BIOCHEMICAL COMPOSITION OF THE COPEPOD EUTERPINA ACUTIFRONS FROM THE COASTAL WATERS OF ALEXANDRIA

By

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ABSTRACT

Protein, carbohydrate and lipid contents were determined in the most numerous and wide spread copepod Euterpina acutifrons collected from the coastal waters of Alexandria. Protein is the major biochemical component of the copepod constituting on the average 35.2 ± 6.7% of the organisms' dry weight followed by carbohydrate (7.9 ± 1.6%) and lipid (3.7 ± 1.9%) contents. Organisms collected from the Eastern Harbor (eutrophic basin) affected by sewage discharge were characterized by high biochemical contents while opposite to landbased sources affected by industrial discharge, the organisms' biochemical components were at a minimum. Compared to other representatives of the zooplankton community, Euterpina retains high protein content indicating its suitability as food for marine fishes.

The stepwise multiple regression equations revealed that the different biochemical components of Euterpina are mostly affected with the occurrence of available food represented by chlorophyll a concentrations. The average Protein/Carbohydrate ratio for E. acutifrons i.e. 4.46 ± 2.3 indicate that the organism is inhabiting a nutrient sufficient environment. Lipid/protein ratios more than 0.2 observed at industrial discharge affected areas indicate pollution impact on protein production.

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INTRODUCTION

The most numerous, important and wide spread group of marine holoplankton, in both inshore and offshore waters, is copepods, constituting usually a large percentage, numerically, of the total zooplankton community. In aquatic environments, copepods play an important role in the transfer of energy from the primary producers to the higher levels in the food chain. Furthermore, they are themselves favorite food items for many animals including economic fishes. Some economic fishes as herring and others are largely dependent on the abundance of copepods as an important food [1-3]. In the south-eastern Mediterranean Sea, El-Rashidy [4] found that most of the fish larvae feed on pelagic copepods. Many copepod species are exclusively carnivorous, the great majority being omnivorous, feeding primarily on diatoms, dinoflagellates and particulate organic matter [1].

Plankton species are useful tools for monitoring certain aspects of the environment such as hydrographic events, eutrophication, pollution and long term changes in the environmental conditions. Marked abnormalities of species composition may indicate the need for more intensive physical and chemical analyses. Most ecological studies showed the appearance of a considerable time lag in the response of the biological population when compared to that of physico-chemical variables.

Berdugo and Kimor [5], Lakiss [6,7] and Pasteur et al. [8] classified Euterpina acutifrons among the main copepod species recorded in the coastal waters of the eastern Mediterranean. In the S.E. Mediterranean, Euterpina acutifrons was recorded along the Egyptian coast [9-15]. Most of these studies showed that this species constitutes not less than 70% of the copepod population in the inshore waters which in turn constitutes more than 60% of the zooplankton community.

The biochemical composition of copepods or even zooplankton community vary within a certain range depending mainly on environmental factors. The increase or decrease in the biochemical parameters of an organism is dependent on some external factors such as the amplitude of annual discharge from land-based sources, absence and presence of food as well as some physical parameters like temperature and light.

Since E. acutifrons is considered as the main food source for the coastal marine fishes in the area and in view of the increasing types and amounts of pollutants discharged to the coastal waters of Alexandria in the last ten years, the present work was attempted to focus and evaluate the fundamental changes in the biochemical composition of the organism in relation to changes in water quality variables.

STUDY AREA

The investigated area extends along the coast of Alexandria about 60 km between Agami in the west (Longitude 29° 45' E and Latitude 31° 08' N) and Abu-Qir Bay (Boughaz El-Maadia) in the east (Longitude 30° 05' and 30° 22' E, and Latitude 31° 16' and 31° 21' N) (Figure 1). The coast between Agami and Abu-Qir extends more or less straight with slight undulations forming embayments [16]. Two of these embayments form the eastern and western harbours.

Apart from the direct impact of land runoff, Agami region (station 1) is an exposed coastal area located 23 Km west of Alexandria (Figure 1). The area is characterized by its oligotrophic nature. Mex bay (stations 2 & 3), receives wastewater from several effluent sources, ex: El- Umam agricultural drain (6 X 10^6 m^3 d^-1), chlor-alkali plant (35 X 10^6 m^3 d^-1), petroleum and cement factories wastes as well as from the Western Harbor (3.1 X 10^6 m^3 d^-1). The Western Harbour (stations 4 & 5) is one of the most important trading harbours in the Mediterranean Sea. The harbour receives 90 X 10^3 m^3 d^-1 of polluted agricultural discharge through Naubaria Canal in addition to wastes and garbage dumped directly from marine vessels as well as indirect discharges from loading and unloading processes rendering its waters highly turbid.

The Eastern Harbour (E.H.) of Alexandria (stations 6-9) is a semi-enclosed basin connected to the Mediterranean Sea through two openings. The harbour receives about 150-200 X 10^3 m^3 d^-1 of untreated sewage from the main metropolitan sewage pump station of the central part of Alexandria discharging 400 m west of the harbour opening (Figure 1). Inside the harbour, several small sewage openings discharge between 10 and 15 X 10^3 m^3 d^-1 of unprocessed sewage. In addition large quantities of waste products of fishing and sailing boats anchoring inside the harbour are also dumped into the north western part.

Abu Qir (AQ) bay, where stations 10-12 (Tabia sector) and 13-15 (Maadia sector) were sampled, is located at the eastern part of Alexandria and bordered from the eastern side by Rosetta estuary. The bay receives about 1.4 X 10^4 m^3 y^-1 of brackish agricultural water from lake Edku in addition to 0.73 X 10^4 m^3 y^-1 of industrial discharge of 36 factories including paper mills, chemicals, dyes, textiles, fertilizers...etc., through Tabia pump station.

MATERIAL AND METHODS

During the period from Summer 1989 to Spring 1990, zooplankton samples were collected during 4 cruises, representing the four successive seasons. In each cruise 15 stations were sampled covering the coastal waters of Alexandria (Figure 1). Stations were chosen to represent different areas varying in their exposure to different types and quantities of pollutants discharged.
Samples were collected using a zooplankton net of 1 m mouth diameter and 120 m mesh size provided with a bucket having a window covered with # 10 nylon mesh. The net was towed for twenty minutes at the lowest speed (about 0.25 m s\(^{-1}\)). After discharging gross contaminating particles, samples were preserved in 250 ml glass containers previously soaked in 3 M pure HCl, washed with DDW and rinsed with filtered seawater before sampling.

Samples were kept in an ice box and were frozen at -4°C immediately upon reaching the lab. In the lab, samples were thawed, placed in petri-dishes and microscopically examined where undamaged Euterina adults were identified, sorted and gently rinsed with DDW [17]. Specimens were dried in an oven at 65°C until constant weight and stored in glass Scintillation vials. All weighings were performed with a Sartorius model 1800 electrobalance having an accuracy of 5 X 10\(^{-4}\) and sensitivity and readability of 0.1 µg.

Stored copepods were pooled to subsamples of about 10 mg dry weight, homogenized at 4°C with 0.6 ml aqua bi-dist. Aliquot of each homogenate was analyzed for protein, total carbohydrates and lipid contents. Total protein was determined according to modified Lowery method [18,19] using the Folin-Cio-Caltean phenol reagent for color development and bovine Serum albumin as standard. Total carbohydrates were determined using the phenol method [20] modified by Herbert et al. [21] using Glucose as reference. The method comprises determination of free as well as bound sugars. Lipids were extracted with Chloroform : methanol : water (1:2:0.8 v/v/v) [22] using Cholesterol as standard.

Water samples from the same sampling sites were collected simultaneously during the study period for temperature, dissolved oxygen, secchi disc and salinity measurements as well as the analyses of chlorophyll a biomass, reactive phosphorus and nitrate-nitrogen [15,23]. Values for chlorophyll a, reactive phosphorus and nitrate-nitrogen in different seasons appear in Figure 2 (a-c).

RESULTS

The reason for studying the levels of major body components is the presence of a correlation with other parameters like body length, dry mass, as well as serving as biomass parameters. Biochemical composition determines the caloric value of the body which are important for bioenergetic studies [24,25]. In addition, during growth and development, changes in relative pro-

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Fig. 2a: Nitrate concentrations (µM) in the coastal waters of Alexandria.
Fig. 2b: Phosphorus concentrations (μM) in the coastal waters of Alexandria.

Fig. 2c: Chlorophyll a concentrations (μg/m3) in the coastal waters of Alexandria.
portions of these components may occur. Knowing the levels of the major biochemical constituents in food and in the animals [26-28] helps to understand the effect of food quality on the dynamics of the population.

Protein Content

Protein is the major biochemical component of E. acutifrons in the coastal waters of Alexandria, with an annual average of $35.2 \pm 6.7\%$ dry weight of the organism. Protein values ranged from 12.6 to 60.3%, recorded at El-Tabia inshore station during spring and mid E.H. station during autumn, respectively (Table 1). Protein during the investigation period, showed clearly elevated values in the E.H. area (stations 6-9) especially during summer and autumn (Figure 3). The inshore stations of Maadia sector, Tabia sector and Mex Bay are characterized by low protein values during the whole study period, when compared with offshore stations. However, significant differences do not appear between both stations sampled in the Western Harbour. On the other hand, located away from land drainage effect, station 1 recorded high protein levels fluctuating between 42.0 and 52.1% with an annual average of 46.3 ± 4.7%.

Protein concentrations of Euterpina in spring (av. $26.5 \pm 8.6\%$) were significantly lower than those reported for other seasons (av. $36.5 \pm 3.4\%$ to $39.5 \pm 7.4\%$). The variation coefficient of the average values reached 30% during autumn due to an extraordinary average of 53.4% appearing in the E.H.. Among different localities both the harbours seemed to record a similar average i.e. 37.6%, while, on the other hand, a slight variation was observed in the annual averages of Mex and Abu Qir Bays (29.4 ± 7.3% for Mex Bay, 28.7 ± 6.0% for El-Tabia sector and 31.3 ± 6.6% for El-Maadia sector).

Carbohydrate content

Carbohydrate is the second important biochemical component of E. acutifrons collected from the coastal waters of Alexandria. The absolute carbohydrate content of the organism as a percent of dry weight fluctuated between 3.4% at Agami in summer and 19.3% at the central E.H. station during autumn, however, the later represented the maximum protein content (Table 1). Likewise protein, the carbohydrate content of Euterpina was also enriched in the E.H. (Figure 3) coinciding with the high average chlorophyll a recorded in the harbour during the same period i.e. 8 mg chlorophyll a/m3. The offshore stations of both Maadia (12.5%) and Tabia (10.9%) sectors showed the maximum levels in the bay during the study period (Table 1). No perceptible difference was observed between W.H. stations during winter and spring. In Mex Bay, the inshore and offshore stations showed an alternation of high and low carbohydrate values during the study period. Moreover, for Agami area, the carbohydrate content increased gradually from 3.4% in summer to 9.7% in spring (Table 1).

The maximum seasonal carbohydrate content of E. acutifrons appeared in winter i.e. $8.8 \pm 1.2\%$ (Figure 3). On seasonal basis, the carbohydrate content showed a highly significant correlation with chlorophyll a values in autumn ($r = 0.94, p < 0.003$) and summer ($r = 0.74, p < 0.003$). Also, on a regional basis, the correlation appeared to be highly significant ($r = 0.90, p < 0.001$) indicating that food is an important factor affecting carbohydrate content, in different sampled areas.

Lipid content

In the coastal waters of Alexandria, lipids of E. acutifrons dry weight, constituted an annual average of $3.7 \pm 1.9\%$, ranging
Biochemical composition of *Euterpina Acutifrons*

Table 1

Protein, carbohydrate and lipid contents of *Euterpina acutifrons* (% DW) in the coastal waters of Alexandria during 1989/1990.

<table>
<thead>
<tr>
<th>Location</th>
<th>AG</th>
<th>MX</th>
<th>WH</th>
<th>EH</th>
<th>AQ (TB)</th>
<th>AQ (MD)</th>
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<td>Station</td>
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<tr>
<td>PR%</td>
<td>42.0</td>
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<td>36.3</td>
<td>39.1</td>
<td>32.9</td>
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<tr>
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<td>6.6</td>
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<td>9.5</td>
<td>7.3</td>
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<tr>
<td>LP%</td>
<td>1.9</td>
<td>2.6</td>
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<tr>
<td>PR%</td>
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<td>28.8</td>
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<tr>
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<td>11.1</td>
<td>12.6</td>
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<tr>
<td>LP%</td>
<td>1.8</td>
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* AG = AGAMI MX = MEX BAY WH = WESTERN HARBOUR EH = EASTERN HARBOUR AQ(TB) = ABU QIRI EL-TAIBIA SECTOR AQ(MD) = ABU QIRI MAADIA SECTOR.

LP = LIPID PR = PROTEIN CR = CARBOHYDRATE

from 1.1 to 10.6% (Table 1). The lipid content shows characteristically high levels in organisms inhabiting the E.H. area (Figure 3); whereas that of W.H. (av. 4.8 ± 2.1%) and Maadia sector (av. 3.8 ± 1.5%) of AQ Bay showed to a less extent relatively moderate lipid contents.

On the contrary, low lipid contents of the organisms were noticed at Tabia sector (av. 2.3 ± 1.0%), Mex Bay (av. 2.6 ± 0.8%) and Agami (av. 1.9 ± 0.3%).

Concerning the seasonal variations in the lipid content of *Euterpina*, the highest averages of Western and Eastern harbours in summer, were reflected on the maximum annual average of lipid during this seasons i.e. 5.1 ± 2.7%. However, variations among other sampling periods was nearly limited. In general, lipid was significantly correlated with chlorophyll a (r = 0.84, p < 0.002).

**DISCUSSION**

Generally, the biochemical composition of *Euterpina acutifrons*, sampled from the coastal waters of Alexandria, is affected by the availability of food, the type of wastes discharged from land-based sources as well as seasonal changes in environmental factors.

The stepwise regression equations (Microstat version 2.0) were used for assessing the impact of different environmental factors reported for each season separately, on the protein, carbohydrate and lipid contents of *Euterpina* (Appendix 1). The biochemical content was used as a dependent variable while salinity, oxygen, light penetration, temperature, phosphorus, nitrate and chlorophyll a as independent variables. Equations indicated that chlorophyll a (phytoplankton biomass index) is an important element affecting solely (dominant case) or in combination with other measured parameters like phosphorus, temperature ..., etc., the biochemical composition of *E. acutifrons*. Temperature is second in importance especially for protein as obvious from winter and spring equations and to a less extent summer's equation. Nitrate appeared in several equations specially for carbohydrate (summer and spring) and lipids (summer) while for protein, nitrate appeared but with a lower significance in summer.

Opposite to both Tabia and Maadia openings, specially at high discharge periods, the lowest biochemical concentrations were recorded during the study period (Figure 3). The discharge of such pollutants not only affects the concentrations of different biochemical components of the organism but also may lead to a complete disappearance of the animal (see station 10 during summer 1989). However, despite the discharge of large amounts of untreated sewage water to the Eastern Harbour basin, the harbour is characterized by extraordinary high biochemical levels. The sewage discharged to the harbour carries sufficient amounts of nutrients capable of elevating the biomass and productivity of the microalgae (diatoms), the basic food source for Euterpina.

Despite their principal role in the marine food web, limited studies have been carried out on the biochemical composition of copepods. Available data dealt with total populations or dominant groups rather than monospecific species. During the present study, the average protein, carbohydrate and lipid contents of...
Euterpina were 35.2 ± 6.7%, 7.9 ± 1.6% and 3.7 ± 1.9%, respectively. Nandakumar et al. [29] observed that protein contents, in zooplankton collected from North central Arabian Sea, were 34.6 ± 4.55%, while those of lipid and carbohydrate were 6.3 ± 2.12% and 6.0 ± 1.29%, respectively. The average carbon content was 34.62 ± 5.47% showing a significant correlation with protein r = 0.72 but insignificant correlations with carbohydrates and lipids.

Protein/carbohydrate ratio has been reported to be a sensitive method or index of detecting changes either in the nutrient availability or metabolic activities [30]. In the present study, the statistical relationship between Protein / Carbohydrate with nutrients was estimated. At times of sufficient nitrogen and phosphorus, an increase was observed in Protein / Carbohydrate ratio. During nutrient sufficiency, cell metabolism is directed towards protein synthesis.

The decrease in protein with diminishing nitrogen resources can be accelerated by various factors, such as temperature [31]. Generally, many authors use the Protein/Carbohydrate ratio as a criterion for predicting the nutritional status of the population. Healey [32] and Healey and Hendzel [33] suggested the use of Protein/Carbohydrate of < 0.7, 0.7-1.2 and > 1.2 as indicators of extreme nutrient deficiency, moderate deficiency and sufficiency, respectively. The average Protein/Carbohydrate ratio for E. acutifrons during the present study is 4.46 ± 2.3, reflecting nutrient sufficiency in inshore waters of Alexandria.

Lipids are important biochemical components in fresh and sea water ecosystems [34-36]. Cavaletto et al. [37] estimated the total lipid content of female L. macrurus to range from 42.3 ± 3.1% to 67.3 ± 3.3% of dry weight while for males total lipid ranged from 44.7 ± 3.0% to 50.0 ± 2.1%. For Senecella calanoides, they recorded a total lipid content of 29.5 ± 3.0%.

Most studies have shown that lipids are important energy reservoirs in many species. Unusually, lipids of copepods varied within wide limits [38,39]. Kattner and Krause [40] observed high lipid content in zooplankton after phytoplankton blooms. Gatten et al. [41] studied the total lipid content and lipid classes of C. helgolandicus and observed high variability from year to year, especially in females.

The seasonal variation of total lipid in E. acutifrons was dependent on environmental conditions such as food supply appearing as phytoplankton blooms (see chlorophyll, Figure 2c), which is highly variable owing to nutrient supply (see nitrogen and phosphorus, Figures 2a & b). Gatten et al. [41] and Kattner et al. [42] reported that copepods are generally the heaviest and rich in lipid shortly after the spring phytoplankton bloom.

Bamstedt et al. [43] estimated the total lipid content of different copepod species in Koster Fjorden, western Sweden. They recorded levels of 15.8% for Calanus finmarchicus (CV), 9.6% for Acartia clausi (female), 11.3% for Acartia clausi (male) and 17.7% for Pseudocalanus spp. (CV), in the surface waters (0-30 meters). However, Herring [44] determined lipid concentrations for different zooplankton species and found values ranging from 0.5 to 6.9%.

The lipid/protein ratio gives a rapid indication of the extent of consumption of either component. The annual average ratio for E. acutifrons was 0.105 with a range of 0.051 to 0.167. Comparison of lipid/protein ratios for different sampled stations indicated that stations occurring within a surplus of food condition i.e. Eastern Harbour (Figure 2c) had a higher ratio (average range 0.17-0.22), while those located under stress from industrial discharge ex: Mex bay and Abu Qir bay, showed 50% lower ratios ranging between 0.07-0.10 and 0.06-0.09, respectively. This finding suggests that industrial pollution has more impact on protein production than on lipid mobilization. In case of nutrients sufficiency, lower lipid/protein i.e. <0.1 were observed confirming enhanced protein synthesis/production during this period. On the contrary, in warm seasons, ratios were much higher i.e. 0.2-0.3 especially in the Eastern and Western Harbours, due to enhanced protein consumption (catabolism) as well as accumulation of lipids (Figure 3).

REFERENCES

Biochemical composition of Euterpina Acutifrons


APPENDIX 1

Stepwise multiple regression equation for different biochemical parameters during the study period

**Summer 1989**
Protein (%) = 46.4 ± 6.42 + 2.99 ± 1.06 chla - 2.02 ± 1.15 S%o + 3.53 ± 2.1 SD + 7.56 ± 5.32 PO₄ - 1.7 ± 1.66 NO₃ + 1.02 ± 1.09 DO + 1.44 ± 1.99 T (r = 0.7432, p < 0.0013).
Carbohydrate (%) = 20.26 ± 0.805 + 1.04 ± 0.11 chla - 0.9125 ± 0.15 NO₃ - 0.398 ± 0.09 S%o + 0.66 ± 0.28 SD (r = 0.885, p < 0.00001).
Lipid (%) = 3.804 ± 1.20 + 0.776 ± 0.104 chla - 0.363 ± 0.118 NO₃ (r = 0.6437, p < 0.0002).

**Autumn 1989**
Protein (%) = 27.5 ± 6.37 + 2.82 ± 0.47 chla (r = 0.8577, p = 0.0004).
Carbohydrate (%) = 5.179 ± 1.51 + 1.10 ± 0.11 chla (r = 0.9613, p = 2x10⁻⁷).
Lipid (%) = 0.7 ± 1.07 + 1.49 + 0.63 PO₄ + 0.08 ± 0.02 chla (r = 0.7930, p = 0.006).

**Winter 1990**
Protein (%) = 3.387 ± 6.54 + 1.96 ± 1.11 T (r = 0.6114, p = 0.011).
Carbohydrate (%) = 2.61 ± 1.78 + 3.146 ± 1.43 chla (r = 0.7212, p = 0.0002).
Lipid (%) = 1.368 ± 1.006 + 0.6839 ± 0.099 chla (r = 0.9418, p = 0.0003).

**Spring 1990**
Protein (%) = 3.38 ± 6.54 + 1.963 ± 1.109 T (r = 0.4405, p = 0.1002).
Carbohydrate (%) = 4.79 ± 1.07 + 1.17 ± 0.43 NO₃ + 0.33 ± 0.99 chla (r = 0.7130, p = 0.0139).
Lipid (%) = 1.97 ± 0.9 ± 0.4538 ± 0.073 chla (r = 0.8639, p = 0.00003).

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