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# TEMPORAL FLUCTUATIONS OF FATTY ACIDS IN *PACHYGRAPSUS CRASSIPES* FROM SOUTHERN CALIFORNIA

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ABSTRACT

The biochemistry of fatty acids (FA) can be affected by a number of factors, including environmental temperature, which may affect the way FAs are physiologically metabolized. In this study, we investigated FAs in *Pachygrapsus crassipes*, in relation to environmental temperatures in southern California. Although there was a trend toward differences in FA abundances in the hepatopancreas of females compared with those found in the hepatopancreas of males, these differences were not significant through most of the year. The sampling month influenced changes in the abundances of both individual FAs (identities) and FA saturation categories (saturated = SAFA, monounsaturated = MUFA, polyunsaturated = PUFA). The abundances of palmitoleic acid, palmitic acid, and docosahexaenoic acid were found to fluctuate significantly over time, although this fluctuation did not appear to be directly influenced by temperature since an increase in FA abundance between February and April preceded an increase in temperature. In all months except for June, PUFAs dominated the FA profile. Changes in FAs may be an acclimatory mechanism used by *P. crassipes* to take advantage of specific biochemical properties of FAs. We conclude that, while temperature may affect FA abundance and composition, other underlying factors, such as changes in day length, food availability, molting, mating and reproduction, may also influence FA abundances in *P. crassipes* from southern California.

KEY WORDS: energy storage, environmental temperature, hepatopancreas, homeoviscous adaptation, physiology, shore crab

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## INTRODUCTION

The striped shore crab, *Pachygrapsus crassipes* (Randall, 1839), is found in the high-intertidal zone of rocky shores, on hard-mud shores, in tidepools, and among mussel beds from Oregon to Baja California, and in the waters of Japan and Korea (Hiatt, 1948; Meinkoth, 1994). Intertidal zones in tropical and temperate areas are unique environments, experiencing wide fluctuations in temperature which can have a major effect on intertidal marine life (Morton and Harper, 1995; Dunbar, 2002). Intertidal temperature fluctuations can occur daily, seasonally, or sporadically with the interaction of warm and cold currents on shore (Willmer et al., 2000). In some areas, temperature fluctuations may range from as low as 15°C in winter to 40°C in summer, and daily from 15°C to 28.5°C (Klugh, 1924; Morris and Taylor, 1983; Huggett and Griffiths, 1986; Morton and Harper, 1995; Chan, 2000; Dunbar et al., 2003). Although high-shore intertidal organisms such as *P. crassipes* are able to withstand current temperature fluctuations, they may be exposed to more extremes if global temperatures change.

Because they may be exposed to changing temperatures, animals employ a variety of adaptations ranging from behavioral to physiological that result in enhanced tolerance to extreme temperatures. These strategies may include homeoviscous adaptation (Hazel and Williams, 1990; Hall et al., 2002; Maskrey et al., 2005), either through altered fatty acid (FA) synthesis, or through the dietary incorporation of saturated FAs in hot weather and unsaturated FAs in cold weather in order to maintain cell membrane fluidity and maximize the efficiency at which energy stores can be metabolized. This selective retention of dietary FAs was suggested in other crustacean species (Perez-Velazquez et al., 2003). Such dietary selectivity may also affect lipid stores (Wang et al., 2007). For example, Farkas and Herodek (1964) observed that cool temperatures were correlated with subsequent elongation of dietary FAs in copepods.

Additional effects of fluctuating temperatures on organism systems include differential growth and feeding rates (Lellis and Russell, 1990; Tong et al., 2000), and changes in membrane lipids through cholesterol:lipid ratios (Cuculescu et al., 1995). In the lobsters, *Panulirus japonicus* (von Siebold, 1824) [Matsuda and Yamakawa, 1997] and *Jasus verreauxi* (Milne Edwards, 1851 [Moss et al., 2001], increasing temperatures caused an increase in molt rate at the phyllosoma stage of development. Behavioral temperature-dependent effects were reported to include certain inter- and intra-species interactions (Bertness et al., 1999), as well as the reproductive timing in lobsters (Velázquez, 2003).

The current study investigated the effects of temperatures on the FA profile in *P. crassipes*, because changes in FA content could be an acclimatory mechanism to take advantage of the biochemical properties of FAs at different

temperatures, and may help to rationalize how *P. crassipes* survives fluctuating temperatures characteristic of the intertidal zone.

## Materials and Methods

### Temperature Data and Specimen Collection

Ocean temperatures were obtained online from the Southern California Coastal Ocean Observing System. Temperatures were logged every minute by an automated shore station located approximately 2 m below the mean lowest low tide at the Newport Beach Pier (N33°35′54″, W117°54′03″). Daily averages were calculated using temperatures at 0000, 0600, 1200, and 1800 hrs.

*Pachygrapsus crassipes* were collected six times between April 2006 and February 2007 (April, June, August, October, December, and February). Collections were carried out at the south end of Little Corona del Mar, Newport Beach, California (N33°35′21″, W117°52′05″). Twenty intermolt-stage crabs (10 males, 10 non-gravid females) weighing from 5-10 ± 0.1 g were gathered during each collecting period. Crabs were determined to be intermolt-stage if the carapace was fully hardened and not soft to the touch (Hiatt, 1948). Specimens were immediately frozen in liquid nitrogen, transported to the laboratory, and stored at −75 ± 5°C to prevent FA fluctuations prior to analysis.

### Hepatopancreas Excision

Data were recorded on the carapace width (CW), carapace length (CL), wet weight and sex of each crab prior to hepatopancreas dissection. Carapace measurements were obtained using handheld calipers and recorded to ± 0.1 mm. As much of the hepatopancreas as possible, averaging 0.21 ± 0.01 g, was dissected from each crab and placed in separate plastic screw-top vials. Samples were weighed to ± 0.001 g and stored at −75 ± 5°C prior to FA extraction.

### Fatty Acid Extraction

Extraction procedures followed modified protocols from Styrishave and Andersen (2000) as detailed by Sjoboen (2007). Each sample was manually homogenized in 21 ml of a 1:2 concentration of chloroform: methanol to extract the FAs, and 1 ml of a 1 mg · ml$^{-1}$ (3.2 mM) internal standard (IS; nonadecanoic acid dissolved in chloroform) added to aid in quantification of total FAs. Preliminary analyses showed no indication of the presence of this FA in our samples, thereby validating its use as an IS. Esterification and extraction of the IS served as an internal control for sample FAs. The sample was evaporated with nitrogen while submerged in a 40 ± 5°C water bath, then combined with 1 ml 0.5 M KOH in methanol and incubated in a water bath at 85 ± 5°C for 35 min. This saponification reduces FA degradation and other artifacts (Gutnikov, 1995). One ml of 20% $BF_3$ in methanol was added to resuspend the sample, and incubated for an additional 15 min in an 85 ± 5°C water bath to methylate the FAs to their corresponding fatty acid methyl esters (FAME) for analysis (Gutnikov, 1995). Two ml of HR-GC hexane (EMD Chemicals, Norwood, OH) were added to the sample, and the upper phase transferred to a titration funnel. The solution was washed two times with 10 ml saturated NaCl and two times with 10 ml saturated $NaHCO_3$ to completely free the lipids from proteins and tissue. The salts in the aqueous phase significantly enhance the partitioning of lipids into the organic phase, ensuring effective extraction of lipids from the tissue sample (Folch et al., 1957). This is helpful since there can be up to a 2% loss of lipids in extractions from liver tissue (Folch et al., 1957). The upper (organic) phase was pipetted into a glass GC vial, which was then sealed and stored at −75 ± 5°C until it was analyzed. Individual FAs extracted by this method were compared to total FAs from each sample.

### Fatty Acid Analysis

Fatty acid data were collected by gas chromatography-mass spectroscopy (GC/MS) using a Hewlett-Packard 5890 series II gas chromatograph attached to an HP 5970 series mass selective detector (MSD). Variability between crab samples could not be significantly decreased with multiple GC/MS analyses; therefore, each sample was analyzed once. Methyl esters were separated with an Ultra 2 capillary column (J & W Scientific; length: 12 m; diameter: 0.2 mm; film thickness: 0.33 µm) using a 1 µl 20:1 split injection with helium as the carrier gas. Standard curves were created using commercial fatty acid methyl ester (FAME) standards (Nu-Chek Prep, Inc., Elysian, MN) at varying concentrations with comparison to methyl nonadecanoate (19:0) serving as an IS. This FA was not present in our samples during preliminary analysis. The elution time of each FAME was compared to the standards to determine identity and concentration. Peak analysis was done using MSD ChemStation D.02.00.275 software (Agilent Technologies).

Fatty acids from each sample were quantified, following esterification, by comparison with the IS, and the abundance ratio of each FAME to IS calculated. If the peak was indistinguishable from background noise (< 5:1 signal-to-noise ratio), it was recorded as being absent from the sample. Likewise, if the signal-to-noise ratio was above 5:1 but without a well-defined peak, the particular FAME was deemed to be below the level of quantification. If there was no detection of a FAME during GC/MS analysis it was reported as 0. While this implies that the FAME was below the detection threshold, it does not mean that it was absent from the sample. Due to variation in inter-specimen hepatopancreas excision, concentrations of extracted FAs were standardized according to the weight of hepatopancreas removed from each specimen. Results are reported in µmoles FA · g$^{-1}$ hepatopancreatic tissue.

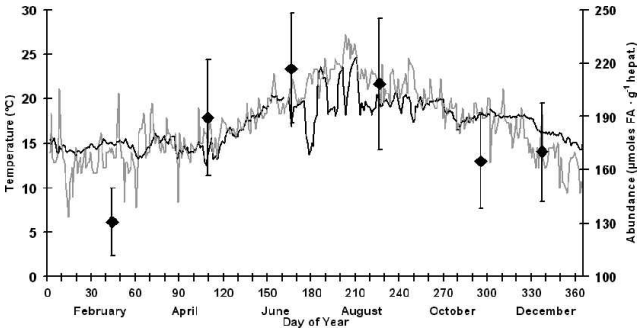


Fig. 1. Overall trend of fatty acid (FA) abundance (◆) in the hepatopancreas of *P. crassipes* for all samples (sexes pooled) in comparison to water (▬) and air (▬) temperatures from Newport Beach, California. Units are in µmole FA · g$^{-1}$ hepatopancreatic tissue ± 1 standard error. $N = 120$ specimens.

### Statistical Analyses

All data were analyzed using the Statistical Package for the Social Sciences (SPSS) with alpha = 0.05. Fatty acid data failed to meet the assumptions of homogeneity and normality and were not improved by log or rank transformation. Data also did not meet sphericity assumptions with Mauchly's test. As a result, original FA data were used for the analyses and some results are reported using the Greenhouse-Geisser adjustment for degrees of freedom.

## Results

### Temperatures

Average daily water temperatures with respect to each month were significantly different in temperature among months (one way MANOVA, $F_{(22,698)} = 53.63$, $P < 0.001$, partial $\eta^2 = 0.628$). Through post-hoc analyses we determined that December-May had significantly lower water temperatures than the rest of the year. Our results revealed that month had a significant influence on water temperatures (ANOVA, $F_{(11,349)} = 164.57$, $P < 0.001$, partial $\eta^2 = 0.83$). Throughout the year, water temperatures ranged between a low of 12.6°C in April and May, and a high of 24.7°C in July (Fig. 1).

### Fatty Acids

When we analyzed width of *P. crassipes* (irrespective of sex), the mass of excised hepatopancreatic tissue, and total FA abundance for each hepatopancreas by Pearson's correlations, we found total FA abundance to be independent of both carapace width and hepatopancreas mass. It was clear, however, that the hepatopancreatic mass varied significantly among months (ANCOVA, $F_{(5,107)} = 5.40$, $P < 0.001$, partial $\eta^2 = 0.201$). This pattern suggests artifactual differences occurring as a result of specimen transport and handling. The hepatopancreas of some specimens was more difficult to remove than that of others, resulting in a variation of total extracted tissue.

To evaluate the possible relationship between HFA content and body condition, we used residuals from the regression of body mass on CW as a measure of relative body condition. Although body mass was affected by leg loss for many specimens (not accounted for in measurements) and differences between sexes and months, there was still a strong association of body mass and CW ($r^2 = 0.55$). Total HFA content (μmoles FA $\cdot$ $g^{-1}$ hepatopancreatic tissue) was then regressed on the residuals (i.e., body condition). The negligible association ($r^2 = 0.02$) suggested that total HFA content corresponded poorly with body condition.

Sex Analyses.—Despite variations in monthly patterns, the difference in the abundance of either individual FAs or FA saturation categories between females and males was not significant throughout the year (Table 1). The only observed difference was a higher concentration of MUFAs in females than males during the month of December, as indicated in Table 1. We pooled the mean FA abundances from both sexes for subsequent analyses, because we found no significant differences in FA abundances between sexes (Table 2).

Temporal Trends.—We found that the individual abundances of palmitoleic, palmitic, and docosahexaenoic acids fluctuated significantly between months (palmitoleic: $F_{(5,114)} = 3.28$, $P = 0.008$; palmitic: $F_{(5,114)} = 3.10$, $P = 0.012$; docosahexaenoic: $F_{(5,114)} = 5.19$, $P < 0.001$; Table 2). Palmitoleic and palmitic acids had significantly higher abundances in April, June and August than in February and October. Docosahexaenoic acid was significantly higher in June and August than in February, October, and December. All three FAs had a significantly higher abundance during warm months than during cold months. It is interesting to note, however, that there is a sharp increase in FA abundance between February and April, while temperatures remain fairly stable (Fig. 1).

Percent composition of each FA with respect to total FAs is shown in Table 3. We found a significant difference of abundances among FA saturation categories, represented by saturated (SAFA), monounsaturated (MUFA), and polyunsaturated (PUFA) FAs ($F_{(1.7,182.8)} = 115.68$, $P < 0.001$; Fig. 2). There was also a significant interaction between FA saturation category and month (each individual category was affected differently over time when compared to one another), where the abundance of FA saturation categories fluctuated significantly among months, with higher overall abundance of FAs seen in warmer months ($F_{(8.5,182.8)} = 4.09$, $P < 0.001$). Therefore, when looking at a particular month it may be possible to predict an approximate abundance of FAs, or by looking at FA abundance it may be possible to predict an approximate time frame (month) in which this abundance could be expected. Analysis of FA saturation categories in terms of percent by concentration of total FAs (Fig. 3) resulted in improved resolution in temporal trends. While percents SAFA and MUFA followed similar trends with both maxima in June, percent PUFA exhibited a reversed pattern with a minimum in June, while dominating at other times. These trends exhibit both temporal and inter-category significance (Table 3).

## Discussion

Throughout this study, we found that water temperatures in Newport Beach were significantly lower during December-May when compared to the rest of the year. We also discovered that HFA content was independent of crab size, corresponded poorly with body condition and that there were no significant sexual differences. Of all the FAs analyzed, three of these (palmitoleic, palmitic, and docosahexaenoic acids) had significantly higher abundance during warm months when compared to their abundance during cold months. There were also differences in abundance between saturation categories, and an overall higher abundance of total FAs in warm months than in cold months.

The decapod hepatopancreas is the primary location of energetic lipid storage, and FA composition is determined by a combination of diet, biosynthesis (O'Connor and Gilbert, 1968), and temperature. In our study, there was a substantial fluctuation in abundance of individual FA identities from month to month. This may suggest crabs are undergoing preferential utilization or accumulation of specific FAs during these periods of time. Guerra-García et al. (2004) found that environmental temperatures and diet influenced FA composition in caprellidean amphipods from the Strait of Gibraltar. As a result of temperature-induced changes, lipid membranes and FA stores undergo homeoviscous adaptation; ideal viscosity for immediate environmental conditions (Hazel and Williams, 1990; Willmer et al., 2000). Desaturase enzymes control homeoviscous adaptation, and are affected by both temperature and ingestion of saturated lipids regardless of temperature. This may occur by FA synthesis or by the oxidation/reduction of existing FAs (Van Handel, 1966). If FAs are too viscous in cold weather, *P. crassipes* may be unable to access valuable FA stores. In the same way, if FAs are insufficiently viscous in warm weather, cell membranes may ''melt'' and FA stores may be inaccessible. Therefore, it may be beneficial for *P. crassipes* to store different types of FAs at different temperatures in order to take advantage of specific biochemical properties of FAs. These FA changes may also help to reveal details of energy utilization (Chapelle, 1986). Lehti-Koivunen and Kivivuori (1998) recognized homeoviscous adaptation occurring in neuronal membranes of the crayfish, *Astacus astacus* (Linnaeus, 1758), with

Table 1. Mean monthly fatty acid (FA) abundance of FA types and FA saturation category compared between sexes. Units are in μmole FA · $g^{-1}$ hepatopancreatic tissue ± 1 standard error. The symbol ∑ indicates total abundance for each category. Bold numbers indicate a significant difference ($P < 0.05$) between females and males. $n = 10$ males and 10 females for each month, $N = 120$.

| | February 2007 | | April 2006 | | June 2006 | | August 2006 | | October 2006 | | December 2006 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male |
| SAFA | | | | | | | | | | | | |
| 14:0 | 9.68 ± 4.21 | 3.04 ± 0.63 | 7.30 ± 2.45 | 7.12 ± 2.03 | 10.12 ± 1.76 | 9.86 ± 3.15 | 10.50 ± 2.56 | 9.85 ± 3.23 | 6.38 ± 1.80 | 7.62 ± 2.61 | 7.80 ± 1.62 | 3.62 ± 1.33 |
| 16:0 | 13.43 ± 2.58 | 9.66 ± 1.81 | 18.17 ± 4.02 | 20.86 ± 4.58 | 26.51 ± 3.45 | 25.47 ± 5.89 | 21.39 ± 4.30 | 18.18 ± 5.29 | 12.88 ± 2.69 | 17.68 ± 4.30 | 21.27 ± 3.63 | 11.44 ± 3.21 |
| 18:0 | 9.24 ± 1.67 | 7.34 ± 1.17 | 9.70 ± 1.48 | 12.88 ± 2.30 | 14.02 ± 1.62 | 12.73 ± 2.11 | 11.78 ± 2.02 | 12.28 ± 3.06 | 7.72 ± 1.37 | 11.16 ± 2.07 | 12.00 ± 1.82 | 8.62 ± 2.09 |
| 20:0 | 2.98 ± 0.94 | 1.44 ± 0.41 | 1.61 ± 0.66 | 1.92 ± 0.56 | 2.48 ± 0.43 | 3.47 ± 2.08 | 1.93 ± 0.57 | 3.15 ± 1.14 | 2.05 ± 0.47 | 2.93 ± 0.81 | 2.78 ± 0.48 | 1.66 ± 1.04 |
| 22:0 | 0.69 ± 0.28 | 0.38 ± 0.23 | 0.35 ± 0.25 | 0.75 ± 0.32 | 0.29 ± 0.20 | 0.00 ± 0.00 | 0.47 ± 0.26 | 1.43 ± 0.91 | 0.33 ± 0.10 | 0.93 ± 0.33 | 1.20 ± 0.33 | 0.73 ± 0.55 |
| 24:0 | 0.40 ± 0.27 | 0.30 ± 0.17 | 0.47 ± 0.26 | 0.84 ± 0.37 | 0.07 ± 0.07 | 0.00 ± 0.00 | 0.33 ± 0.22 | 1.06 ± 0.75 | 0.06 ± 0.04 | 0.49 ± 0.28 | 0.94 ± 0.31 | 0.81 ± 0.65 |
| ∑SAFA | 36.42 ± 8.51 | 22.17 ± 4.22 | 37.59 ± 8.81 | 44.37 ± 9.71 | 53.49 ± 6.89 | 51.52 ± 10.97 | 46.41 ± 9.53 | 45.95 ± 13.17 | 29.41 ± 6.27 | 40.81 ± 9.91 | 45.98 ± 7.29 | 26.88 ± 8.19 |
| MUFA | | | | | | | | | | | | |
| 14:1 | 1.23 ± 0.35 | 0.41 ± 0.15 | 1.55 ± 0.91 | 1.45 ± 0.48 | 1.59 ± 0.51 | 1.43 ± 0.60 | 2.02 ± 0.67 | 2.54 ± 0.91 | 1.05 ± 0.38 | 1.29 ± 0.62 | 1.60 ± 0.47 | 0.57 ± 0.31 |
| 16:1 | 16.60 ± 3.41 | 7.97 ± 2.30 | 23.38 ± 6.87 | 25.91 ± 7.36 | 35.08 ± 5.46 | 31.48 ± 9.72 | 26.03 ± 5.99 | 25.05 ± 7.86 | 14.48 ± 3.97 | 18.39 ± 6.08 | 24.05 ± 4.72 | 10.96 ± 4.39 |
| 18:1 | 34.50 ± 6.96 | 22.87 ± 5.05 | 36.21 ± 9.26 | 45.66 ± 11.89 | 51.50 ± 7.62 | 57.48 ± 15.60 | 40.08 ± 8.27 | 44.92 ± 13.06 | 27.47 ± 6.83 | 37.65 ± 9.98 | 49.47 ± 8.69 | 23.23 ± 7.24 |
| 20:1 | 4.74 ± 1.11 | 3.71 ± 1.11 | 4.37 ± 1.23 | 6.27 ± 1.71 | 4.73 ± 0.60 | 3.61 ± 1.00 | 4.65 ± 1.07 | 5.37 ± 1.76 | 2.72 ± 0.65 | 4.46 ± 1.10 | 5.72 ± 1.61 | 3.27 ± 1.26 |
| 24:1 | 0.13 ± 0.09 | 0.13 ± 0.10 | 0.00 ± 0.00 | 0.40 ± 0.19 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.07 ± 0.07 | 0.68 ± 0.55 | 0.00 ± 0.00 | 0.32 ± 0.22 | 0.63 ± 0.19 | 0.43 ± 0.41 |
| ∑MUFA | 57.21 ± 11.66 | 35.09 ± 8.37 | 65.51 ± 18.00 | 79.69 ± 20.74 | 92.90 ± 12.81 | 94.01 ± 83.53 | 72.85 ± 15.52 | 78.56 ± 23.60 | 45.73 ± 11.63 | 62.11 ± 17.55 | **81.46 ± 14.27** | **38.45 ± 13.26** |
| PUFA | | | | | | | | | | | | |
| 18:2 | 20.29 ± 4.07 | 11.59 ± 2.34 | 20.43 ± 6.11 | 20.80 ± 4.63 | 19.81 ± 2.15 | 17.94 ± 4.51 | 19.79 ± 4.77 | 20.70 ± 5.92 | 19.96 ± 3.75 | 23.39 ± 5.71 | 26.97 ± 3.75 | 17.45 ± 5.51 |
| 18:3 | 10.80 ± 2.10 | 7.06 ± 1.44 | 11.92 ± 3.70 | 11.84 ± 2.89 | 11.17 ± 1.59 | 9.94 ± 3.08 | 15.19 ± 4.46 | 12.90 ± 3.99 | 12.21 ± 2.62 | 15.33 ± 4.16 | 16.58 ± 2.72 | 10.37 ± 3.35 |
| 20:4 | 19.02 ± 2.92 | 19.02 ± 2.88 | 17.05 ± 3.94 | 18.26 ± 3.61 | 13.61 ± 2.29 | 14.52 ± 4.94 | 20.15 ± 4.83 | 22.68 ± 4.75 | 20.83 ± 2.47 | 28.85 ± 4.75 | 29.79 ± 4.07 | 16.84 ± 4.49 |
| 20:3 | 3.19 ± 0.77 | 2.20 ± 0.60 | 2.69 ± 0.76 | 3.69 ± 0.91 | 2.18 ± 0.35 | 1.65 ± 0.63 | 3.22 ± 0.84 | 2.97 ± 0.72 | 2.43 ± 0.43 | 2.72 ± 0.67 | 4.60 ± 0.76 | 3.00 ± 1.42 |
| 20:2 | 5.22 ± 1.34 | 3.93 ± 1.00 | 5.16 ± 1.22 | 7.16 ± 1.76 | 5.20 ± 0.53 | 4.72 ± 1.34 | 5.55 ± 1.24 | 6.26 ± 1.78 | 3.59 ± 0.67 | 4.59 ± 0.87 | 6.48 ± 1.30 | 4.37 ± 1.75 |
| 22:6 | 5.14 ± 1.35 | 3.12 ± 0.92 | 10.16 ± 3.57 | 22.17 ± 6.19 | 22.19 ± 6.17 | 19.30 ± 6.91 | 20.52 ± 5.76 | 23.08 ± 9.00 | 6.65 ± 1.62 | 11.55 ± 3.68 | 4.86 ± 1.01 | 6.17 ± 2.97 |
| ∑PUFA | 63.65 ± 11.81 | 46.92 ± 8.64 | 67.42 ± 18.24 | 80.87 ± 18.71 | 74.16 ± 11.21 | 68.07 ± 20.46 | 84.41 ± 20.85 | 88.58 ± 24.75 | 65.67 ± 10.52 | 86.43 ± 17.94 | 89.28 ± 12.46 | 58.21 ± 18.47 |
| ∑FA | 157.3 ± 27.2 | 104.2 ± 18.7 | 170.5 ± 39.8 | 204.9 ± 43.1 | 220.6 ± 26.7 | 213.6 ± 50.8 | 203.7 ± 41.3 | 213.1 ± 53.9 | 140.8 ± 24.6 | 189.3 ± 39.6 | 216.7 ± 29.5 | 123.5 ± 36.2 |

Table 2. Mean monthly fatty acid (FA) abundance of FA identity and FA saturation category for pooled sexes. Three FAs fluctuated among months (palmitic acid = 16:0, palmitoleic acid = 16:1, docosahexaenoic acid (DHA) = 22:6), with significantly higher abundance during the months of April, June and August compared to February, October and December. Bold numbers indicate the FAs which fluctuated significantly. Units are in µmole FA · $g^{-1}$ hepatopancreatic tissue ± 1 standard error. The symbol ∑ indicates total abundance for each category. $N$ = 120 specimens.

| | February | April | June | August | October | December |
|---|---|---|---|---|---|---|
| SAFA | | | | | | |
| 14:0 | 6.36 ± 3.32 | 7.21 ± 0.09 | 9.99 ± 0.13 | 10.18 ± 0.32 | 7.00 ± 0.62 | 5.71 ± 2.09 |
| 16:0 | **11.54 ± 1.89** | **19.51 ± 1.35** | **25.99 ± 0.52** | **19.78 ± 1.60** | **15.28 ± 2.40** | **16.36 ± 4.92** |
| 18:0 | 8.29 ± 0.95 | 11.29 ± 1.59 | 13.37 ± 0.65 | 12.03 ± 0.25 | 9.44 ± 1.72 | 10.31 ± 1.69 |
| 20:0 | 2.21 ± 0.77 | 1.76 ± 0.16 | 2.97 ± 0.49 | 2.54 ± 0.61 | 2.49 ± 0.44 | 2.22 ± 0.56 |
| 22:0 | 0.54 ± 0.15 | 0.55 ± 0.20 | 0.14 ± 0.14 | 0.95 ± 0.48 | 0.63 ± 0.30 | 0.96 ± 0.23 |
| 24:0 | 0.35 ± 0.05 | 0.65 ± 0.19 | 0.03 ± 0.03 | 0.70 ± 0.36 | 0.27 ± 0.22 | 0.87 ± 0.06 |
| ∑SAFA | 29.3 ± 9.9 | 41.0 ± 9.1 | 52.5 ± 8.3 | 46.2 ± 10.3 | 35.1 ± 9.7 | 36.4 ± 12.6 |
| MUFA | | | | | | |
| 14:1 | 0.82 ± 0.41 | 1.50 ± 0.05 | 1.51 ± 0.08 | 2.28 ± 0.26 | 1.17 ± 0.12 | 1.08 ± 0.52 |
| 16:1 | **12.29 ± 4.31** | **24.65 ± 1.26** | **33.28 ± 1.80** | **25.54 ± 0.49** | **16.44 ± 1.95** | **17.50 ± 6.54** |
| 18:1 | 28.69 ± 5.82 | 40.93 ± 4.73 | 54.49 ± 2.99 | 42.50 ± 2.42 | 32.56 ± 5.09 | 36.35 ± 13.12 |
| 20:1 | 4.23 ± 0.51 | 5.32 ± 0.95 | 4.17 ± 0.56 | 5.01 ± 0.36 | 3.59 ± 0.87 | 4.50 ± 1.23 |
| 24:1 | 0.13 ± 0.00 | 0.20 ± 0.20 | 0.00 ± 0.00 | 0.38 ± 0.30 | 0.16 ± 0.16 | 0.53 ± 0.10 |
| ∑MUFA | 46.2 ± 15.1 | 72.6 ± 19.0 | 93.5 ± 18.6 | 75.7 ± 18.1 | 53.9 ± 16.1 | 60.0 ± 26.6 |
| PUFA | | | | | | |
| 18:2 | 15.94 ± 4.35 | 20.62 ± 0.18 | 18.87 ± 0.94 | 20.24 ± 0.46 | 21.67 ± 1.72 | 22.21 ± 4.76 |
| 18:3 | 8.93 ± 1.87 | 11.88 ± 0.04 | 10.55 ± 0.62 | 14.04 ± 1.15 | 13.77 ± 1.56 | 13.48 ± 3.10 |
| 20:4 | 19.02 ± 0.00 | 17.65 ± 0.61 | 14.07 ± 0.45 | 21.41 ± 1.27 | 24.84 ± 4.01 | 23.32 ± 6.47 |
| 20:3 | 2.70 ± 0.50 | 3.19 ± 0.50 | 1.92 ± 0.26 | 3.09 ± 0.12 | 2.57 ± 0.15 | 3.80 ± 0.80 |
| 20:2 | 4.57 ± 0.64 | 6.16 ± 1.00 | 4.96 ± 0.24 | 5.91 ± 0.35 | 4.09 ± 0.50 | 5.43 ± 1.05 |
| 22:6 | **4.13 ± 1.01** | **16.17 ± 6.00** | **20.75 ± 1.45** | **21.80 ± 1.28** | **9.10 ± 2.45** | **5.51 ± 0.66** |
| ∑PUFA | 55.3 ± 13.0 | 74.1 ± 18.1 | 71.1 ± 15.1 | 86.5 ± 20.6 | 76.1 ± 17.4 | 73.8 ± 22.1 |
| ∑FA | 130.7 ± 19.3 | 189.3 ± 32.5 | 217.1 ± 31.1 | 208.4 ± 37.1 | 165.1 ± 26.3 | 170.1 ± 27.5 |

maintenance of membrane fluidity by increasing the content of unsaturated lipids during cooler temperatures and decreasing it in warmer temperatures. Similarly, Lahdes et al. (2000) observed changes in the fluidity of membrane lipids of the *Gammarus* spp. amphipod corresponding to changes in habitat temperatures. Perez-Velazquez et al. (2003) looked at FAs in male *Litopenaeus vannamei* (Boone, 1931) shrimp held at 26°C and 32°C. They noticed a higher percentage of more saturated FAs at 32°C than at 26°C, and more unsaturated FAs at 26°C than 32°C as would be expected if the FAs were attaining a conformation suitable to the surrounding temperatures. Because of such FA adaptation, *P. crassipes* may be more able to remain active in temperature extremes to avoid predation, acquire food, and locate mates.

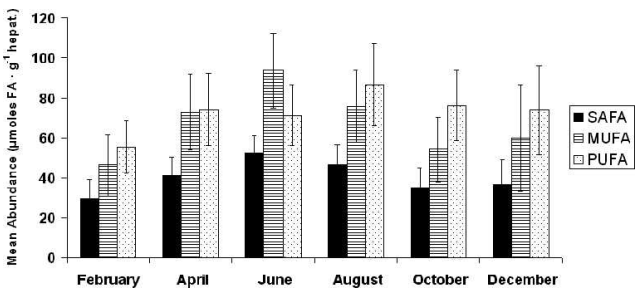


Fig. 2. Comparison of the abundance of fatty acid (FA) saturation categories (saturated FA (SAFA) = ■, monounsaturated FA (MUFA) = ▤, polyunsaturated FA (PUFA) = ⬚). Units are in µmole FA · $g^{-1}$ hepatopancreatic tissue ± 1 standard error. $N$ = 120 specimens.

Other possible contributors to the fluctuations in FA identities we observed in *P. crassipes* may include food availability and molt cycle. If metabolic cost is not met by an increase in food intake, stored fat resources may be utilized and would be reflected by a decrease in stored FAs, such as that seen in the body composition of the amphipod,

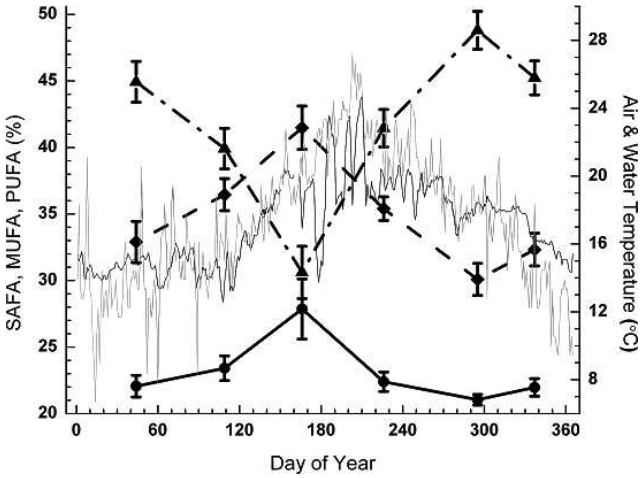


Fig. 3. Seasonal changes in the relative proportions of fatty acid (FA) categories. The FA categories are represented by percent saturated FAs (SAFA = ●), monounsaturated FAs (MUFA = ◆) and polyunsaturated FAs (PUFA = ▲). The percentages were calculated for each crab and then averaged for each sampling time. Units are in % by concentration (µmole FA·$g^{-1}$ hepatopancreatic tissue) ± 1 standard error. $N$ = 120 specimens. Water (▬) and air (▬) temperatures are presented for Newport Beach, California.

Table 3. Percentage total fatty acids (FA) for each FA with pooled sexes. Percentage was determined by the equation: $\% = 100\left(\frac{[FA]}{\sum [FA]}\right)$. Units are percentages ± 1 standard error. $n = 20$ specimens for each month, $N = 120$.

| | February | April | June | August | October | December |
|---|---|---|---|---|---|---|
| Myristate | 4.54 ± 1.23 | 3.85 ± 0.74 | 4.60 ± 0.37 | 4.89 ± 0.83 | 4.28 ± 0.80 | 3.26 ± 0.56 |
| Palmitate | 8.91 ± 1.01 | 10.34 ± 1.36 | 11.97 ± 0.72 | 9.52 ± 1.40 | 9.24 ± 1.25 | 9.54 ± 1.31 |
| Stearate | 6.46 ± 0.67 | 5.94 ± 0.60 | 6.16 ± 0.33 | 5.77 ± 0.74 | 5.69 ± 0.62 | 6.26 ± 0.82 |
| Arachidate | 1.64 ± 0.31 | 0.93 ± 0.20 | 1.37 ± 0.14 | 1.21 ± 0.26 | 1.50 ± 0.23 | 1.32 ± 0.37 |
| Behenate | 0.40 ± 0.12 | 0.28 ± 0.09 | 0.06 ± 0.04 | 0.45 ± 0.20 | 0.37 ± 0.09 | 0.57 ± 0.20 |
| Lignocerate | 0.27 ± 0.10 | 0.34 ± 0.10 | 0.02 ± 0.01 | 0.33 ± 0.16 | 0.15 ± 0.07 | 0.54 ± 0.23 |
| Myristoleate | 0.59 ± 0.12 | 0.80 ± 0.25 | 0.70 ± 0.10 | 1.09 ± 0.23 | 0.72 ± 0.18 | 0.60 ± 0.14 |
| Palmitoleate | 9.10 ± 1.36 | 13.09 ± 2.27 | 15.32 ± 1.15 | 12.27 ± 2.00 | 10.00 ± 1.80 | 9.98 ± 1.78 |
| Oleate | 21.95 ± 2.77 | 21.59 ± 3.33 | 25.13 ± 1.70 | 20.38 ± 3.11 | 19.70 ± 3.02 | 20.81 ± 3.04 |
| 11-Eicosenoate | 3.29 ± 0.54 | 2.79 ± 0.46 | 1.92 ± 0.14 | 2.40 ± 0.41 | 2.14 ± 0.32 | 2.64 ± 0.53 |
| Nervonate | 0.10 ± 0.05 | 0.10 ± 0.05 | 0.00 ± 0.00 | 0.18 ± 0.12 | 0.08 ± 0.05 | 0.32 ± 0.14 |
| Linoleate | 12.01 ± 1.46 | 10.99 ± 1.80 | 8.69 ± 0.47 | 9.71 ± 1.53 | 13.26 ± 1.71 | 13.29 ± 2.03 |
| Linolenate | 6.82 ± 0.81 | 6.34 ± 1.10 | 4.86 ± 0.34 | 6.75 ± 1.23 | 8.38 ± 1.22 | 8.02 ± 1.26 |
| Arachidonate | 15.17 ± 1.63 | 9.39 ± 1.23 | 6.49 ± 0.51 | 10.27 ± 1.38 | 15.02 ± 1.30 | 13.69 ± 1.73 |
| Homogamma linolenate | 2.07 ± 0.32 | 1.68 ± 0.26 | 0.88 ± 0.08 | 1.49 ± 0.23 | 1.58 ± 0.20 | 2.28 ± 0.51 |
| 11,14-Eicosadienoate | 3.55 ± 0.55 | 3.24 ± 0.47 | 2.28 ± 0.12 | 2.83 ± 0.44 | 2.49 ± 0.28 | 3.26 ± 0.65 |
| Docosahexaenoate | 3.13 ± 0.52 | 8.31 ± 1.66 | 9.55 ± 1.23 | 10.45 ± 2.15 | 5.41 ± 0.97 | 3.62 ± 1.09 |
| SAFA | 22.06 ± 0.82 | 23.41 ± 0.93 | 27.86 ± 2.26 | 22.39 ± 0.73 | 21.04 ± 0.40 | 21.97 ± 0.66 |
| MUFA | 32.90 ± 1.55 | 36.45 ± 1.20 | 41.49 ± 1.63 | 35.40 ± 0.89 | 30.08 ± 1.20 | 32.33 ± 1.23 |
| PUFA | 44.93 ± 1.54 | 39.92 ± 1.53 | 30.61 ± 1.97 | 41.45 ± 1.41 | 48.81 ± 1.43 | 45.23 ± 1.28 |

*Monoporeia affinis* (Lindstrom, 1855) [Lehtonen, 1996], and the sardine, *Sardina pilchardus* (Walbaum, 1792) [Bandarra et al., 1997], due to changes in food availability. Barry and Ehret (1993) discovered that while *P. crassipes* has a definite preference for certain types of algae, they will modify their diet according to availability. Hopkins et al. (1993) found that the diet of the prawn, *Pandalus borealis* (Krøyer, 1838), affected lipid composition of the animal. This would particularly be noticeable by the consumption of various algae species, since different algal species vary in FA composition (Dembitsky et al., 2003). Molt cycle also affects FA composition, as Jeckel et al. (1990) demonstrated in females of the shrimp, *Pleoticus muelleri* (Bate, 1888). They showed that pre-molt accumulation and synthesis of FAs, and FA metabolism during the molting period, contributed to variations in FA abundance in the digestive gland.

The effect of the average monthly temperatures may also partially explain the difference in FA saturation categories. Diet and available food abundance (Hopkins et al., 1993; Goedkoop et al., 2000) may contribute toward this monthly variance in FA saturation category. Changes in algal and meat availability may affect fluctuations in FAs, since *P. crassipes* is known to modify its diet to correspond with food abundance (Barry and Ehret, 1993). Aguilar-Rosas et al. (2002) observed that there was a higher diversity of algal species during autumn months than during summer months in the Gulf of Santa Clara, Mexico. Types of lipid and lipid abundance in algae also fluctuate seasonally. Accumulation of lipids occurs in algae when there is high illumination and little available nitrogen, which together permits photosynthesis and inhibits protein synthesis and growth (R. Lewin, personal communication). In addition, Dembitsky et al. (2003) showed differences in the FA profile of various algae. Therefore, as is likely, since algal species and abundances in the intertidal zone fluctuate with environmental temperatures and other seasonal factors (Cubit, 1984; Aguilar-Rosas et al., 2002), the hepatopancreatic fatty acid (HFA) composition of *P. crassipes* may fluctuate seasonally as a result of algal consumption. Fatty acid composition differs interspecifically in animals as well (Çelik et al., 2004), and therefore variations in consumed meat sources may also contribute to FA differences in *P. crassipes*. While the relationship of diet to FA content was not analyzed in this study, we did observe a difference in FAs throughout the year. Comparing these changes to diet in future studies may reveal more about the lifestyle and adaptive capabilities of *P. crassipes* and their relationship to the intertidal environment. However, some changes in FA abundance may not be evident due to high inter-individual variation, a pattern also seen in other marine crustaceans (Heath and Barnes, 1970).

During warm months (June, August, October), we found that *P. crassipes* exhibited a higher abundance of FAs compared with cold months (February, April, December), particularly noticeable in the PUFAs. We found that, except for June when MUFAs were most abundant, PUFAs in *P. crassipes* were more abundant than both SAFAs and MUFAs. Styrishave and Andersen (2000) found similar results in *Carcinus maenas* (Linnaeus, 1758), with significantly higher concentrations of PUFAs than SAFAs and MUFAs. They attributed this difference to the lifestyle of the crab, where PUFAs are retained during energetically taxing events and occasions of limited feeding, such as during the summer molting and reproductive periods. The abundance of PUFAs relies directly on the diet of the organism. Polyunsaturated FAs are most affected by changes in temperature and possible associated dietary modifications since they are not synthesized de novo by the crab (Brett and Müller-Navarra, 1997). Instead, they must be consumed and converted into needed FAs by elongation, desaturation, or oxidation.

Temperature has broad reaching effects on the physiology and behavior of animals, such as seen in growth rates of the lobster, *Jasus edwardsii* (Hutton, 1875) [Thomas et al., 2000]. Roberts (1957b) reported that the activity rate of

*P. crassipes* is slower during cooler temperatures than in warmer temperatures, most likely resulting in a lower foraging rate and less food consumption in cool months. Hiatt (1948) observed that 18.3°C was a transitional temperature for *P. crassipes*, since below this temperature, crabs were slower than at temperatures above 18.3°C. Monthly temperatures throughout the present study period averaged at or above 18.4°C between June and September, corresponding with the crab's potential increase in foraging and activity intensity. In *P. crassipes*, metabolism was shown to increase, beginning approximately two weeks before undergoing a molt, and was maintained at that rate throughout the molting process (Roberts, 1957a). Since *P. crassipes* fasts while molting, it is necessary to build up energy stores prior to the energetically demanding molt cycle. Because a higher molting rate is found in warm months than cool months (Roberts, 1957b), there is likely to be a lower abundance of FAs during the warm months than may be anticipated from the high activity and foraging rate of *P. crassipes*. For our study, only individuals in intermolt stage were sampled; therefore, inferences about the effect of molting on stored FAs are beyond the scope of this work.

In addition to diet and molting, mating may also be a factor in fluctuating FA abundance. Mating in *P. crassipes* occurs once or twice a year between February and October, but is most frequent in warm months with a peak in June (Hiatt, 1948; Ricketts et al., 1985). Mating activity in other crustaceans, such as the spiny lobster, *Panulirus interruptus* (Randall, 1840), also exhibits a temporal trend, with higher frequency during warm temperatures than cool temperatures (Velázquez, 2003). Because this period is physically demanding, lifestyle is expected to contribute to the HFA profile at any given point in time. It is not known whether these changes in FA profile are also physiologically affected by environmental temperatures, either as a primary effect, e.g., trigger for activating transcription of desaturase enzymes within the crab (Aguilar et al., 1999), or a secondary effect, e.g., change in food source (Cubit, 1984), environmental stimuli for molting (Roberts, 1957b) or migration (Asakura and Kikuchi, 1984).

Homeoviscous adaptation is not an immediate response. If the changes we saw in FAs were a direct result of homeoviscous adaptation, we may expect a lag time for changes in FA saturation to occur, as seen in the compound eyes of the crab, *Hemigrapsus sanguineus* (de Haan, 1835) [Eguchi et al., 1994]. However, if changes in FAs were the result of behavioral temperature adaptations, i.e., dietary modification, we might expect a more rapid adjustment in HFA. Maskrey et al. (2005) found that FAs in the barnacle, *Balanus perforatus* Bruguiére, 1789, followed a pattern suggestive of homeoviscous adaptation. They observed changes in the FA saturation ratio to fluctuate from a higher saturated/unsaturated ratio during warm summer months to a lower saturated/unsaturated ratio during cool winter months. Our data suggest the FA profile of *P. crassipes* may be partially due to change in behavior, since abundance in FAs fluctuated somewhat independently of environmental temperatures, and may precede fluctuations in water temperature, possibly as a result of changing day length. Trends of PUFA, MUFA or SAFA, as percents of total FAs, appear to be leading the environmental temperature changes rather than responding to them (Fig. 3). Thus, the change in behavior may be an increase in activity and foraging during summer months (Roberts, 1957b), contributing to an increase in HFA. In contrast, a decrease in activity and foraging during winter months would likely result in reduced HFA. These conclusions are warranted since the activity level of many crustacean species fluctuates with light conditions (Lagerspetz and Vainio, 2006). For example, Hoang et al. (2003) observed that light intensity and photoperiod affected growth rates in the prawn, *Fenneropenaeus merguiensis* (De Man, 1888). Lipcius and Herrnkind (1985) showed that behavior of spiny lobsters was affected by both photoperiod and temperature, such that under conditions of longer day length or warmer temperatures, the lobsters mated with an increased frequency. Smith et al. (1999) also found activity of the lobster, *Homarus gammarus* (Linnaeus, 1758), to vary seasonally based primarily on temperature, but also on illumination. They observed that lobster activity was positively correlated to water temperature and negatively correlated to illumination.

In summary, we found a monthly change in the HFA of *P. crassipes*, but that this change did not appear to be solely related to fluctuations in the environmental temperatures of the study site. This implies that although temperatures differ across months, this difference may not be sufficiently large to directly influence changes in FA storage. However, changing day light length, resulting in subsequent temperature changes, may be a distinct environmental factor triggering changes in activity or dietary preference, or it may directly affect the abundance of particular food sources. Evidence from our study suggests differences in temperature of the narrow range experienced on rocky shores of southern California are insufficient to directly lead to large fluctuations in HFA in this species. Nevertheless, the effects of temperature may be indirect, influencing behavioral responses or modifications in lifestyle activities, such as food choice, mating, and molting, leading to fluctuations in FA abundance.

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