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A BIOCHEMICAL APPROACH FOR THE ESTIMATION OF FOOD PROVISION FOR HETEROTROPHIC ORGANISMS OF THE BLACK SEA

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Abstract. Some biochemical indices were applied in order to understand the state of food provision (or supply) for Black Sea pelagic animals during May 1991 and September 1996. Total lipid content and the main lipid fractions (especially wax esters) of *Calanus euxinus* females strongly correlated ($P<0.005$) with mean integrated chlorophyll-a of the euphotic zone. In September 1996 a trend (albeit not statistically high, $r=0.79$, $P<0.1$) was also observed between total lipid content of female *Calanus* and glycogen content of its predator, the ctenophore *Pleurobrachia rhodopis*.

Comparison of sea regions differing in water dynamics for the biochemical indices used was of special interest. Animals from the cyclonic regions (where the main pycnocline is located at 100-120 m) contained almost double the lipid and glycogen content than those obtained from the anticylonic regions (where the main pycnocline is located at 160-190 m). The euphotic zone of cyclonic regions was also richer in nutrients (phosphate and nitrate) and chlorophyll (indicating phytoplankton biomass and thereby the food of *Calanus*) compared to anticyclonic regions.

Long-term observations on the abundance and lipid content of the sprat *Sprattus sprattus* revealed the importance of river inflow for the nutrition of this pelagic fish species. As a result of the increased transport of riverborne nutrients, the lipid content of this fish had risen from 9% to 16%. It can be concluded that these biochemical indices may be successfully used for monitoring the condition (well being) of dominant heterotrophic species in the pelagic ecosystem of the Black Sea.

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1. Introduction

As food is one of the most important factors limiting the growth and reproduction of marine animals, studies related to food provision (or supply) in their natural environment are of much significance. Food also provides the main biotic link between organisms and the environment and sets a limit to productivity.

However, it is a very difficult task to quantify the relationship between the available forage and its consumers in the sea. At present, not a single method reliably evaluates the quantity of food organisms and their predators on one hand, and the amount of food they are consuming in the water body on the other hand. Physiological methods devised to assess the food provision of aquatic organisms have also proved to be imperfect.

In strict terms, food provision (F_p) is understood as a relationship between food consumed(F_c) and the food demand of the organism (F_d):

$$F_p = F_c / F_d.$$

Current methods for the estimation of food consumed (F_c) through the index of fullness (amount of food in the digestive tract; [1, 2]), the rate of digestion [3, 4] and the activity of digestive enzymes [5, 6] are imperfect. The same refers to the indirect methods such as energy and substance balances (e.g. oxygen consumption and nitrogen excretion; [2, 7]). Obviously, it is simply impossible to imitate the totality of diverse natural factors in experiments.

The evaluation of food demand (F_d), seems to be even more complicated. The absolute value of the food demand is the total of all elements in energy balance equation (i.e., the amount of substance or energy used for both somatic and generative growth, and for basal and active metabolism). This value is strongly influenced by body weight and physiological condition (primarily, the stage of development and maturation) of the organism, ambient temperature, availability of food and other factors. Certainly, these dynamic components can not all be taken into account during examination of specific populations and species.

Unfortunately, the indirect approach to the study of food provision is also inefficient. One can not estimate the amount of food supplied from the total biomass of food organisms alone, since there are always other consumers feeding on these food organisms and their composition will certainly affect the food uptake of the organisms being studied. Besides, one must be aware that the process of food consumption depends not only on the presence of competitors for food but also on the pressure of predators [8, 9].

Abiotic factors like temperature, light, turbidity, stratification, current velocities and other factors have also considerable impact on the availability of food organisms. These may be described by the following equation:

$$F_p = f(A_b, A_{c1} \ldots A_{cn}, T, L, \ldots, x_1, \ldots x_n)$$

where A_b is the biomass of food organisms, $A_{c1} \ldots A_{cn}$ are the biomass of consumers, T is the temperature, L is the light, and $x_1 \ldots x_n$ are the numerous other factors.

An alternative approach entails methods of direct estimation of the effect of consumption. The effect of consumption is considered as the integral characteristics of the influence of forage on organisms investigated. As growth (or production) and energy accumulation are the elements of the organism's life cycle, their rates are directly proportional to the food provision [10].

In quantitative description of these processes (i.e. growth and energy accumulation) biochemical indicators are widely applied. These allow us to assess the "degree of well-being" (i.e. the state) of organisms, populations and species on the whole [10, 11, 12, 13, 14, 15]. For many fishes and planktonic crustaceans the meaningful parameter is the content of neutral lipids (triacylglycerols and wax esters) in the body, while for ctenophores and molluscs it is the content of glycogen.

The Black Sea is a suitable experimental basin for application of the aforementioned methods in the estimation of food provision in diverse marine animals. This arises firstly from the fact that for this sea there exists a large data series suitable for the investigation of spatial and temporal variations in lipid and glycogen contents of some dominant species including commercial fishes. Secondly, long-term studies on the physical, chemical and biological characteristics of the Black Sea have made it possible to evaluate the link between food provision and the current ecological situation in specific localities or in the whole of the basin.

The present study focused on three dominant cold-water pelagic species of the Black Sea, namely the copepod *Calanus euxinus*, the ctenophore *Pleurobrachia rhodopis* and the fish *Sprattus sprattus phalericus*. *Calanus euxinus* is the most important fodder zooplankton species of the Black Sea ecosystem, for both commercially valuable small fishes including *Sprattus* and the other competing carnivores such as the ctenophore *Pleurobrachia rhodopis*. During the warm months of the year, the *Calanus* population is made up of two sub-populations: an actively feeding, migrating sub-population and a nonmigrating, nonfeeding (deep) sub-population. Being the main food component of *Pleurobrachia* and *Sprattus*, the biomass and condition of *Calanus euxinus* are the most important factors affecting the level of food provision in the ctenophore and the fish. In turn, the biomass as well as the condition of the feeding population of *Calanus* are determined by measuring the concentration and composition of phytoplankton (being the main food for the copepod) in the water column. Adult *Calanus* are observed to feed on a wide range of microphytoplankton species (i.e. >10-50 μ). So the condition of these heterotrophs must be correlated within the food chain of phytoplankton - *Calanus* - *Pleurobrachia* and *Sprattus*. In the present study the validity of this approach has been examined.

2. Material and Methods

In this study, we used materials obtained during the following research cruises:

1) the R/V Professor Vodyanitsky (Institute of Biology of the Southern Seas, IBSS Sevastopol, Ukraine) cruise to the northern deep-sea area of the Black Sea in May 1991. The task was to elicit quantitative relationships between the index of food provision and the nutritive base of *C. euxinus*. The latter was estimated through the content of chlorophyll-a in the sea water, which is used as an indicator of phytoplankton biomass.

2) the R/V Bilim (Institute of Marine Sciences, Erdemli, Turkey) cruise to the southern Black Sea in September 1996. The task was to link data on the food provision of *C. euxinus* and *P. rhodopis* with hydro-physical and hydro-chemical factors determining the nutritive base of Black Sea heterotrophs.

3) numerous research cruises of R/V's of IBSS and AzCherNIRO (Azov and Black Seas Research Institute of Fisheries and Oceanography, Kerch, Ukraine) to the north-western and some other regions of the Black Sea. The resulting sets of data cover more than three decades, from the 1960's to the present day. Analysis of these data sets may reveal the relationship between the dynamics of food provision and the riverine inflow.

Material for the study of food provision in planktonic crustaceans was collected from 15 stations located in the northern Black Sea and from 6 stations in its southern region (Fig. 1).

The survey conducted in the northern Black Sea (May - June 1991) involved the collection of *Calanus* by vertical hauls with a Jome plankton net (0.8 m mouth diameter, equipped with 200 μm mesh) at each station. Sampling was carried out at night (21:00h - 00:00h) in surface waters (from 30-50 m to 0 m) at temperatures of 17-21 ^{0}C. It is known that during these hours *Calanus* ascend from deeper waters towards the sea surface for feeding [16, 17] which was confirmed by the microalgal remains in the intestines of captured copepods.

Alongside sampling, measurements of chlorophyll-a concentrations in the euphotic zone (0-75 m) at 6-8 different depths for all stations using the fluorimetric extraction technique were taken [18]. Physical characteristics of the water column were measured using a Nielsen-Brown CTD probe attached with 12 l plastic water bottles and a fluorometer. Design of the depth selection was aimed at imitating the in situ fluorescence profile most closely and taking the best possible number of samples from the layer of maximum temperature gradients.. The lower euphotic boundary was determined from the fluorescence minimum. Averages of vertically integrated chlorophyll concentrations in the euphotic layer were calculated at each station.

For the lipid content analysis 50 female copepods from a size range of 3.3-3.5 mm total length were used for each of two replicates. They were placed in the chloroform - methanol (2:1 v/v) preparation for lipid extraction as described by Folch *et al.* [19]. Resulting extracts were stored at -20 ^{0}C for further qualitative and quantitative determinations of lipid content at the laboratory. The sulfovanillin technique [20] was applied to define the content of total lipids in extracts. Lipids extracted from 200 female *Calanus* according to Folch *et al.* [19] and determined using weighing technique as described by Kates [21] were accepted as standard. Total lipids were divided into groups by means of thin-layer chromatography on Silufoll UW254 plates (Kavalier Ltd. Czechoslovakia) and a series of solvents with the inverse polarity gradient (e.g. chloroform, hexane : diethyl ether (9:1 v/v) and hexane [22]. Adaptation of the chromatography chamber (three chambers successively resting on each other) allowed us to attain the effect of the polarity gradient. The innermost chamber was filled with chloroform, the intermediate with hexane-ether mixture and outermost with hexane. Prior to lipid separation, plates were activated at 105 ^{0}C and treated with a 10% solution of

phosphomolibdenum acid in ethanol; after separation plates were developed at 110 ^{0}C. Determination of individual lipid classes was made using an ERS-65 densitometer (Karl Zeiss, Germany). On examination of the corresponding lipid standards (phospholipids, triacylglycerols, sterols, sterol esters, wax esters) it was ensured that identical conditions were maintained. Total lipid content and lipid fractions were described as μg per individual.

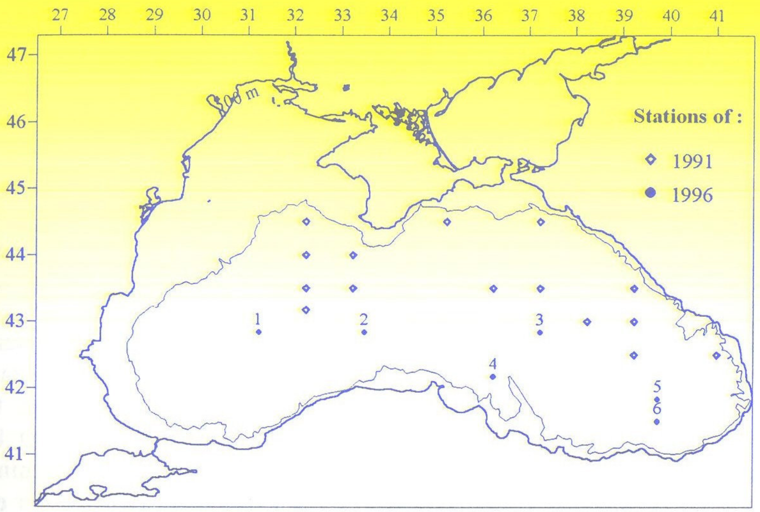

Figure 1. Sampling stations in June 1991 and September 1996 (numbered).

During the survey in the southern Black Sea (25 September - 4 October 1996) copepods (*Calanus euxinus*) were captured at night in surface waters using a Nansen plankton net (112 μm mesh size, 0.7 m diameter) and the ctenophore *Pleurobrachia rhodopis* was caught with a Hensen net (300 μm mesh size, 0.7 m diameter) from the entire aerobic water column. The boundaries of the aerobic water column were determined for each station from the density depth of δ_t =16.20 [23, 24] calculated from temperature and salinity profiles defined by means of a Sea Bird CTD probe. Ascend in the δ_t =16.20 to 100-120 m depth was indicative of cyclonic activity and descend to 160 - 200 m depth was anticyclonic one . The sea surface temperature was 18-21 ^{0}C.

Along with copepods and ctenophores, mesozooplankton samples were taken from throughout the entire aerobic layer. Samples were treated with buffer solutions of formaldehyde, and enumeration, species identification and size measurements were carried out under a binocular microscope using a Bogorov chamber. The biomass of individual organisms was evaluated by the computation technique as described by Petipa [25, 26].

Ctenophores with an aboral length of 9-11 mm and a weight of 400-500 mg made up the largest proportion of the population and were used for determination of polysaccharide and glycogen contents. After capture and size measurement ctenophores were kept in containers for 2-3 h in order to empty their guts.

Polysaccharide and glycogen content determinations were made in individual samples applying the spectrophotometric method [27]. The polysaccaride content was calculated as μg per g of wet weight, glycogen in μg of wet weight and as a percentage of the total polysaccharide content.

At each station concentrations of chlorophyll, nitrates and phosphates were measured at depth intervals of 15-20 m from the surface down to the δ_t = 16.50 density level. Profiles of chlorophyll, nitrate and phosphate contents were drawn, and integrated concentrations for the euphotic zone evaluated.

Data from the research cruises undertaken by IBSS, YugNIRO (former AzCherNIRO), and YugRyb-Promrazvedka (Fishery Observation Service) and information provided by fishing boats contributed to the knowledge of food provision in the fish species sprat. Research cruises were carried out during the late feeding season of sprat (June-July) when fat content of the fish was maximal. The main bulk of data was obtained in the north-western Black Sea where sprat stocks were the most abundant.

Fish caught with the trawl net were divided into size groups of 5 mm increments. In each group there ranged from 10 to several dozens of fish. These were examined for lipid content. For this purpose fish were chopped in a mincing-machine; then a sub-sample from the minced flesh was put into a laboratory dish and dried at 105 ^{0}C until reaching a constant weight. Following this the lipid content was determined (in dried batches) using ethyl-ether extraction in a Soxhlet apparatus. Ethyl ether extraction permits the isolation of mainly neutral lipids and partially polar lipids [20]. At the same time, in the ship laboratory total lipids were extracted from wet (undried) batches according to Folch *et al.* [19] in order to provide further analysis of lipid content by means of the weighing method. This mode of extraction yields the most reliable results. The relationship between dry (y) and wet extraction (x) is as follows [28]:

$$y = 0.83\ x.$$

This relationship makes it possible to compare data obtained using either of these two methods during a long-term monitoring study.

Along with lipid content, the abundance and biomass of sprat were also examined during a survey undertaken in May by the R/Vs of AzCherNIRO employing the Simrad Scientific Hydroacoustic instrument.

The hypothesis that the in situ lipid content of the copepod *Calanus euxinus* and glycogen content of the ctenophore *Pleurobrachia rhodopis* is a function of food provision was tested using Simple Linear Correlation analysis [29].

3. Results

Previously, for the 1991 cruise data, a highly significant correlation (r = 0.82; n= 9; P<0.005) was shown to exist between the chlorophyll-a concentration and biomass of phytoplankton (determined microscopically) within the size range of 10-50μm [15].

This indicates that the chlorophyll-a measurements alone may be sufficient for the evaluation of food availability for female *Calanus*. Indeed, highly significant correlations were found between concentrations of (integrated mean) chlorophyll-a and of total lipid or its fractions (wax esters, phospholipids, triacylglycerols) of the feeding (and migrating) female *Calanus* (sampled at the surface layer during night-time). The relationship between mean chlorophyll-a concentration and the content of wax esters

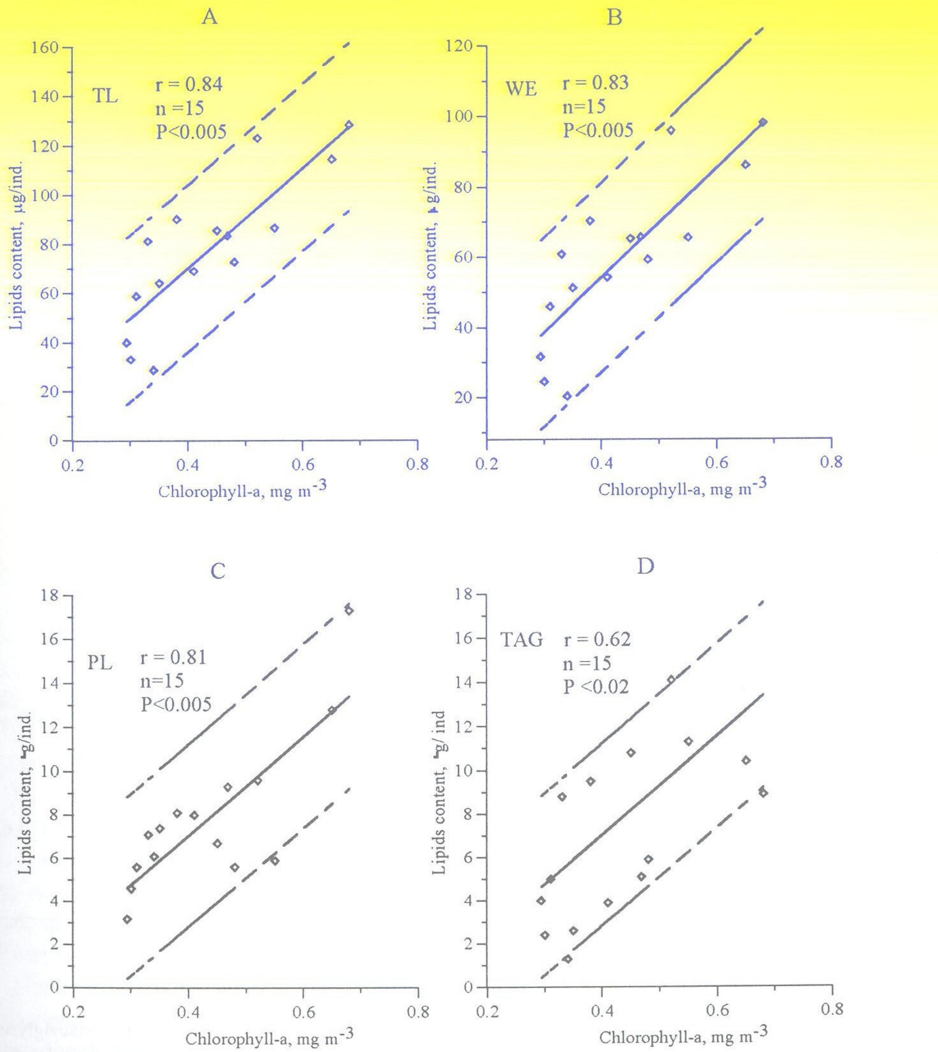

Figure 2. Relationship between lipid (TL- total lipids, WE- wax ester, PL- phospholipids, TAG- triacylglycerols) contents of female *Calanus euxinus* and mean integrated chlorophyll-a concentration in the northern Black Sea during 1991 cruise.

(WE; being the major fraction of lipid in *Calanus*) is shown in Fig. 2B where r = 0.83 (P<0.005). Respective correlation values were r = 0.81 (P<0.005) for the phospholipids (Fig. 2C), r = 0.62 (P<0.02) for the triacylglycerols (Fig 2D) and r = 0.84 (P<0.005) for the total lipids (Fig. 2A). It is worth noting that the lipids of *Calanus* females consisted mainly of wax esters (77.5%), phospholipids (10.4%) and triacylglycerols (8.4%) [15].

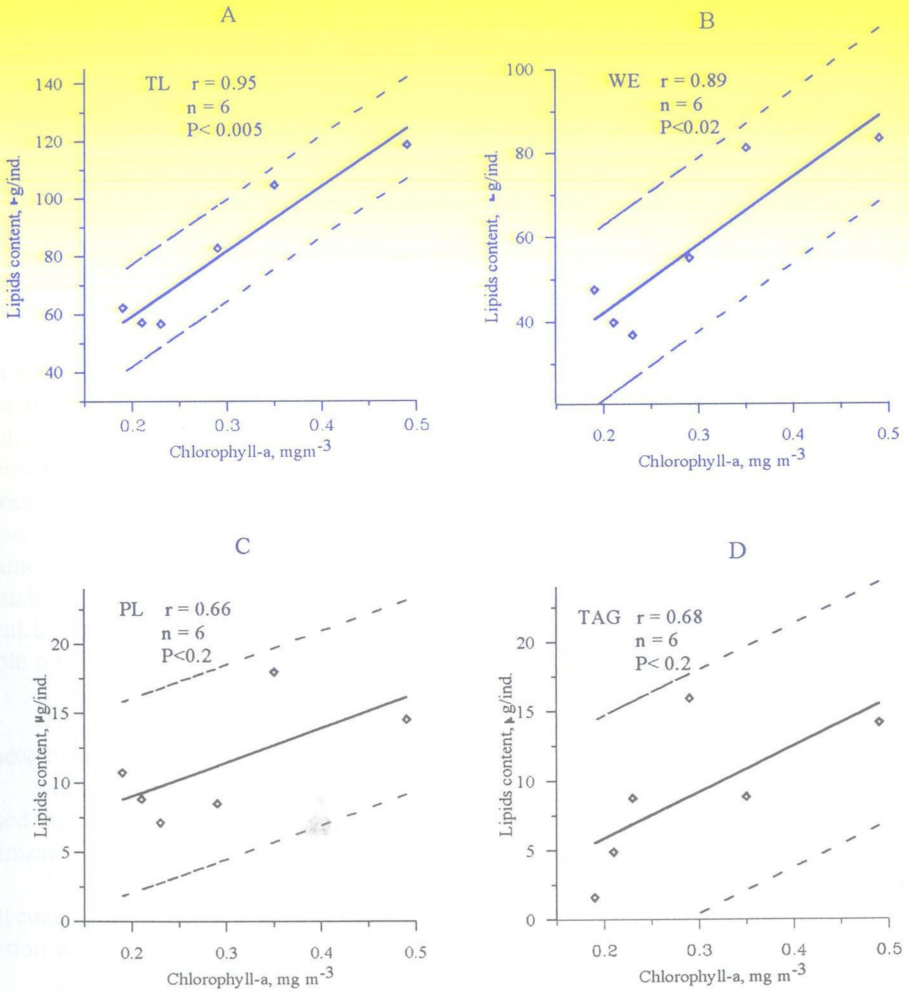

Figure 3. Relationship between lipid (TL- total lipids, WE- wax ester, PL- phospholipids, TAG- triacylglycerols) contents of female *Calanus euxinus* and mean integrated chlorophyll-a concentration in the southern Black Sea during 1996 cruise.

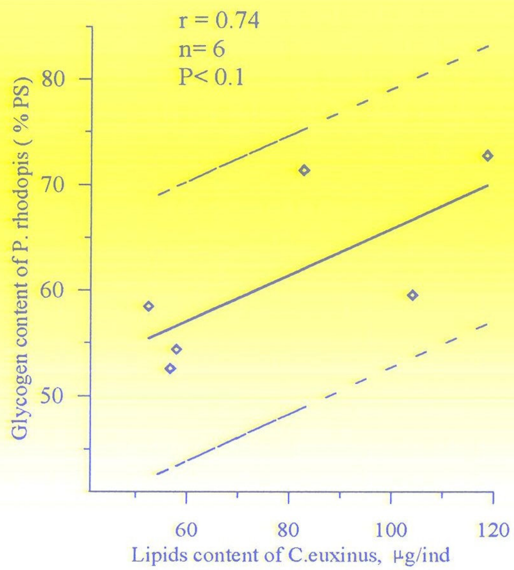

Figure 4. Glycogen content (% of total polycacharides) of *Pleurobrahia rhodopis* versus total lipid content (µg/ind) of *Calanus euxinus* females in September 1996.

In the 1996 cruise, wax esters again made up the majority of total lipid (average 72%, standard deviation 6.25%) in feeding (and migrating) female *Calanus*. The mean chlorophyll-a concentrations also correlated well both with wax ester content (r = 0.89; n = 6; P<0.02; Fig. 3B) and total lipid content (r = 0.95; n = 6; P<0.005; Fig. 3A) in *Calanus*. With an average of 13% (standard deviation 3%), phospholipids were the second most important fraction of total lipids. Neither phospholipids nor triacylglycerols showed a significantly strong relationship with chlorophyll-a, but they both displayed positive trends (Fig. 3C, D).

Thus, the level of lipids stored by *Calanus* clearly responds to changes in the chlorophyll-a concentration which, in turn is related to the food availability.

Similar to the chlorophyll-a - *Calanus* lipid relationships, a trend was also observed between the lipid content of *Calanus* and the glycogen content of *Pleurobrachia*. However the correlation coefficient for this relationship obtained from the 1996 cruise data was not significant (r = 0.74; P<0.1; Fig. 4). Overall, the mean glycogen content of the ctenophore was 38.5 µg/g wet weight within the range of 29.4 and 51.6 µg/g wet weight.

Not only biotic but also abiotic factors influence the accumulation of energy in *C. euxinus* and *P. rhodopis*. The effect of abiotic factors manifests itself distinctly at the stations located in areas differing in water column stability. At stations situated in the cyclonic region (stations 1-3 in Fig.1; where δ_t = 16.2 density level is at 100-120 m depth), higher concentrations of nutrients (i.e. NO_3 and PO_4) and chlorophyll-a were measured as compared to those in the anticyclonic region (stations 4-6 in Fig. 1; δ_t = 16.2 density level is at 160-190 m depth) (Fig. 5 and Table 1). Similar to nutrients and chlorophyll concentrations, the lipid content in *C .euxinus* and glycogen content in *P. rhodopis* were higher in the cylonic region. From the three stations located in the anticyclonic region, the average lipid content of *Calanus* was 58.8 μg ind^{-1} (SD=3.1 μg ind^{-1}) which is only about half of the value for the cyclonic region (average 101.9 μg ind^{-1} with a SD=18.0 μg ind^{-1}).

TABLE 1. Physical and chemical measurements at the stations in the cyclonic and anticyclonic regions during September 1996. T^{0} and S‰ are mean values in the upper quasi-homogeneous (in temperature) "mixed layer"; NO_3, PO_4 and chlorophyll-a are mean integrated values in photic zone.

Stations	T^{0}C	S	Depth, m of σ_t = 16.20	NO_3 mM/m^3	PO_4 mM/m^3	Chl-a mg/m^3
Cyclonic region						
1	18.55	18.35	105	1.326	0.423	0.49
2	18.88	18.41	113	0.871	0.275	0.35
3	18.47	18.29	123	0.790	0.200	0.29
Mean	18.63	18.35	114	0.996	0.299	0.38
Anticyclonic region						
4	17.85	18.24	160	0.532	0.109	0.21
5	21.61	18.08	193	0.371	0.032	0.19
6	21.72	18.01	180	0.412	0.042	0.23
Mean	20.39	18.11	178	0.438	0.061	0.21

As opposed to the lipid content of *Calanus*, the total mesozooplankton biomass, (akin to the biomass of *C. euxinus* being one of the principal contributors to the total mesozooplankton biomass) displayed no significant correlation (or even trend) with the integrated chlorophyll-a concentration (Fig. 6).

However, the biomass of some mesozooplankton organisms may provide important information in relation to the investigated subject. For instance, although not significant, a clear trend for an inverse correlation has manifested itself between the lipid content of *Calanus* and the biomass of *Noctiluca* (Fig. 7). It is known that whilst developing their greatest abundance in autumn, *Noctiluca* feed predominantly on phytoplankton [30]. During the 1996 cruise *Noctiluca* was also observed to be feeding heavily on phytoplankton. This luminous protozoan rapidly increases in biomass and when its energy expenditure is low and when nutritive organisms are widely available, *Noctiluca* is a strong competitor to *C. euxinus*, diminishing the copepod's food source. The inverse relationship (though not statistically significant) between *Noctiluca*'s biomass and chlorophyll-a as given in Fig. 8 provides the evidence of this situation. Long-term studies initiated in 1960, have shown that the fatness and biomass of sprat increased considerably (from 9 to 16% and from 400 to 1600 thousand tonnes, respectively; Fig. 9) as the river inflow capacity into the Black Sea rose. The drastically

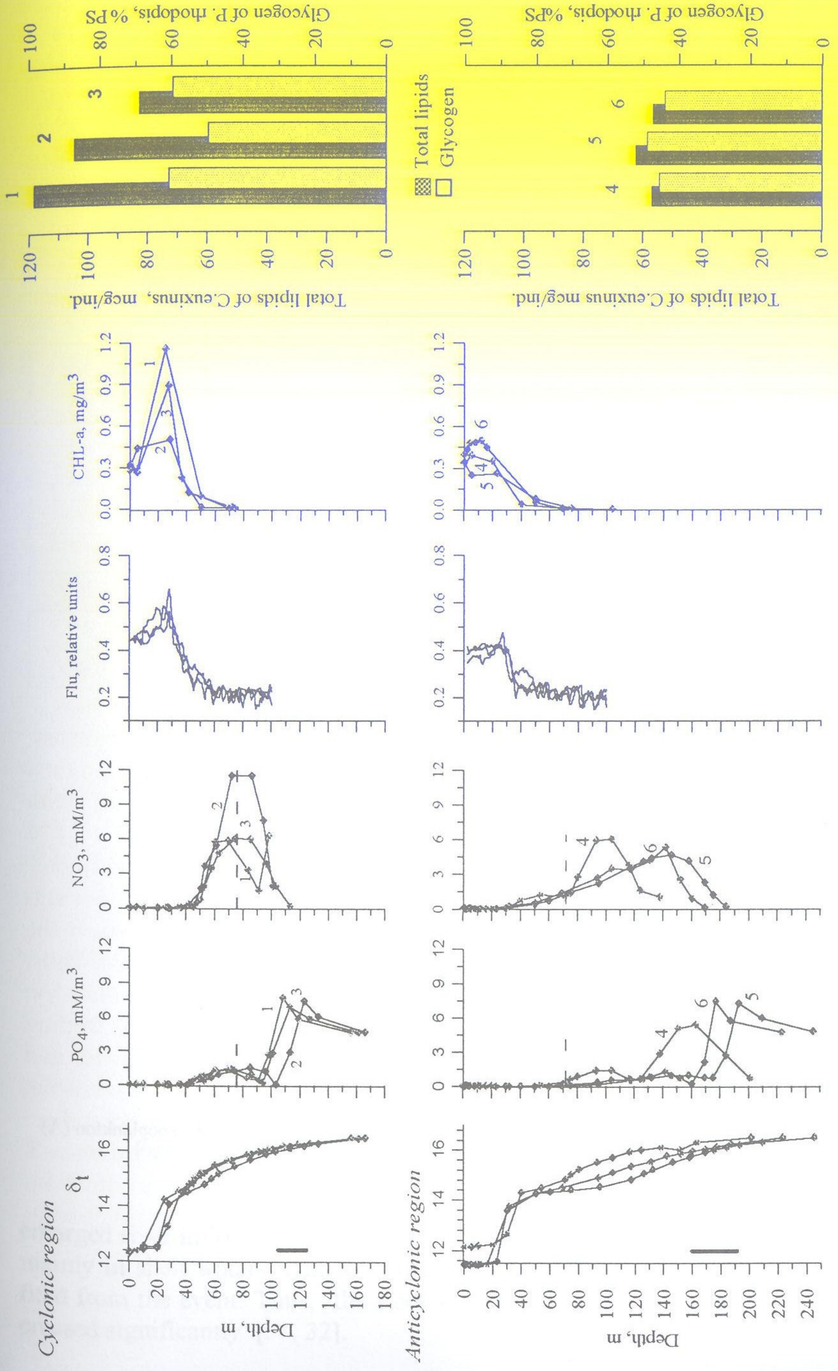

Figure 5. Vertical profiles of sigma-t, phosphate, nitrate, in-situ fluorescence, chlorophyll-a concentrations and total lipids of *Calanus euxinus* females and glycogen content of *Pleurobrachia rhodopis* in cyclonic region (stations 1, 2 & 3) and in anticyclonic region (stations 4, 5 & 6) in the southern Black Sea. Bold lines on sigma-t profiles show oxygen minimum layer being the diapausing layer (sigma-t = 15.4 - 16.2) for *Calanus*. Dashed lines on PO_4 & NO_3 profiles indicate bottom border of the euphotic zone. PS = Polysaccharides.

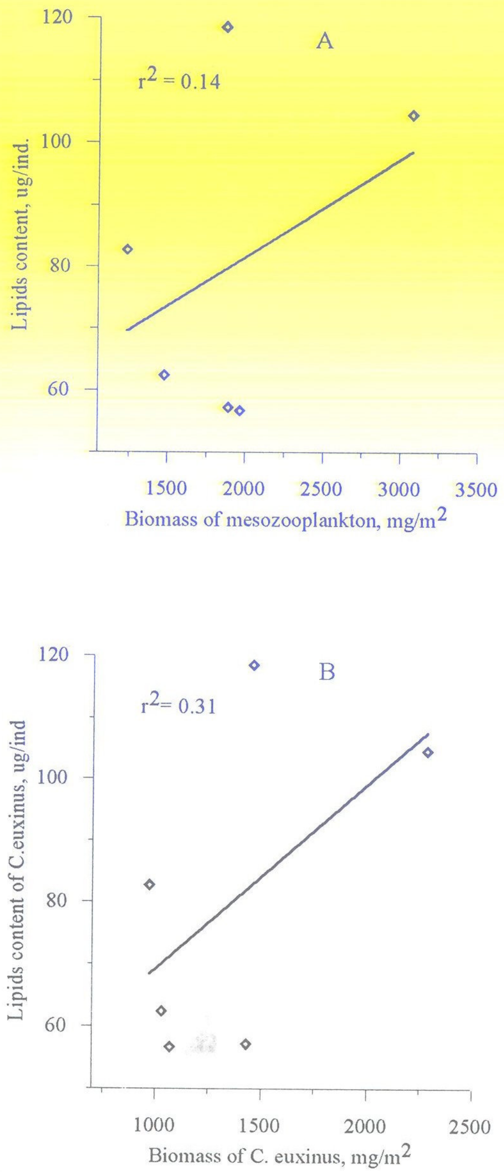

Figure 6. Relationship between lipids content of *C. euxinus* females and biomass of total mesozooplankton (A) and biomass of *C. euxinus* (B).

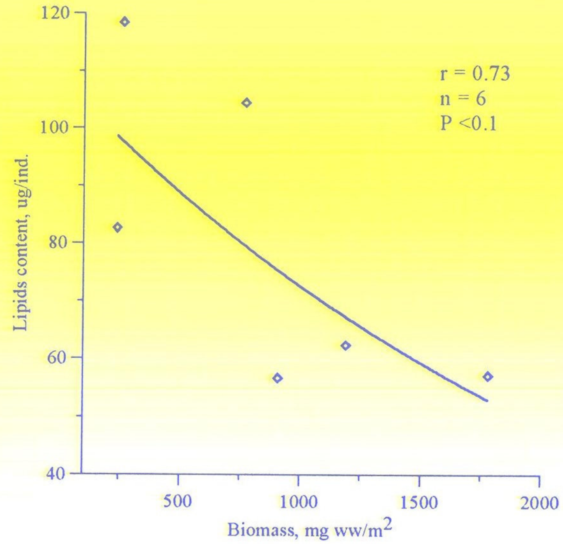

Figure 7. Relationship between lipids content of C.euxinus females and biomass of Noctiluca miliaris during September 1996.

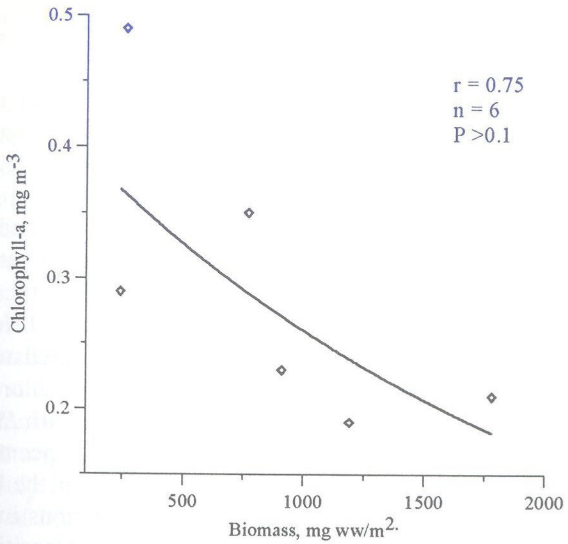

Figure 8. Relationship between mean integrated in euphotic zone chlorophyll-a concentration and biomass of Noctiluca miliaris during September 1996.

enlarged river inflow carrying an enormous amount of nutrients caused eutrophication mainly in shelf waters. Paradoxically, many pelagic organisms including sprat benefited from the event. Thus, the stock of pelagic fishes such as anchovy and sprat increased significantly [31, 32].

4. Discussion

Biochemical indicators showing the extent and rate of energy accumulation in marine organisms were applied for studying the food provision as early as the 1950-1960's in the Black Sea. At that time the organisms under investigation were Azov and Black Sea anchovy, Azov Sea kilka and sprat. The approach proved to be reliable and has been widely used in studying the condition of these fishes [10, 11, 31, 33, 34, 35, 36, 37]. Long-term observations have revealed the "well-being" of populations and stocks of mass fishes of the Azov and Black Seas to be dependent on the condition of their nutritive base.

Since the 1980's studies concerned with the food provision of fish have been expanded to other seas. They have focused mostly on the relation between the relevant lipid characteristics of fish fry (e.g. Atlantic herring, [38, 39]; Atlantic cod, [40] and Californian anchovy, [41, 42]) and the nutritive conditions, i.e. the biomass of food phytoplankton.

Similar studies were also conducted on marine invertebrates which were predominantly planktonic crustaceans. These studies showed a direct relationship between the content of waxes and other lipid fractions stored in the body of *Calanus* species from the Pacific [43, 44, 45] and the Atlantic Ocean [2, 46, 47] and the condition of their food base expressed in terms of maximum or integrated chlorophyll concentration in the euphotic zone, phytoplankton biomass or primary production. A similar relationship was also seen in other planktonic organisms namely *Rhinocalanus nasatus, Euphausia pacifica* and chaetognaths [45].

The present study has also shown that the total lipid content or individual lipid fractions in *Calanus euxinus*, the glycogen content *in Pleurobrachia rhodopis* and the content of neutral lipids in *Sprattus sprattus phalericus* are indicative of the abundance of food available to these organisms.

Furthermore, the results obtained reveal the importance of physical and chemical factors in describing the spatial variation of food available to *C. euxinus* and *P. rhodopis* throughout the Black Sea. Phosphates and nitrates provide the chemical base for the growth of phytoplankton which, in turn, enables the production of heterotrophic organisms; in particular, the primary consumer *C. euxinus*, and its predator *P. rhodopis*. In cyclonic regions where high concentrations of nutrients and chlorophyll are found in euphotic zone, *C. euxinus* displays an elevated lipid content with *P. rhodopis* containing high glycogen levels. In anticyclonic zones with lower concentrations of both nutrients and chlorophyll, there was a corresponding decline in the body lipid content of copepods and glycogen of ctenophores. Even small alterations in the chlorophyll content may cause marked changes in the biochemical composition of the animals. Measurements of integrated chlorophyll content which were made during the research cruise in 1991 showed the range of variation to be as wide as from 0.31 to 0.58 mg m^{-3} . Content of waxes and other lipid fractions changed accordingly [15].

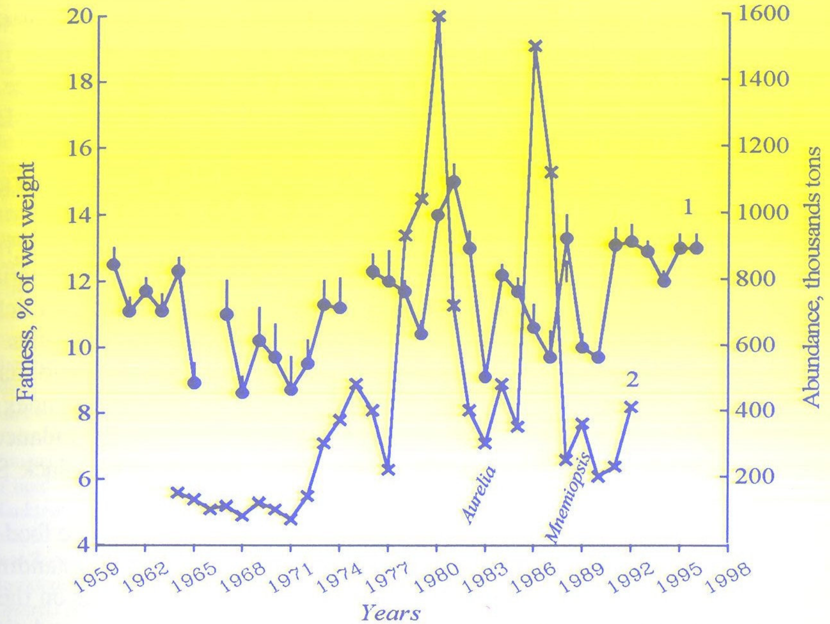

Figure 9. Long-term variation in fatness (1) and abundance (2) of Black Sea sprat. 1- for the northwestern Black Sea, 2 - for the entire Black Sea. Data on fatness are given with their standard errors. *Aurelia*: Indicating the outburst in early 1980's. *Mnemiopsis*: Indicating the outburst in late 1980's.

Hakanson [41, 42, 44] demonstrated spatial variation in food provision of zooplankton and fish larvae in the Californian current and validated that deeper waters of near-shore areas were richer in food for heterotrophic plankton than off-shore areas. Similarly it is shown here that a correlation between lipid characteristics and chlorophyll content was present, whilst it did not exist between lipids and total zooplankton biomass. Bamstedt *et al.* [2] reported that in Kosterfjorden (on the western coast of Sweden) the food provision of the copepod species *Calanus finmarchicus, Acartia clausi* and *Pseudocalanus sp.* also displayed spatial variability which was deduced from the content of total lipids and individual lipid fractions . Those estimates depended strongly on the content of nitrates, dissolved organic matter, chlorophyll and bacterioplankton production. The spatial heterogeneity in the food provision of planktonic organisms is evidently due to nutrient flow and organic matter transportation not only horizontally, but also vertically. A suitable example is known as "the profusion of life" for the Peruvian anchovy with an enormous biomass of fish. Albeit on a lesser scale, a similar event does exist in the open Black Sea where cyclonic activity induces the ascent of nutrient-rich deep water up to the euphotic zone.

The "horizontal input" of nutrients with river inflow into the Black Sea has an even more expressed influence on the food provision of heterotrophs. Nutrients are of special importance in the food provision of mass pelagic fishes (i.e. sprat and anchovy) of the Black Sea. Anthropogenic pressure on the environment has sharply increased since the mid 1970's, resulting in river inflow bringing more and more nutrients into the Black Sea [23, 48]. As a result the concentration of nitrates in the north-western

Black Sea increased from 154 to 340 thousand tons and phosphates from 14 to 55 thousand tons. Consequently, primary and secondary production rates became much higher and stimulated the development of phyto- and zooplankton abundance and biomass. For example, the concentration of phytoplankton in the north-western Black Sea increased from 52 to 1000 g m^{-3}.

The resulting high organic matter content of Black Sea pelagic waters had grave consequences: in the early 1980's mass development of the medusa *Aurelia aurita* took place, and in the late 1980's an even larger outbreak was recorded for the introduced ctenophore *Mnemiopsis sp.* (Fig. 9). Being trophic deadlocks, these gelatinous organisms are very strong competitors for food with the sprat and anchovy which resulted in a sharp drop in the food provision of these two main pelagic fish species. The fatness index of sprat fell to 9% and the stock was reduced to a level similar to that for registered for the 1970's. A similar decline in the stock level and fatness index was also observed for the anchovy. Such fluctuations in the condition and abundances of pelagic fishes provide strong evidence for the disturbed stability of the Black Sea's pelagic ecosystem.

It may be concluded that biochemical methods can reliably estimate the food provision of heterotrophic organisms. This would contribute to a better understanding of the relations between organisms, populations and resources of mass species on the one hand and environmental factors on the other, and may allow continuous and simple monitoring of the condition of mass species of the Black Sea as well as the state of its ecosystem.

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