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# Nucleic acids and fatty acids of the common octopus, *Octopus vulgaris*, in relation to the growth rate

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## Abstract

The aim of this study was to investigate changes in the ribonucleic acid (RNA)/deoxyribonucleic acid (DNA) ratio and the fatty acid composition of cultured *Octopus vulgaris* (50–750 g) in relation to recent (last 30 days) specific growth rate (SGR). Wild animals (80–500 g), collected in the field throughout the year (Aegean Sea, Mediterranean), were also examined for the aforementioned biochemical parameters. Octopuses were reared in a closed seawater system at three different temperatures (15, 20 and 25 °C). The octopuses were fed on squid (*Loligo vulgaris*). The cultured animals showed a high n-3 (33–52.9%) and n-6 (3.3–13.7%) polyunsaturated fatty acid content, but with a high variation for 22:6n-3 [docosahexaenoic acid (DHA)], 20:5n-3 [eicosapentaenoic acid (EPA)] and 20:4n-6 [arachidonic acid (AA)]. The proportion of these fatty acids (% total fatty acids) and the RNA/DNA ratio were linearly ($P < 0.0001$) related to SGR. Specifically, RNA/DNA (0.5–1.9) and AA (2.7–10.7%) increased, while EPA (10.4–19.7%) and DHA (20.8–31.9%) decreased, with increasing SGR (0.4–1.7% day$^{-1}$). The highest levels of SGR, RNA/DNA and AA were detected in small (50–150 g) octopuses reared at 20 and 25 °C and in large (500–750 g) animals reared at 15 °C. Similar RNA/DNA levels and fatty acid percentages were found in wild octopuses. It is concluded that RNA/DNA, DHA, EPA and AA could be used as biochemical indices for predicting the growth rate of *O. vulgaris*.

**Keywords:** *Octopus vulgaris*, fatty acid composition, RNA, DNA, growth rate

## Introduction

*Octopus vulgaris* meets many of the criteria for intensive aquaculture. These include high fecundity (Mangold 1983), fast growth and easy adaptation to captivity conditions (Iglesias, Sánchez, Otero & Moxica 2000), high feed efficiency (Mangold & von Boletzky 1973) and high market price (Vaz-Pires, Seixas & Barbosa 2004). Common octopus ongrowing has recently begun to develop in Mediterranean countries, either in tanks (García García & Aguado Giménez 2002; Miliou, Fintikaki, Kountouris & Verriopoulos 2005; García García & Cerezo Valverde 2006) or in floating cages (Rodríguez, Carrasco, Arronte & Rodríguez 2006).

Mediterranean cephalopods contain large amounts of polyunsaturated fatty acids (PUFAs; 52.9–56.3% of total fatty acids) (Sinanoglou & Miniadis-Meimaroglou 1998). The most characteristic PUFAs of cephalopods were found to be docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and arachidonic acid (AA; 20:4n-6), the latter showing a large variation (from 1–2% up to 9.8% of total fatty acids) in octopuses (Culkin & Morris 1970; Gibson 1983; Sinanoglou & Miniadis-Meimaroglou 1998).

Passi, Cataudella, Di Marco, De Simone and Rastrelli (2002) found an n-3/n-6 ratio of more than one in cephalopods from the Mediterranean Sea, confirming their great importance as a significant dietary source of n-3 PUFA for humans. It is believed that EPA and DHA reduce cardiovascular mortality and attenuate inflammatory responses (Ramesha & Pickett 1986). However, in cultured octopuses the n-3 PUFA levels are correlated with the dietary input and influence the growth rate (Navarro & Villanueva

 

2000, 2003). Squid-fed common octopuses showed a fatty acid profile with a high content of polyunsaturates (51.32–57.62% total fatty acids), particularly n-3 highly unsaturates (38.01–52.73% total fatty acids), which varied with temperature and body weight (Miliou, Fintikaki, Tzitzinakis, Kountouris & Verriopoulos 2006). Apart from its importance in evaluating the nutritional value of octopuses, fatty acid composition may be useful in predicting their growth rate, which is also related to body weight and temperature (Miliou *et al.* 2005).

Changes in tissue mass (growth) can be assessed by a relatively simple measurement of tissue weight but this is a highly nonspecific measurement. Macromolecular content, and ratio, were sensitive and provided some insight into how growth is affected (Barron & Adelman 1984). Growth is largely a function of changes in cell size, number and metabolic activity. Growth processes are influenced by tissue deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) concentrations; thus, quantitative analysis of nucleic acids provides a means of estimating growth (Dagg & Littlepage 1972; Bulow 1987; Mustafa, Lagardere & Pastoureaud 1991). The quantity of DNA per cell is constant in somatic tissue within a given species (Bulow 1987), and total DNA is generally used as an index of the number of cells within a tissue (Buckley 1980; Valente, Gomes & Fauconneau 1998).

Ribonucleic acid is directly involved in protein synthesis (Buckley 1980; Bulow 1987). Its use as a growth indicator is based on the premise that the amount of RNA in cell varies in proportion to the amount of protein synthesis (Bulow 1987). The RNA/DNA ratio is considered to be a more accurate index of metabolic activity than RNA concentration alone, because the ratio is not affected by differences in cell numbers (Buckley 1984; Bulow 1987; Mustafa *et al.* 1991). The RNA/DNA ratio has been determined to be an effective index of the nutritional status in fish larvae (Buckley 1984; Clemmesen 1994; Ferron & Leggett 1994; Kawakami, Machioka, Kimura & Nakazono 1999). In addition, the RNA/DNA ratio has been used as an indicator of growth of fish larvae and juveniles in toxicological studies (Barron & Adelman 1984), fisheries oceanography studies (Buckley, Caldarone & Ong 1999) and aquaculture research (Carter, Seeto, Smart, Clarke & van Barneveld 1998).

Temperature and food availability are the main factors affecting the growth and the RNA/DNA ratios of fish larvae (Buckley 1980, 1982, 1984; Clemmesen 1994). It is known that temperature has a positive effect on the growth rate but a negative effect on the RNA/DNA ratio (Buckley 1982; Goolish & Adelman 1984; Buckley *et al.* 1999). According to Ferron and Leggett (1994), changes in temperature or food availability are reflected first on the RNA/DNA ratio, but their effects on growth rates lag behind. Because the RNA/DNA ratio is temperature dependent, care must be exercised in assessing nutritional condition (Ramírez, Cortés, García & Carpena 2004). Kawakami *et al.* (1999) suggested that for a better understanding of the metabolic process, studies on the lipid composition in parallel to RNA/DNA ratios would be necessary.

The aim of this study was to investigate changes in the RNA/DNA ratio and the fatty acid composition of *O. vulgaris* in relation to the growth rate. A comparison of these biochemical parameters between cultured and wild octopuses was also performed. The use of nucleic acids and fatty acids as growth indicators was evaluated.

## Materials and methods

The octopuses, *O. vulgaris* Cuvier, 1797 (order Octopoda, suborder Incirrata), were collected throughout the year by free diving in the Saronicos Gulf (Aegean Sea, Eastern Mediterranean). The animals were transferred to the laboratory immediately after collection. The animals were reared in a closed seawater (37.5–38.5 g $L^{-1}$) system with temperature control (15, 20 and 25 °C). The diurnal variation in water temperature was $\pm$ 0.5 °C. Photoperiod was set at 12 h light:12 h dark.

Thirty animals were reared at 20 °C for different time periods: 30, 60, 90, 120, 150 and 180 days after collection in the field (five animals for each rearing period). In addition, 15 octopuses were maintained at 15 °C and another 15 at 25 °C for 60 days after collection in the field. Specimens were kept apart and hand-fed on squid (*Loligo vulgaris*), once a day, at midday. Previously, batches of squid were frozen to be used as food. The ration was 3% of body weight per day, and the animals were weighed every 30 days. At the end of each rearing period, octopuses were weighed and then placed on ice for immediate death. Before weighing, specimens were deprived of food for 24 h. Then, each animal was homogenized (after removal of the viscera), freeze dried and preserved at $-$ 80 °C until analysis. The same procedure was followed for 16 wild octopuses immediately after their transfer to the laboratory. All specimens were immature, according to the descriptions given by Nigmatulin (1977) for the six stages of maturity.

The specific growth rate (SGR) for each octopus (% $day^{-1}$) was estimated for the last 30 days of rearing

  

and calculated as SGR = 100(ln$W_t$—ln$W_O$)$t^{-1}$, where $W_t$ is the final weight (g), $W_O$ is the initial weight (g) and $t$ = 30 days. Ribonucleic acid and DNA were quantified according to the method described by Holland and Hannant (1973). Calibration curves were drawn with DNA from calf thymus (Serva, Heidelberg, Germany) and RNA from yeast (Sigma, Athens, Greece).

Lipids were extracted in chloroform:methanol (2:1, v:v) using the method of Folch, Lees and Sloane-Stanley (1957). Samples were stored under nitrogen at − 80 °C before analysis. Fatty acid composition was determined for each octopus (three replicates). Fatty acid methyl esters (FAMEs) were prepared by transesterification with anhydrous methanol containing 2% sulphuric acid and 0.01% (w/v) butylated hydroxytoluene (BHT) for 16 h at 50 °C (Christie 1982). Fatty acid methyl esters were separated by gas liquid chromatography (GLC) on a Perkin Elmer gas chromatograph (Autosystem XL, Perkin-Elmer, Waltham, MA, USA) equipped with a flame ionization detector and a split/splitless injector fitted to a capillary column of fused silica (length: 30 m, i.d.: 0.32 mm, film thickness: 0.25 μm; FameWax, Restek, Bellefonte, PA, USA). Helium was used as the carrier gas. Injector and detector temperatures were set at 225 and 250 °C respectively. The oven temperature was programmed to increase from 130 to 175 °C at a rate of 5 °C min$^{-1}$ and then to 225 °C at a rate of 20 °C min$^{-1}$, where it was held for 10 min. Peak areas were integrated using TURBOCHROM NAVIGATOR Software (Version 4.1). Fatty acid methyl esters were identified by reference to a well-characterized fish oil and to authentic standards (Alltech, Sigma and Larodan).

Regression analysis was performed between the proportion of fatty acids or the RNA/DNA ratio and the SGR. The significance of differences among means ($P < 0.05$) was tested by ANOVA (LSD multiple range test). For comparing the means among octopuses reared for different time periods, ANCOVA (body weight as covariate) was conducted. Previously, normality of distributions ($\chi^2$-test, Kolmogorov–Smirnov $D$ and Anderson–Darling $A^2$) and homogeneity of variances (Cohran's $C$ and Barlett's tests) were assessed. Where appropriate, data were transformed [asin $(1 - x/100)$]. Untransformed means ± standard error (SE) are presented. Discriminant analysis was used for classifying cultured octopuses into groups.

## Results

The proportion of certain fatty acids (% total fatty acids) in cultured octopuses was related to SGR. Specifically, an inverse linear relationship ($P < 0.0001$) was found between DHA (Fig. 1a) or EPA (Fig. 1b) and SGR. On the contrary, AA was regressed on SGR with a positive linear relationship ($P < 0.0001$; Fig. 1c). In addition, 16:0, 18:1 (including 18:1n-9, 18:1n7 and 18:1n-5), 18:2n-6, 20:2n-6, 22:4n-6 and 22:5n-6 increased with increasing SGR ($P < 0.0001$). Furthermore, RNA/DNA increased as SGR increased ($P < 0.0001$; Fig. 1d).

Analysis of covariance analysis proved that rearing time did not significantly ($P > 0.05$) affect SGR, RNA/DNA and the proportions of fatty acids, including those related to SGR (Fig. 2).

Docosahexaenoic acid (20.8–31.9%), EPA (F10.4–19.7%) and AA (2.7–10.7%) varied significantly and discriminant analysis proved that of the 60 cultured octopuses, 100% were correctly classified into three groups as shown in Fig. 3. Group A included small animals (50–150 g) reared at 20 and 25 °C and large animals (500–750 g) reared at 15 °C. Group C contained small animals (50–150 g) reared at 15 °C and large animals (500–750 g) reared at 20 and 25 °C. Group B included octopuses of intermediate body weight (200–450 g) maintained at the three temperatures.

The 16:0, 20:4n-6, 22:4n-6 and 22:5n-6 levels were higher in group A and the lowest in group C ($P < 0.0001$; Table 1). The opposite was observed for the 20:5n-3 and 22:6n-3 ($P < 0.0001$) levels. In addition, the 18:1, 18:2n-6, 20:2n-6 levels and DHA/EPA ratios (1.6–2) were significantly higher in group A compared with the other two groups. The n-3/n-6 ratio of cultured octopuses (2.4–15.4%) was the lowest in group A and the highest in group C ($P < 0.0001$). Wild animals (80–500 g) showed a fatty acid composition similar to that of reared octopuses belonging to group A.

The RNA/DNA ratio was significantly ($P < 0.0001$) higher in wild animals, as well as in group A cultured octopuses (Fig. 4a). Furthermore, group A exhibited the highest SGR and group C the lowest ($P < 0.0001$) (Fig. 4b).

## Discussion

The RNA/DNA ratio has been used to reflect protein synthesis on the assumption that DNA per cell remains constant, whereas RNA is proportional to protein synthesis (Pelletier, Dutil, Blier & Guderley 1994). Fluctuations observed in the RNA/DNA ratio showed that the fast-growing octopuses are more metabolically active and have a larger capacity for

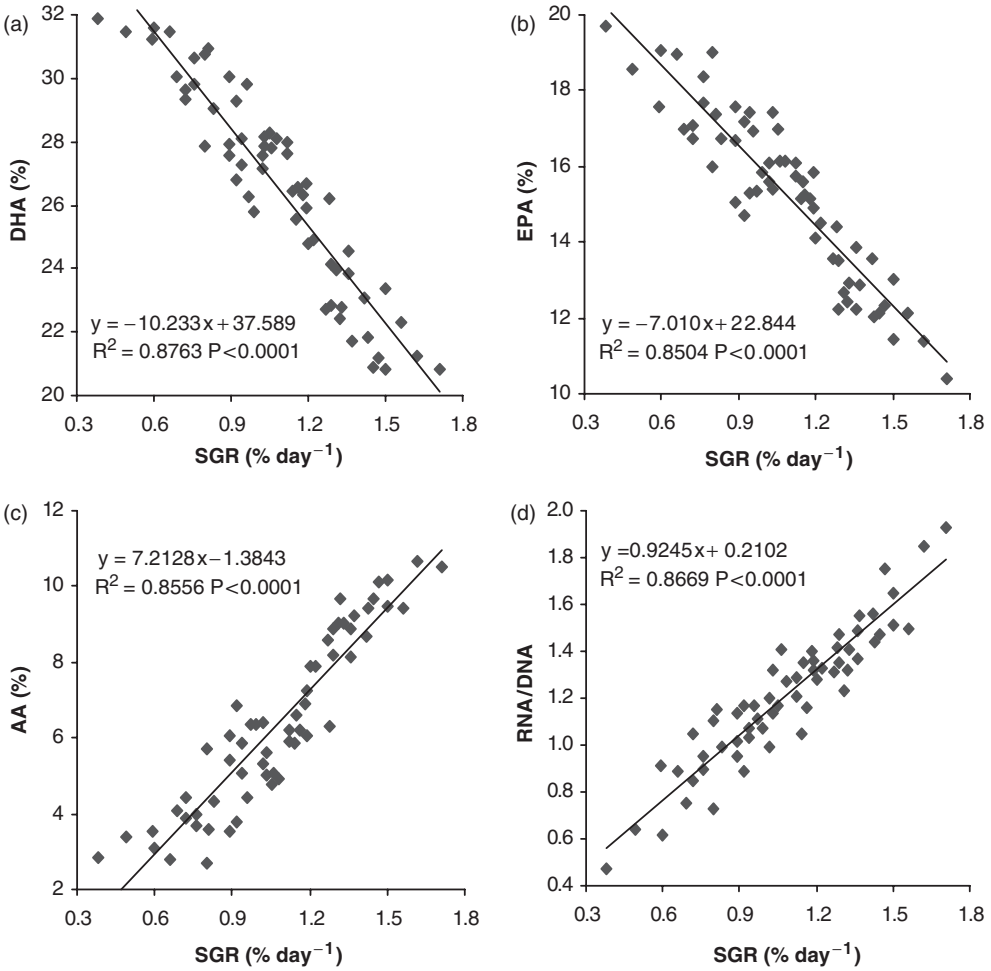


**Figure 1** Regression lines of (a) docosahexaenoic acid (DHA) (b) eicosapentaenoic acid (EPA) (c) arachidonic acid (AA) (% total fatty acids) and (d) ribonucleic acid (RNA)/deoxyribonucleic acid (DNA) vs. specific growth rate (SGR) of *Octopus vulgaris*. Linear equations and regression coefficients ($R^2$) shown.

synthesizing protein and growth. Several authors have found a negative relationship between the RNA/DNA ratio and temperature (Buckley *et al.* 1999). Goolish and Adelman (1984) have suggested that the elevated RNA/DNA levels observed at low temperatures are part of a compensatory mechanism for reduced RNA activity at low temperatures. In this study, RNA/DNA was related to SGR of *O. vulgaris*, irrespective of the rearing temperature. Miliou *et al.* (2005) found that the variations in SGR in octopuses of different body weight reared at 15–25 °C were consistent with those for feed efficiency, protein and energy retention. Thus, the nutritional condition of the common octopus can be estimated from the RNA/DNA ratio.

In this study, the concentration (% of total fatty acids) of EPA and DHA was related to the growth rate of *O. vulgaris*. Specifically, EPA and DHA decreased with increasing SGR, showing lower levels in smaller octopuses reared at the higher temperatures (20–25 °C) and in larger animals at the lower temperature (15 °C). It has been reported that the growth rate of the common octopus increases with increasing body weight at 15 °C, but the opposite occurred at 20–25 °C (Miliou *et al.* 2005). It appears that the observed decreases in EPA and DHA reflect a higher demand for membrane synthesis in faster growing octopuses.

Palmitic acid (16:0), EPA and DHA were the most abundant fatty acids found in the lipids of *O. vulgaris*, as has been reported for many cephalopod species (Culkin & Morris 1970; Sinanoglou & Miniadis-Meimaroglou 1998; Passi *et al.* 2002). The squid-fed octopuses showed a DHA/EPA ratio ranging from 1.6 to 2, which is above those detected (0.4–0.6) in the

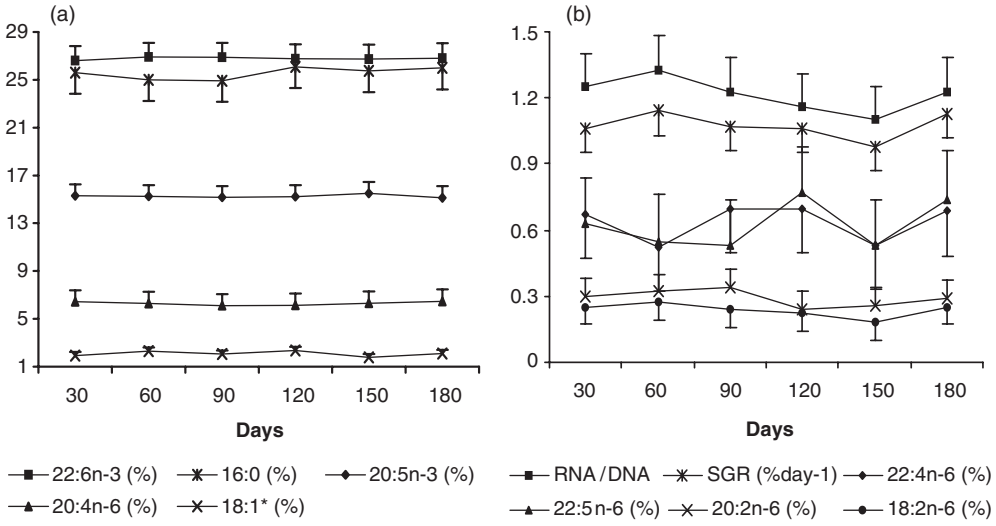


**Figure 2** Selected fatty acids (% total fatty acids), ribonucleic acid (RNA)/deoxyribonucleic acid (DNA) and specific growth rate (SGR) (last 30 days) estimated (ANCOVA using body weight as a covariate) in octopuses reared for different time periods at 20 °C. Values are least square mean with a 95% LSD confidence interval. * 18:1n-9, 18:1n-7 and 18:1n-5.

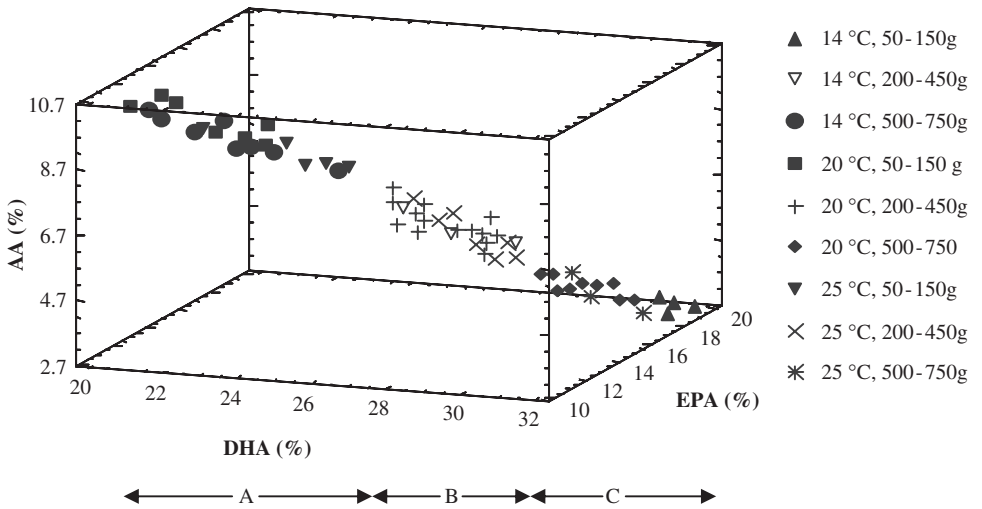


**Figure 3** Scatterplot of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) (% total fatty acids) discriminating cultured octopuses in three groups A, B and C, with respect to rearing temperature and final body weight.

cultured paralarvae of the common octopus fed on *Artemia* only (Navarro & Villanueva 2000, 2003). Okomura, Kurihara, Iwamoto and Takeuchi (2005) found a DHA/EPA ratio equal to 1.5 in common octopus paralarvae fed on *Artemia* and flakes of Pacific sandeel, and suggested that this ratio is a necessary condition for the normal growth and development of these paralarvae. In this study, the faster growing octopuses showed an increased average DHA/EPA ratio (1.8) and generally a fatty acid composition similar to that of wild octopuses (Navarro & Villanueva 2000, 2003; present study).

Decreases observed in the n-3 HUFA of cultured octopuses were accompanied by increases in 18:1. Marine finfish fatty acid profiles exhibited an increase in 18:1n-9, which, in general, is one of the characteristics of essential fatty acid deficiency (Ibeas, Cejas, Gómez, Jeres & Lorenzo 1996). An

**Table 1** Total lipid fatty acid composition (% of total fatty acids) estimated in wild and three groups (see Fig. 3) of cultured octopuses (mean ± SE)

| | | Cultured | | | |
|---|---|---|---|---|---|
| **Fatty acid** | **Wild ($n = 16$)** | **Group A ($n = 20$)** | **Group B ($n = 24$)** | **Group C ($n = 16$)** | ***P*** |
| <14:0 | 1.025 ± 0.116 | 1.055 ± 0.104 | 1.020 ± 0.095 | 0.890 ± 0.116 | NS |
| 14:0 | 0.779 ± 0.066 | 0.848 ± 0.059 | 0.898 ± 0.054 | 0.840 ± 0.066 | NS |
| 14:1 | 0.237 ± 0.012 | 0.236 ± 0.011 | 0.207 ± 0.010 | 0.210 ± 0.012 | NS |
| 15:0 | 0.391 ± 0.051 | 0.459 ± 0.046 | 0.462 ± 0.042 | 0.381 ± 0.051 | NS |
| 16:0 | 27.639 ± 0.446$^{c}$ | 28.335 ± 0.399$^{c}$ | 25.414 ± 0.364$^{b}$ | 24.150 ± 0.446$^{a}$ | *** |
| 16:1n-7 | 0.231 ± 0.017 | 0.222 ± 0.015 | 0.242 ± 0.014 | 0.203 ± 0.017 | NS |
| 16:2n-4 | 1.036 ± 0.050 | 1.032 ± 0.044 | 1.005 ± 0.041 | 0.963 ± 0.050 | NS |
| 17:0 | 1.378 ± 0.152 | 1.200 ± 0.136 | 1.188 ± 0.124 | 1.106 ± 0.152 | NS |
| 16:4n-1 | 0.080 ± 0.008 | 0.082 ± 0.007 | 0.087 ± 0.006 | 0.081 ± 0.008 | NS |
| 18:0 | 10.116 ± 0.318 | 9.959 ± 0.284 | 9.738 ± 0.260 | 9.215 ± 0.318 | NS |
| 18:1* | 2.566 ± 0.131$^{b}$ | 2.787 ± 0.117$^{b}$ | 1.912 ± 0.107$^{a}$ | 1.958 ± 0.131$^{a}$ | *** |
| 18:2n-6 | 0.331 ± 0.023$^{b}$ | 0.291 ± 0.021$^{b}$ | 0.171 ± 0.019$^{a}$ | 0.146 ± 0.023$^{a}$ | *** |
| 18:3n-6 | 0.144 ± 0.010 | 0.123 ± 0.009 | 0.123 ± 0.008 | 0.127 ± 0.010 | NS |
| 18:3n-3 | 0.104 ± 0.009 | 0.098 ± 0.008 | 0.099 ± 0.008 | 0.088 ± 0.009 | NS |
| 20:0 | 0.118 ± 0.020 | 0.111 ± 0.018 | 0.115 ± 0.016 | 0.101 ± 0.020 | NS |
| 20:1n-9 | 2.373 ± 0.100 | 2.428 ± 0.090 | 2.611 ± 0.082 | 2.539 ± 0.100 | NS |
| 20:2n-6 | 0.368 ± 0.026$^{b}$ | 0.357 ± 0.023$^{b}$ | 0.274 ± 0.021$^{a}$ | 0.222 ± 0.026$^{a}$ | *** |
| 20:4n-6 | 9.426 ± 0.209$^{c}$ | 9.176 ± 0.187$^{c}$ | 5.925 ± 0.170$^{b}$ | 3.628 ± 0.209$^{a}$ | *** |
| 20:3n-3 | 0.372 ± 0.027 | 0.345 ± 0.024 | 0.307 ± 0.022 | 0.368 ± 0.027 | NS |
| 20:5n-3 | 12.416 ± 0.256$^{a}$ | 12.666 ± 0.229$^{a}$ | 15.757 ± 0.209$^{b}$ | 17.844 ± 0.256$^{c}$ | *** |
| 22:0 | 0.105 ± 0.012 | 0.091 ± 0.011 | 0.096 ± 0.010 | 0.081 ± 0.012 | NS |
| 22:1n-11 | 0.725 ± 0.069 | 0.562 ± 0.061 | 0.619 ± 0.056 | 0.656 ± 0.069 | NS |
| 22:1n-9 | 0.859 ± 0.062 | 0.753 ± 0.055 | 0.785 ± 0.050 | 0.834 ± 0.062 | NS |
| 21:5n-3 | 0.221 ± 0.020 | 0.227 ± 0.018 | 0.215 ± 0.016 | 0.206 ± 0.020 | NS |
| 22:4n-6 | 1.076 ± 0.055$^{c}$ | 0.959 ± 0.050$^{c}$ | 0.673 ± 0.045$^{b}$ | 0.288 ± 0.055$^{a}$ | *** |
| 22:5n-6 | 0.946 ± 0.057$^{c}$ | 0.971 ± 0.051$^{c}$ | 0.634 ± 0.047$^{b}$ | 0.322 ± 0.057$^{a}$ | *** |
| 22:5n-3 | 1.101 ± 0.085 | 1.000 ± 0.076 | 1.159 ± 0.070 | 1.054 ± 0.085 | NS |
| 22:6n-3 | 22.660 ± 0.288$^{a}$ | 22.711 ± 0.258$^{a}$ | 27.158 ± 0.235$^{b}$ | 30.439 ± 0.288$^{c}$ | *** |
| 24:1n-9 | 1.119 ± 0.107 | 0.925 ± 0.096 | 1.110 ± 0.087 | 1.062 ± 0.107 | NS |
| DHA/EPA | 1.834 ± 0.020$^{b}$ | 1.797 ± 0.018$^{b}$ | 1.725 ± 0.016$^{a}$ | 1.709 ± 0.020$^{a}$ | |
| n-3/n-6 | 3.063 ± 0.333$^{a}$ | 3.150 ± 0.298$^{a}$ | 5.848 ± 0.272$^{b}$ | 11.008 ± 0.333$^{c}$ | *** |

Means in the same row with different superscript letters are significantly different.
NS, not significant ($P > 0.05$); DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SE, standard error.
***$P < 0.0001$. *Includes 18:1n-9, 18:1n-7 and 18:1n-5; DHA: 22:6n-3; EPA: 20:5n-3.

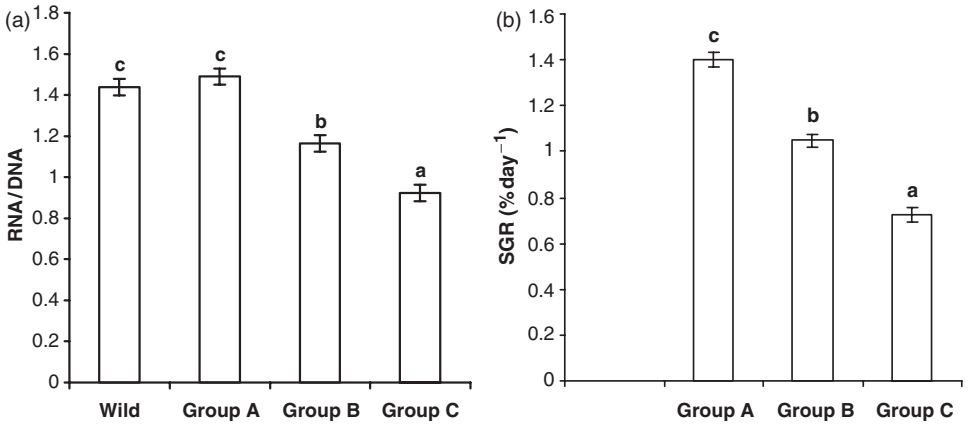


**Figure 4** Mean values (± SE) of (a) ribonucleic acid (RNA)/deoxyribonucleic acid (DNA) and (b) specific growth rate (SGR) in three groups of cultured octopuses (as shown in Fig. 3) and in wild animals only for the former.

increase in monounsaturated fatty acids, particularly in 18:1 isomers, has been observed in cultured *O. vulgaris* paralarvae fed on n-3 HUFA-deficient diets (Navarro & Villanueva 2003). However, in this study, the diet provided was rich in n-3 HUFA and the highest 18:1 levels were observed in the fastest growing octopuses. It appears that the increases in 18:1 levels were associated with the maintenance of the membrane unsaturation index, compensating for the decreases in DHA and EPA. It has been shown that the role of 18:1n-9 and n-3 HUFA in modulating the membrane unsaturation index is fundamental in fish tissues (Sargent, Henderson & Tocher 1989).

The AA content was elevated in the fastest growing octopuses with relatively low EPA levels. Competition between EPA and AA during phospholipid esterification has been suggested to occur in fish, particularly for phosphatidylinositol (PI), where both fatty acids are major components (Bell, Castell, Tocher, MacDonald & Sargent 1995). A decrease in EPA has been observed in gilthead sea bream with the increase in dietary AA (Fountoulaki, Alexis, Nengas & Venou 2003; Van Anholt, Koven, Lutzky & Wendelaar Bonga 2004). In this study, AA levels showed a large variation (from 2.7% to 10.7%) in total fatty acids, although the diet (squid) offered to all specimens had the same fatty acid composition and moreover was poor in AA. Thus, the relatively high level of AA in octopuses appears to be an inherent characteristic of *O. vulgaris*, because it does not correlate with dietary input. In addition, *O. vulgaris* paralarvae fed on a diet poor in AA maintained this fatty acid at the levels found in hatchlings (Navarro & Villanueva 2000).

Increase in 20:2n-6 in octopuses with increased AA and decreased n-3 PUFA levels indicates preferential desaturation and elongation of 18:2n-6 (Durazo-Beltrán, D'Abramo, Toro-Vazquez, Vasquez-Paláez & Viana 2003). The specific accumulation of 22:4n-6 and 22:5n-6, which were present in the diet in small amounts, suggests activation of Δ6 desaturase when the n-3 HUFA levels in octopus decreased. Increased levels of 20:4n-6, 22:4n-6 and 22:5n-6 have been found in trout fed on low n-3 HUFA diets (Buzzi, Henderson & Sargent 1997). However, in marine fish, 18:2n-6 to AA conversion rates, as well as those of 18:3n-3 to EPA and DHA, are low, negligible or zero (Sargent, Bell, McEnvoy, Tocher & Estévez 1999). As far as marine molluscs are concerned, the ability for enzymatic bioconversion of C18 precursors to AA has been reported for some species, such as the abalones *Haliotis fulgens* (Durazo-Beltrán *et al.* 2003), *H. laevigata* (Dunstan, Baillie, Barrett & Volkman 1996) and *H. discus hannai* (Uki, Sugiura & Watanabe 1986).

Ribonucleic acid/DNA and fatty acid composition of the wild octopuses sampled in this study did not vary with body weight and predict a relatively high growth rate, indicating that the animals in the field were found at their optimal conditions. Katsanevakis and Verriopoulos (2004) reported that small octopuses preferred warm waters, while large ones preferred cold waters (deeper areas) in the Aegean Sea. However, the variation in AA levels reported for wild octopuses (Culkin & Morris 1970; Gibson 1983; Sinanoglou & Miniadis-Meimaroglou 1998; Passi *et al.* 2002) may be associated with the growth rate, as arachidonic acid seems to be independent of dietary input.

In conclusion, the fatty acid composition of *O. vulgaris* fluctuated with growth rate, but with an n-3/n-6 PUFA ratio of more than 2.4. Cultured octopuses could be an excellent source of arachidonic acid, along with a high DHA/EPA content, particularly the fastest growing animals. It is concluded that RNA/DNA, DHA, EPA and AA could be used as biochemical indices for predicting the growth rate of *O. vulgaris*, irrespective of ambient temperature. This may prove important for the development of mariculture and reduced handling of octopuses.

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## References

Barron M.G. & Adelman I.R. (1984) Nucleic acid, protein content and growth of larval fish sublethally exposed to various toxicants. *Canadian Journal of Fisheries and Aquatic Sciences* **41**, 141–150.

Bell J.G., Castell J.D., Tocher D.R., MacDonald F.M. & Sargent J.R. (1995) Effects of different dietary arachidonic acid: docosahexaenoic acid ratios on phospholipid fatty acid compositions and prostaglandin production in juvenile turbot (*Scophthalmus maximus*). *Fish Physiology and Biochemistry* **14**, 139–151.

Buckley L.J. (1980) Changes in ribonucleic acid, deoxyribonucleic acid, and protein content during ontogenesis in winter flounder, *Pseudopleuronectes americanus*, and effect of starvation. *Fishery Bulletin* **77**, 703–708.

Buckley L.J. (1982) Effects of temperature on growth and biochemical composition of larval winter flounder *Pseudopleuronectes americanus*. *Marine Ecology Progress Series* **8**, 181–186.

Buckley L.J. (1984) RNA–DNA ratio: index of larval fish growth in the sea. *Marine Biology* **80**, 291–298.

Buckley L., Caldarone E. & Ong T.-L. (1999) RNA–DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. *Hydrobiologia* **401**, 265–277.

Bulow F.J. (1987) RNA–DNA ratios as indicators of growth in fish: a review. In: *Age and Growth of Fish* (ed. by R.C. Summerfelt & G.E. Hall), pp. 45–64. Iowa State University Press, Ames, IA, USA.

Buzzi M., Henderson R.J. & Sargent J.R. (1997) Biosynthesis of docosahexaenoic acid in trout hepatocytes proceeds via 24-carbon intermediates. *Comparative Biochemistry and Physiology* **116B**, 263–267.

Carter C.G., Seeto G.S., Smart A., Clarke S. & van Barneveld R.J. (1998) Correlates of growth in farmed juvenile southern bluefin tuna *Thunnus maccoyii* (Castelnau). *Aquaculture* **161**, 107–119.

Christie W.W. (1982) *Lipid Analysis*. Pergamon, Oxford, 207 pp.

Clemmesen C. (1994) The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. *Marine Biology* **118**, 377–382.

Culkin F. & Morris R.J. (1970) The fatty acids of some cephalopods. *Deep-Sea Research* **17**, 171–174.

Dagg M.J. & Littlepage J.L. (1972) Relationships between growth rate and RNA, DNA, protein and dry weight in *Artemia salina* and *Euchaeta elongata*. *Marine Biology* **17**, 162–170.

Dunstan G.A., Baillie H.J., Barrett S.M. & Volkman J.K. (1996) Effect of diet on the lipid composition of wild and cultured abalone. *Aquaculture* **140**, 115–127.

Durazo-Beltrán E., D'Abramo L.R., Toro-Vazquez J.F., Vasquez-Paláez C. & Viana M.T. (2003) Effect of triacylglycerols in formulated diets on growth and fatty acid composition in tissue of green abalone (*Haliotis fulgens*). *Aquaculture* **224**, 257–270.

Ferron A. & Leggett W.C. (1994) An appraisal of condition for marine fish larvae. *Advances in Marine Biology* **30**, 217–303.

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

Fountoulaki E., Alexis M.N., Nengas I. & Venou B. (2003) Effects of dietary arachidonic acid (20:4n-6), on growth, body composition, and tissue fatty acid profile of gilthead bream fingerlings (*Sparus aurata* L.). *Aquaculture* **225**, 309–323.

García García B. & Aguado Giménez F. (2002) Influence of diet on ongrowing and nutrient utilization in the common octopus (*Octopus vulgaris*). *Aquaculture* **211**, 171–182.

García García B. & Cerezo Valverde J. (2006) Optimal proportions of crabs and fish in diet for common octopus (*Octopus vulgaris*) ongrowing. *Aquaculture* **253**, 502–511.

Gibson R.A. (1983) Australian fish – an excellent source of both arachidonic acid and n-3 polyunsaturated fatty acids. *Lipids* **18**, 743–752.

Goolish E.M. & Adelman M.G. (1984) Thermoacclimatory and response of nucleic acid and protein content of carp muscle tissue: influence of growth rate and relationship to glycine uptake by scales. *Canadian Journal of Zoology* **62**, 2164–2170.

Holland V.M. & Hannant P.J. (1973) Addendum to a microanalytical scheme for the biochemical analysis of marine invertebrate larvae. *Journal of the Marine Biological Association of the United Kingdom* **53**, 833–838.

Ibeas C., Cejas J., Gómez T., Jeres S. & Lorenzo A. (1996) Influence of dietary n-3 highly unsaturated fatty acids on juvenile gilthead seabream (*Sparus aurata*) growth and tissue fatty acid composition. *Aquaculture* **142**, 221–235.

Iglesias J., Sánchez F.J., Otero J.J. & Moxica C. (2000) Culture of octopus (*Octopus vulgaris*, Cuvier): present knowledge, problems and perspectives. Recent advances in Mediterranean aquaculture finfish species diversification. *Cahiers Options Méditerranéennes* **47**, 313–322.

Katsanevakis S. & Verriopoulos G. (2004) Abundance of *Octopus vulgaris* on soft sediment. *Scientia Marina* **68**, 553–560.

Kawakami Y., Machioka N., Kimura R. & Nakazono A. (1999) Seasonal changes of the RNA/DNA ratio, size and lipid contents and immigration adaptability of Japanese glass-eels, *Anguilla japonica*, collected in northern Kyushu, Japan. *Journal of Experimental Marine Biology and Ecology* **238**, 1–19.

Mangold K.M. (1983) *Octopus vulgaris*. In: *Cephalopod Life Cycles*, Vol. I (ed. by P.R. Boyle), pp. 335–364. Academic Press, London, UK.

Mangold K.M. & von Boletzky S.V. (1973) New data on reproductive biology and growth of *Octopus vulgaris*. *Marine Biology* **19**, 7–12.

Miliou H., Fintikaki M., Kountouris T. & Verriopoulos G. (2005) Combined effects of temperature and body weight on growth utilization of the common octopus, *Octopus vulgaris*. *Aquaculture* **249**, 245–256.

Miliou H., Fintikaki M., Tzitzinakis M., Kountouris T. & Verriopoulos G. (2006) Fatty acid composition of the common octopus, *Octopus vulgaris*, in relation to rearing temperature and body weight. *Aquaculture* **256**, 311–322.

Mustafa S., Lagardere J.P. & Pastoureaud A. (1991) Condition indices and RNA: NA ratio in overwintering European sea bass, *Dicentrarchus labrax*, in salt marshes along the Atlantic coast of France. *Aquaculture* **96**, 367–374.

  

Navarro J.C. & Villanueva R. (2000) Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. *Aquaculture* **183**, 161–177.

Navarro J.C. & Villanueva R. (2003) The fatty acid composition of *Octopus vulgaris* paralarvae reared with live and inert food: deviation from their natural fatty acid profile. *Aquaculture* **219**, 613–631.

Nigmatulin CH.M. (1977) Proisvodstvo i biologuicheskikh nabludéni v more i ikh obrabotka i ofermlénié. *Kalingrand* 56–78.

Okumura S., Kurihara A., Iwamoto A. & Takeuchi T. (2005) Improved survival and growth in *Octopus vulgaris* paralarvae by feeding large type *Artemia* and Pacific sandeel, *Ammodytes personatus*. *Aquaculture* **244**, 147–157.

Passi S., Cataudella S., Di Marco P., De Simone F. & Rastrelli L. (2002) Fatty acid composition and antioxidant levels in muscle tissue of different Mediterranean marine species of fish and shellfish. *Journal of Agricultural and Food Chemistry* **50**, 7314–7322.

Pelletier D., Dutil J.-D., Blier P. & Guderley H. (1994) Relation between growth rate and metabolic organisation of white muscle, liver and digestive tract in cod, *Gadus morhua*. *Journal of Comparative Physiology* **164B**, 179–190.

Ramesha C.S. & Pickett W.C. (1986) Platelet-activating factor and leukotriene biosynthesis is inhibited in polymorphonuclear leukocytes depleted of arachidonic acid. *Journal of Biological Chemistry* **261**, 7592–7595.

Ramírez T., Cortés D., García A. & Carpena A. (2004) Seasonal variations of RNA/DNA ratios and growth rates of the Alboran Sea sardine larvae (*Sardina pilchardus*). *Fisheries Research* **68**, 57–65.

Rodríguez C., Carrasco J.F., Arronte J.C. & Rodríguez M. (2006) Common octopus (*Octopus vulgaris* Cuvier, 1797) juvenile ongrowing in floating cages. *Aquaculture* **254**, 293–300.

Sargent J., Henderson R.J. & Tocher D.R. (1989) The lipids. In: *Fish Nutrition* (ed. by J.E. Halver), 2nd edn, pp. 153–217. Academic Press, New York, USA.

Sargent J., Bell G., McEnvoy L., Tocher D. & Estévez A. (1999) Recent development in the essential fatty acid nutrition of fish. *Aquaculture* **177**, 191–199.

Sinanoglou V.J. & Miniadis-Meimaroglou S. (1998) Fatty acid of neutral and polar lipids of (edible) Mediterranean cephalopds. *Food Research International* **31**, 467–473.

Uki N., Sugiura M. & Watanabe T. (1986) Requirements of essential fatty acids in the abalone *Haliotis discus hannai*. *Bulletin of the Japanese Society of Scientific Fisheries* **52**, 1013–1023.

Valente L.M.P., Gomes E.F.S. & Fauconneau B. (1998) Biochemical growth characterization of fast and slow-growing rainbow trout strains: effect of cell proliferation and size. *Fish Physiology and Biochemistry* **18**, 213–224.

Van Anholt R.D., Koven W.M., Lutzky S. & Wendelaar Bonga S.E. (2004) Dietary supplementation with arachidonic acid alters the stress response of gilthead seabream (*Sparus aurata*) larvae. *Aquaculture* **238**, 369–383.

Vaz-Pires P., Seixas P. & Barbosa A. (2004) Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): a review. *Aquaculture* **238**, 221–238.