaturation of fatty acids with a decrase in temperature during the overwintering period. The greatest difference in PUFA fatty acid composition during the cold period was observed by far in 18:3n-3 (283%) and 20:5n-3 (39.4%) in the muscle and 20:5n-3 (219%) and 22:6n-3 (51.1%) in the hepatopancreas. Under cold-stress, the animals tended to mostly consume 18:0, 18:1n-7 in the muscle and 16:0, 18:0, 18:1n-7, 18:1n-9, 18:2n-6, 20:4n-6 and 18:3n-3 in the hepatopacreas. Chapelle (1978) acclimated the crab *C. maenas* to temperatures of 7, 14 and 27°C and observed the greatest differences in the relative content of 16:0, 16:1, 18:0, 18:1, 20:5 and 22:6 fatty acids in relation to temperature. It is suggested by many authors that acclimation temperature involves in the degree of unsaturation of cellular lipids in order to maintain fluidity of cell membranes essential for biological activities (Sinensky, 1974; Dey, Buda, Wiik, Halver, & Farkas, 1993; Perez-Velazquez *et al.*, 2003; Schlechtriem *et al.*, 2006; Mika, Skorkowski, & Stepnowski, 2014).

The most influenced fatty acids in the muscle and hepotapancreas of *P. semisulcatus* during the overwintering and recovery periods were found to be 18:3n-3 (79.59%) and 22:6n-3 (62.96%) and 18:3n-3 (68.8%), 20:3n-3 (25%) and 22:6n-3 (%63), respectively. The 18:3n-3 level increased by about three-folds during the overwintering period, whilst decreased about four-folds during the recovery period (P<0.01). Similarly, but

to a lesser extent, EPA also rose 50% during the cold-stress, but declined 44% during re-feeding. The level of DHA did not vary significantly during overwintering, but increased about 60% during the recovery period. In the present study, it was clearly seen that DHA was retained better than EPA during the cold exposure, suggesting that *P. semisulcatus*, like other crustaceans, conserved the more valuable n-3 FA during starvation and/or cold period. The data show that 18:0 and, to a lesser extent, 18:1n-7 of the SFA in the muscle, on the other hand, 18:1n-7 and 18:1n-9 of the MUFA in the hepatopancreas were the most exhausted fatty acids during the overwintering period but were regained significantly during the 2-weeks recovery stage. All these results proved that the shrimp tended to pre-dominantly catabolise SFA and to a lesser extent MUFA for their energy requirement during the long overwintering period, when oxidative catabolism of FA was high. On the other hand, the levels of n-6 PUFA were conserved while n-3 PUFA and LC-PUFA levels were magnified in both the muscle and hepatopacreas during the cold-stress. At reduced temperatures, in their study with C. crangon, Mika *et al.* (2014) reported similar response as also did also Perez-Velazquez *et al.* (2003) with L. vannamei. Despite partial starvation coupled with the cold-stress, the significant accumulations of PUFA and LC-PUFA recorded in the tissues of *P. semisulcatus* might be due to the vital role of these fatty acids in membrane structures of shrimp (Farkas, 1979; Schlechtriem *et al.*, 2006; Werbrouck *et al.*, 2016a). The results of Çiçek *et al.* (2013), in which a 21% increase in PUFA levels during even short-starvation (1-3 weeks) followed by a 40% decrease during the two-weeks of refeeding period, also support the above hypothesis. Similar to our results, Wen *et al.* (2006) also found a significant exhaustion of muscle SFA (14:0 and 16:0) but a noteworthy increase in PUFA, especially 18:2n-6 and 18:3n-3 in the 70-day starved-crabs (*Eriocheir sinensis*). The above findings validate that shrimps preserve or even elevate the levels of PUFA during starvation and/or cold-stress, but once the stressors are no longer effective, the levels are dropped. The strategy of preserving or even elevating the levels of especially n-3 PUFA, essential in the structuring of the biological membranes during starvation, food shortages and/or under reduced water temperatures is considered to be a logical biochemical strategy for survival of poikilothermic animals (Wen *et al.*, 2006).

In line with suggestion of Perez-Velazquez *et al.*(2003), who studied fatty acids in association with low temperature in L. vannamei, we also think that high levels of PUFA observed during the long cold-exposure under partial food consumption might be the results of both selective retention of these long-chain fatty acids and, to some extent, some exogenous contribution from dietary source. Werbrouck *et al.* (2016a) was also proposed the selective retention concept in copepods under food deprivation at various temperatures. These authors stated that ambient temperature affected the levels of FA depletion as well as FA mobilization and that a mixed diet could reduce the impact of heat stress on the copepod's membrane structure. Bendiksen and Jobling (2003) also reported that both temperature and feed composition influenced deposition and retention of n-3 and n-6 PUFA in Atlantic salmon (*Salmo salar*) parr and that at low temperature, a very high retention of n-3 PUFA was evident in the fish. In this respect, an adequate provision of PUFA and HUFA-rich diet might be needed for *P. semisulcatus* to support better toleration to critical low thermal extremes during the long overwintering period. However, further studies are needed to clarify the relationship between dietary fatty acid sources and tolerance to low critical temperatures that might be confronted by penaeid shrimps during winter months. It is commonly agreed that such high retention rates of n-3 PUFA in crustaceans and fish is suggested to be a thermal acclimation response, and the lower retention of n-6 PUFA as well as MUFA is taken to imply greater catabolic degradation in low temperatures. In line with this, we also found that *P. semisulcatus* juveniles consumed SFA and MUFA to meet their energy requirement while preserving or even elevating PUFA as well as LC-PUFA for membrane structures during the long cold exposure.

In sub-tropical climates, inclusion of the overwintering strategy in shrimp cultivation can help to prevent crop losses, protect stocks or allow farmers to continue their production cycle in winter. In addition, it can provide fexibility to extend marketing time, enlarge the marketable shrimp size, and offer the opportunity to produce more than one crop per year. The overwintering strategy may provide additional benefits as a heathier food source for human nutrition, as the shrimp can be enriched for n-3 LC-PUFA, by simply keeping them under cold temperature (10-15°C) for a specified period of time with minimal feed cost.

Acknowledgment

This study was supported by the Scientific Research Fund of Cukurova University (Grant No: FYL-2015-5064) and General Directorate of Agricultural Research and Policies (TAGEM-13/AR-GE/34) of the Ministry of Food Agriculture and Livestock of Turkey.

References

AOAC-Association of Official Analytical Chemists (1995). Official Methods of Analysis. 16th ed. Association of Analytical Chemists, Arlington, Virginia, USA.

Barclay, M.C., Dall, W., & Smith, D.M. (1983). Changes in lipid and protein during starvation and the moulting cycle in the tiger prawn, *Penaeus esculentus* Haswell. *Journal of Experimental Marine Biology and Ecology*, 68(3), 229-244. https://doi.org/10.1016/0022-0981(83)90055-2

Bendiksen, E.Å., & Jobling, M. (2003). Effects of temperature and feed composition on essential fatty acid (n-3 and n-6) retention in

Atlantic salmon (*Salmo salar L.)* parr. *Fish Physiology and Biochemistry*, 29(2), 133-140. https://doi.org/10.1023/B:FISH.0000035937.68098.83

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry*, 226(1), 497-509.

Çiçek, D., & Kumlu, M. (2013). Effects of starvation periods on total lipid and fatty acid composition of green tiger shrimp (*Penaeus semisulcatus*). *Çukurova Üniversitesi Fen ve Mühendislik Bilimleri Dergisi*, 29(4), 95-104.

Chapelle, S. (1978). The influence of acclimation temperature on the fatty acid composition of an aquatic crustacean (*Carcinus maenas*). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 204(3), 337-346. https://doi.org/10.1002/jez.1402040304

Cha**pell**e, S., Zwingelstein, G., Meister, R., & Brichon, G. (1979). The influence of acclimation temperature on the phospholipid metabolism of an aquatic crustacea (*Carcinus maenas*). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 210(3), 371-379. https://doi.org/10.1002/jez.1402100303

Chen, K., Li, E., Gan, L., Wang, X., Xu, C., Lin, H., Qin, J.G., & Chen, L. (2014). Growth and lipid metabolism of the pacific white shrimp *Litopenaeus vannamei* at different salinities. *Journal of Shellfish Research,* 33(3), 825-832. https://doi.org/10.2983/035.033.0317

Czesny, S.,& Dabrowski, K. (1998). The effect of egg fatty acid concentrations on embryo viability in wild and domesticated walleye (*Stizostedion vitreum*). *Aquatic Living Resources*, 11(6), 371-378. https://doi.org/10.1016/S0990-7440(99)80002-3

Dey, I., Buda, C., Wiik, T., Halver, J.E.,& Farkas, T. (1993). Molecular and structural composition of phospholipid membranes in livers of marine and freshwater fish in relation to temperature. *Proceedings of the National Academy of Sciences*, 90(16), 7498-7502.

Farkas, T. (1979). Adaptation of fatty acid compositions to temperature a study on planktonic crustaceans. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 64(1), 71-76. https://doi.org/10.1016/0305-0491(79)90185-8

Goss, L.B.,& Bunting, D.L. (1983). *Daphnia* development and reproduction: responses to temperature. *Journal of Thermal Biology*, 8(4), 375-380. https://doi.org/10.1016/0306-4565(83)90025-6

Hoang, T., Lee, S.Y., Keenan, C.P., & Marsden, G.E. (2002). Cold tolerance of the banana prawn *Penaeus merguiensis* de Man and its growth at different temperatures. *Aquaculture Research*, 33(1), 21-26. https://doi.org/10.1046/j.1355-557X.2001.00638.x

Kir, M. (2004). Cold tolerance and overwintering possibility of the Green Tiger Shrimp (*Penaeus semisulcatus*) (PhD Thesis). University of Çukurova, Adana, Turkey.

Kumlu, M.,& Kır, M. (2005). Food consumption, moulting and survival of *Penaeus semisulcatus* during overwintering. *Aquaculture Research*, 36(2), 137-143. https://doi.org/10.1111/j.1365-2109.2004.01196.x

Kumlu, M., Kır, M., Yılmaz, Ö., Göçer, M.,& Eroldoğan, O. T. (2010a). Growth of Overwintered and Pre-seasonally Produced Post-larvae of *Penaeus semisulcatus* in the Subtropics. *Turkish Journal of Fisheries and Aquatic Sciences,* 10(2). http://dx.doi.org/10.4194/trjfas.2010.0216

Kumlu, M., Kumlu, M.,& Turkmen, S. (2010b). Combined effects of temperature and salinity on critical thermal minima of pacific white shrimp *Litopenaeus vannamei* (Crustacea: Penaeidae). *Journal of Thermal Biology*, 35(6), 302-304. https://doi.org/10.1016/j.jtherbio.2010.06.008.

Kumlu, M., Türkmen, S., Kumlu, M., & Eroldoğan, O.T. (2011). Off-season maturation and spawning of the Pacific white shrimp *Litopenaeus vannamei* in sub-tropical conditions. *Turkish Journal of Fisheries and Aquatic Sciences*, 11(1), 15-23. http://dx.doi.org/10.4194/trjfas.2011.0103

Metcalfe, L.D., & Schmitz, A.A. (1961). The rapid preparation of fatty acid esters for gas chromatographic analysis. *Analytical Chemistry*, 33(3), 363-364. https://doi.org/10.1021/ac60171a016

Mika, A., Skorkowski, E.,& Stepnowski, P. (2014). Effect of seasonal and experimental temperature on de novo synthesis of fatty acids in *Crangon crangon*. *Bioscience, Biotechnology, and Biochemistry*, 78(9), 1529-1536. https://doi.org/10.1080/09168451.2014.925778

Pollero, R.J., Baró, M. D.R.G.,& Irazú, C.E. (1991). Lipid classes consumption related to fasting and thermal stress in the shrimp *Macrobrachium borellii*. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 99(1), 233-238. https://doi.org/10.1016/0305-0491(91)90034-B

Perez-Velazquez, M., González-Félix, M.L., Lawrence, A.L.,& Gatlin, D.M. (2003). Changes in lipid class and fatty acid composition of adult male *Litopenaeus vannamei* (Boone) in response to culture temperature and food deprivation. *Aquaculture Research*, 34(13), 1205-1213. https://doi.org/10.1046/j.1365-2109.2003.00931.x

Pruitt, N.L. (1990). Adaptations to temperature in the cellular membranes of crustacea: membrane structure and metabolism. *Journal of Thermal Biology*, 15(1), 1-8. https://doi.org/10.1016/0306-4565(90)90040-O

Schlechtriem, C., Arts, M.T.,& Zellmer, I.D. (2006). Effect of temperature on the fatty acid composition and temporal trajectories of fatty acids in fasting *Daphnia pulex* (Crustacea, Cladocera). *Lipids*, 41(4), 397-400. https://doi.org/10.1007/s11745-006-5111-9

Seidman, E.R.,& Issar, G. (1988). The culture of *Penaeus semisulcatus* in Israel. *Journal of the World Aquaculture Society*, 19(4), 237-247. https://doi.org/10.1111/j.1749-7345.1988.tb00785.x

Sinensky, M. (1974). Homeoviscous adaptation a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 71(2), 522-525.

Wen, X., Chen, L., Ku, Y.,& Zhou, K. (2006). Effect of feeding and lack of food on the growth, gross biochemical and fatty acid composition of juvenile crab, *Eriocheir sinensis*. *Aquaculture*, 252(2), 598-607. https://doi.org/10.1016/j.aquaculture.2005.07.027

Werbrouck, E., Van Gansbeke, D., Vanreusel, A.,& De Troch, M. (2016a). Temperature affects the use of storage fatty acids as energy source in a benthic copepod (*Platychelipus littoralis*, Harpacticoida). *PloS one*. https://doi.org/10.1371/journal.pone.0151779

Werbrouck, E., Van Gansbeke, D., Vanreusel, A., Mensens, C.,& De Troch, M. (2016b). Temperature-induced changes in fatty acid dynamics of the intertidal grazer *Platychelipus littoralis* (Crustacea, Copepoda, Harpacticoida): insights from a short-term feeding experiment. *Journal of Thermal Biology*, 57, 44-53. https://doi.org/10.1016/j.jtherbio.2016.02.002