Commission on the Protection
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Trophic Relationships and Food Supply of Heterotrophic Animals in the Pelagic Ecosystem of the Black Sea

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Trophic Relationships and Food Supply of Heterotrophic Animals in the Pelagic Ecosystem of the Black Sea

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Istanbul, Turkey
2009

Preface

During recent decades, the Black Sea has been affected by many negative factors that strongly changed the condition of its ecosystem. Especially trophic relationships in the Black Sea pelagic system became very vulnerable influencing the food supply, productivity and abundance of many species and populations of this marine basin. Food is one of most important link between biota and its environment.

In this monograph, the role and variability of trophodynamic processes that effect the well-being (health) of main heterotrophic components of ecosystem were analysed in detail for a few key species as indicators for estimation of ecosystem condition in whole. These are most significant mass species of the Black Sea pelagic ecosystem. Among copepods this is *Calanus euxinus* that dominates the mesozooplankton which makes up the fodder base of planktivorous fishes. Among gelatinous these are medusa *Aurelia aurita* and the alien ctenophores *Mnemiopsis leidyi* and *Beroe ovata* which affected strongly mesozooplankton composition. Lastly among fishes the anchovy *Engraulis encrasicolus ponticus* and sprat *Sprattus sprattus phalericus* that dominate small pelagic fishery.

We considered in this monograph:

- Diel feeding behavior, *in situ* feeding rate of *Calanus euxinus* and impact of mesozooplankton on primary production and phytoplankton biomass.

- The effect of vertical migrations on energy budget and its components in *C. euxinus*; metabolic substrates used in catabolic processes under both aerobic and hypoxic conditions, the role of reserve lipids and effect of abiotic factors on individual growth and population structure of this species.

- The intensity and efficiency of ingestion and energy transformation in three gelatinous species (jellyfish *Aurelia aurita*, ctenophores *Mnemiopsis leidyi* and *Beroe ovata*) and their predatory impact on zooplankton community.

- Nutritional condition and food supply of anchovy and sprat in the close interaction with natural biotic and abiotic and anthropogenic factors.

- Tendencies in this interaction during long time space: since 1960 s till present years.

- Estimation of population condition of these species and its long-term change.

This monograph is the collective work of Ukrainian and Turkish scientists studying complex hydrobiological problems of the Black Sea. Its aim is to reveal the significance of nutritional factors on the ecology of Black Sea biota, including changes which have already occurred, as well as offering some insight into changes that may happen in the future. Our joint investigations started in the first half of the 1990s, when conditions for the close cooperation of researchers from the two countries were suitable after the collapse of the Soviet era. This spirit continues to the present day.

Professor Ümit Umluata, Director of Erdemli Institute of Marine Sciences (Middle East Technical University, Ankara) was of paramount importance in organising and fostering the work undertaken. We would like to devote this monograph to the memory of him, who died so prematurely. We are also grateful to Academician Professor V. N. Eremeev, Director of the Sevastopol Institute of Biology of the Southern Sea (National Academy of Sciences of Ukraine), and to the directors of Erdemli Institute of Marine Sciences (Professor Ilkay Salihoglu, Prof*essor* Sukru Besiktepe and Professor Ferit Bingel) who also made significant contributions to the Ukrainian–Turkish collaboration. We are grateful to Dr Bill Parr from the Black Sea Ecosystem Recovery Project for his valuable efforts in improving earlier drafts.

All these investigations were carried out within the framework of the following five NATO linkage-grants:

- Pelagic animal food supply in the unstable Black Sea environment,

- Will the new alien ctenophore *Beroe ovata* control the plankton community in the Black Sea?

- Grazing, growth and production of *Calanus euxinus* in the Black Sea,

- Bioindicators for assessment of Black Sea ecosystem recovery,

- Adaptability and vulnerability of marine species in changing environments.

And four TUBITAK - NASU joint projects:

- Quantification of the recent ctenophore invader *Beroe ovata* impact in the Black Sea

- Monitoring of the Black Sea anchovy and sprat,

- Salinity tolerance as a key factor of invasion success of the copepods of *Calanus genus* into the Sea of Marmara,

- Salinity tolerance as a key factor of invasion success of the mesozooplankton species into the Sea of Marmara.

We hope that this publication will make a substantial contribution to future studies of the Black Sea ecosystem and offers further understanding of those features regulating biological processes in this unique marine basin.

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1. DIEL FEEDING BEHAVIOUR AND GRAZING OF MESOZOOPLANKTON

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1.1 Introduction

Feeding activities play an important role in the transfer of the materials and energy within marine ecosystems. The quantification of feeding is crucial for understanding of food-chain dynamics. Feeding or ingestion rate may be defined as the amount of food eaten by an organism per unit of time. Another measure of expressing an organism's feeding rate is its clearance rate, expressed as the volume of water swept clear of food particles by a consumer per unit of time (Bamstedt et al., 2000).

The basic parameters influencing feeding are well established in the literature. Food abundance is an important factor regulating the feeding rate (Frost, 1972; Houde and Roman 1987; Vanderploeg 1990; Bamstedt *et al*., 1999; Besiktepe and Dam 2002). The functional response explains how the ingestion rate is related to food abundance. As food abundance increases, predators increase their ingestion. Without limiting factors, ingestion rate increases linearly with food abundance. However, there are also some mechanical and physiological constraints, such as volume of the digestive system and time required for digestion. Because of these limitations, the linear relationship reaches to a maximum point (satiation point), then plateaus-out, with ingestion rate increasing little further, despite an increase in food abundance increase (Bamstedt *et al*., 2000; Valiella, 1995).

Food quality is another important factor affecting zooplankton feeding. However, the determination of food quality, in contrast to food concentration, is rather difficult. Food quality is a complex function of many variables, including food size, taste, shape, nutritional composition, stoichiometry, motility and toxicity. In the literature, several studies have identified the role of food quality on the feeding rate of copepods. It is shown that certain algae are preferred in comparison to others of the same size and at the same concentration, algae are preferred to non-biological particles, and live algae to dead ones (Harris, 1996; Paffenhofer and Van Sant, 1985; Ayukai, 1987). It is also reported that particle capture efficiency increases with increasing particle size to an optimum food size (Nival and Nival, 1976; Berggren *et al.*, 1988; Stottrup and Jensen, 1990; Besiktepe and Dam, 2002). Among meso-zooplankton, copepods are generally considered as selective feeders. Laboratory studies have revealed that they examine their food particles individually and they can discriminate particles in terms of taste, size, nutritional quality and toxicity (DeMott, 1988; Kerfoot and Kirk, 1991; Bundy and Vanderploeg, 2002). Copepods live in a nutritionally dilute environment, and to increase encounter rates with food particles they use many strategies, including chemoreception, mechanoreception and direct interception (Price, 1988; Bundy and Vanderploeg, 2002). Bundy and Vanderploeg (2002) suggested that chemoreception mechanisms play a significant role in prey selection. Mayzaud *et al.* (1998) exhibited that *Acartia*-type copepods optimise their nitrogen or protein uptake by chemoreception, mediated selectivity with internal control mechanisms. Marine herbivores have a variety of responses to toxic phytoplankton species. Tolerance to the toxicity of certain algae varies among organisms; toxicity affects both feeding behaviour and survival of grazers (Teegarden and Cembella, 1996; Turner *et al.*, 1998; Turner and Tester, 1997; Frangoulos *et al.*, 2000; Lincoln *et al.*, 2001; Schmidt *et al.*, 2002; Bargu *et al.*, 2003; Maneiro *et al.*, 2005). Among phytoplankton, several diatom species have been shown to have negative effects on copepod feeding, fecundity and survival (Miralto *et al.*, 1999; Lincoln *et al.*, 2001; Colin and Dam, 2002; Irigoien *et al.*, 2002; Olson *et al.*, 2006; Frost, 2005; Pohnert, 2005; Maneiro *et al.*, 2005; Ask *et al.*, 2006). Although the exact causes of these negative impacts are still under discussion, both toxicity and nutritional deficiency have been considered.

Organism body size is a further factor affecting ingestion. This is related to metabolic requirements. Metabolic rate is related with the body mass and this relationship is explained by a well-known curvilinear function (Bamstead *et al*., 2000).

Among environmental factors, turbulence and temperature have effects on the ingestion and clearance rate of zooplankton. Some laboratory studies have shown that, small scale turbulence can increase the encounter rate of zooplankton with their food items (Saiz *et al*., 1992; Kiorboe and Saiz, 1995). Metabolic activities are related directly to ambient temperature (McLaren, 1963, 1966; Ikeda, 1970). Digestion activities increases with temperature, and this leads to an increase in ingestion. The relationship between ingestion and temperature in copepods is well described by Dam and Peterson (1988).

A relationship also exists between feeding and the ambient light regime. Earlier studies showed that feeding is higher at night (Huntley, *et al*., 1987; Morales *et al*., 1993), but the significance of this relationship is still not clear, and is possible that such differences between day and night result from endogenous rhythms (Dam and Peterson, 1993).

There are several direct methods to examine zooplankton feeding rates. The most common method is that of determining concentrations of prey cells through a microscope or an electronic counting device at the beginning and the end of an experiment. The feeding rate of individual zooplankters and the zooplankton community are also estimated from the short-term uptake of tracer particles, including inert particles and radioisotopes (Bamsteat *et al*., 2000).

The gut fluorescence method, developed by Mackas and Bohrer (1976), is also considered an *in situ* method of determining zooplankton feeding rates. It is based on the 100% molar conversion of chlorophyll to phaeopigment. One of the main criticisms of this approach is the variable degree of pigment destruction during gut passage (Head and Harris, 1992).

1.2 Food Environment in the Black Sea

Zooplankton can feed on different food sources from phytoplankters, small heterotrophic organisms to detritus in the marine environment. Phytoplankton is an important food source for zooplankton, and their temporal and spatial distributions are well studied in the Black Sea. Changes in species composition and quantity of phytoplankton in the Black Sea have been reported since the damming of the lower reaches of the Danube River in 1972. Changes in nutrient ratios of the Black Sea favoured small flagellates and coccolithophores over large diatoms (Humborg *et al.*, 1997; Moncheva and Krastev, 1997; Mikaelyan, 1997; Uysal *et al.*, 1998; Yunev *et al.*, 2007). Small flagellates and coccolithophores bloomed during the late spring and summer months (Bologa, 1986; Benli, 1987; Uysal *et al.*, 1998; Eker *et al.*, 1999; Eker-Develi and Kideys, 2003; Cokacar *et al.*, 2004; Mikaelyan *et al.*, 2005). Mikaelyan *et al*. (2005) showed that *Emiliania huxleyi* was the dominant species during June-July 2004 in the northeastern Black Sea. Eker-Develi and Kideys (2003) showed that the coccolithophorid *Emiliania huxleyi* was also the most abundant species during June-July 1996, March-April and September 1998 in the southern Black Sea. However in terms of biomass, diatoms formed the bulk of the phytoplankton in spring and autumn, while dinoflagellates in summer. Among the diatoms *Proboscia alata* and *Pseudosolenia calcar-avis* were the abundant species in spring and autumn, respectively. These investigators also observed an important amount of *Pseudonitzschia pseudodelicatissima* during spring and autumn period. Among the dinoflagellates, *Gymnodinium* sp. was the abundant species in summer (Eker-Develi and Kideys, 2003). Along the Bulgarian coast, *Gyrodinium fusiforme*, *Heterocapsa triquatra* and *Prorocentrum minimum* were the dominant dinoflagellate species during April and July (Moncheva *et al*., 2003). Among the diatoms, *Licmophora ehrenbergii* was the only species common in April and July. *Alexandrium monilatum* was observed in July samples. In Romanian shelf waters, *Prorocentrum cordatum* and *Skeletonema costatum* are the two main bloom-forming species (Yunev *et al*., 2007).

Intensive and frequent harmful algal blooms have been recorded in Romanian and Bulgarian waters, especially during summer (Moncheva and Krastev, 1997; Bodeanu *et al*., 1998; Petranu *et al*., 1999; Moncheva *et al*., 2001). Dinoflagellates *Alexandrium minutum* and *A. tamarense*, causing paralytic shellfish poisoning, were found along the northeastern coast of the Black Sea (Vershinin *et al*., 2006). A domoic acid-producing diatom *Pseudo-nitszchia calliantha* was recorded for the first time in Turkish waters of the Black Sea by Bargu *et al*., (2002). The culture isolated from the Karadag, on the southern coast of the Crimea, was ascribed to *P. pseudodelicatissima* by Davidovich and Bates (1998), but after re-examination of the culture the species was identified as *P. calliantha* (Lundholm *et al*., 2003). Domoic acid production by *P. calliantha* isolated from Sevastopol Bay was confirmed by Besiktepe *et al*. (2008).

Phytoplankton biomass in the Black Sea decreases with depth (Moncheva *et al*. 1998). Around half of the phytoplankton biomass is found in the surface homogeneous layer, about 40% in the thermocline and around 15% of the biomass was in the cold intermediate layer.

Using a long-term data set, Yilmaz *et al*., (1998) identified three precise peaks of chlorophyll-a in the surface waters of the shelf region: a winter peak in January-February; a spring-early summer increase in May-June; and an autumn maximum in September-November. The annual cycle of primary production both in coastal and open waters usually displays 3-4 peaks, depending on nutrient transport to the euphotic zone (Stelmakh *et al*., 1998): one in spring; one or two peaks in summer; and a further one in autumn. The main production is in spring, observed in February-March in open waters, and in March-June in coastal waters (Stelmakh *et al*., 1998). They described the coastal waters of the Black Sea as being eutrophic to mesotrophic, and open waters as being mesotrophic in terms of primary production.

Microzooplankton is another important food source for zooplankton. Recently a species list of tintinnids has been recorded by Galikova and Dolan (2008), concurrent with effects of ecosystem shift in the Black Sea. Since he 1990s there has been an increase in the number of new species, as well as obvious losses of tintinnid species. Ciliates are found at all depths of the Black Sea down to H_2S layer, especially in the suboxic zone (Bird and Karl, 1991; Zubkov *et al*., 1992). Bird and Karl (1991) observed a ciliate maximum at 83 m depth where the sulphide layer begins. Bouvier *et al*., (1997) found the maximum biomass of mixotrophic ciliates occurring in the low nutrient-high food particle concentration water mass of the northwestern Black Sea during summer 1995. They showed that mixotrophic ciliates consume preferably bacteria while mixotrophic dinoflagellates feed mainly on nanoflagellates.

1.3 Vertical Migration and Feeding of Mesozooplankton

Diel vertical migration (DVM) is a general feature of both marine and fresh water zooplankton. Zooplanktonic organisms, generally, ascend to the food-rich upper waters at dusk and stay there during night time to gain food, and then descend to deeper waters with little light and low food. There are several hypotheses to explain DVM (Enright, 1977; Kerfoot, 1985; Lampert, 1993). Among these hypotheses, the ‘predator avoidance hypothesis’ has recently received considerable support.

Zenkevich (1963) distinguished three groups in the Black Sea plankton according to their vertical distribution characteristics. One group demonstrates similar distributions during winter and summer. The bulk of them are usually found at a depth of between 15 and 50 m. Their vertical distribution is affected slightly by variations of temperature and light, observed throughout the seasons. *Acartia clausi* and *Paracalanus parvus* are included in this group of organisms (Zenkevich, 1963).

The second group is represented by cold water stenothermal organisms found in winter at all depths; in summer they sink to the greater depth. This group includes *Calanus euxinus, Pseudocalanus elongatus, Oithona similis* and *Sagitta euxina*. The upper optimum temperature limit for the distribution of *Sagitta* is 10-11°C, for *Calanus*, 13°C for *Pseudocalanus*, and for *Oithona* 14°C (Zenkevich, 1963).

The third group of individuals develops only in summer, and they are in the warm upper layer of water. When temperature decreases they become gradually scarcer, and disappear completely from the plankton during the winter months. This group includes *Centropages kroeyeri*. The lower temperature limit of this species is around 10-14°C (Zenkevich, 1963).

Diel vertical migrations have been observed for a number of species in the Black Sea (Zenkevich, 1963; Zagorodynyaya, 1970; Vinogradov *et al*., 1985, 1986, 1990, 1992a,b; Erkan *et al*., 2000; Svetlichny *et al*. 2000; Besiktepe *et al*., 1998; Besiktepe and Unsal, 2000; Besiktepe, 2001; Mutlu and Bingel, 1999; Mutlu, 1999, 2003). Besiktepe (2001) stated that vertical distribution of the common copepod species in the Black Sea appeared to be related to season, species, stages of species and the oxygen concentration in the water column. The zooplankton samples were collected from 5 depth strata according to major biogeochemical characteristics of the water column (Fig. 1.1) and it was observed that among small species, *A. clausi* and *P. parvus* are mostly in the upper 100 m, while *O. similis* is found largely in the oxygen minimum zone (OMZ). Low oxygen concentration does not appear to restrict the vertical distribution of *O. similis*. Roman *et al.*, (1993) showed that *O. similis* is tolerant of low oxygen concentrations. Differences in vertical distributions of different stages of *C. euxinus* and *P. elongatus* have been observed. Female, CV, CIV stages of *C. euxinus* and female *P. elongatus* showed strong diel vertical migration from the surface waters to the OMZ, while the majority of the other stages remained in the upper 100 m (Besiktepe, 2001).

The copepodite stage of V *Calanus* undergoes seasonal migration during summer and early autumn (during warmer period) in the Black Sea. While some of them were found in the upper food-rich layers during night time, others remain in the OMZ during the diapause period. In June around 50% of the CV population was in the diapause period, while in September only 13% of the population was observed in diapause period. Vinogradov *et al.* (1990) reported that 60-75% of the stage V *Calanus* remained at the lower limit of the oxygenated layer at night time in August. The OMZ may be used as a refuge from predation by both diapausing stages and vertical migrants as pointed out before by Alldredge *et al*., (1984) and Vinogradov *et al.*, (1986).

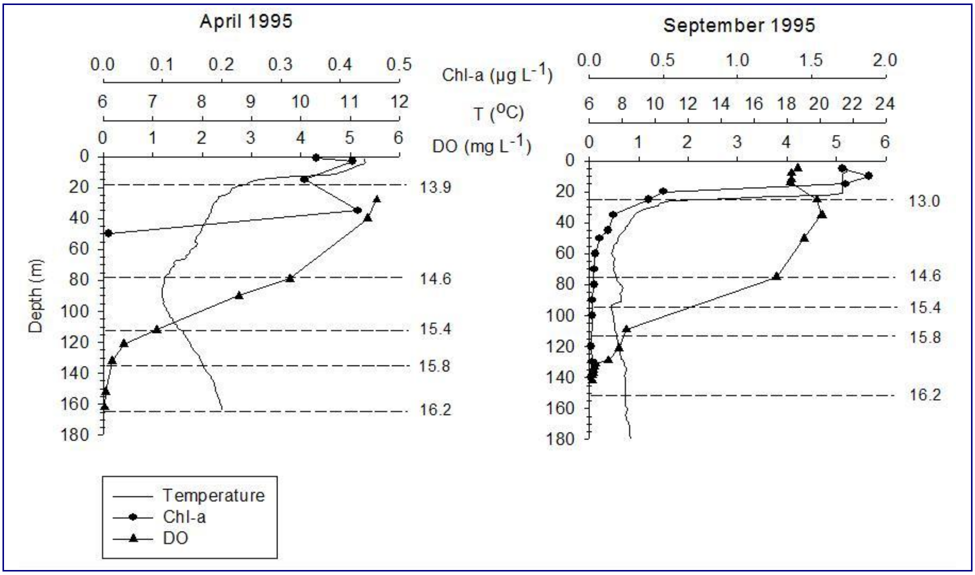

Figure 1.1. Potential temperature, Chl-*a* and dissolved oxygen concentration are plotted against depth for 2 sampling months. Horizontal lines indicate the sampling depths and the corresponding sigma-theta values.

Night and daytime vertical distribution of total copepod assemblages in the water column differed slightly with the season (Fig. 1.2). In April, the difference of vertical distribution between night and daytime appeared more clearly due to the high contribution of strong vertical migrants (i.e. *C. euxinus* and *P. elongatus*) in copepod abundance. In September and June, most of the copepods were present above the OMZ, day and night, and showed small-scale periodic migration. The variability of copepod abundance between day and night samples probably resulted from lateral intrusion of neighbouring water masses.

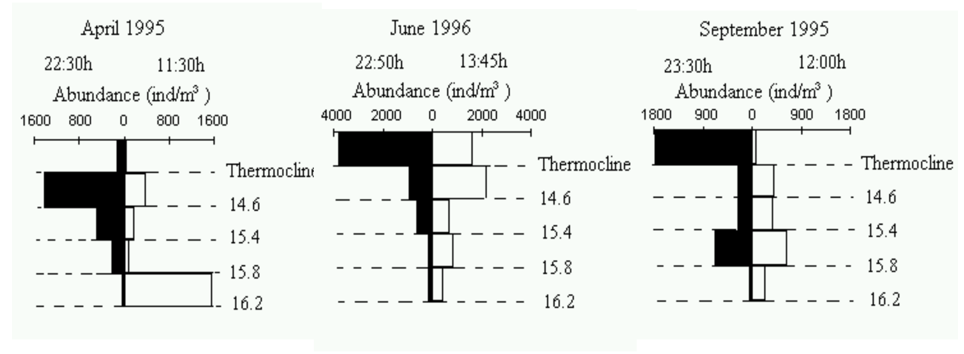

Figure 1.2. Day (white bars) and night (black bars) vertical distribution of copepod assemblages in April and September 1995 and June 1996. Horizontal dashed lines indicate the sampling depths and the corresponding sigma-theta values.

The diurnal feeding behaviour of female of *C. euxinus* throughout the oxic water column is shown in Fig. 1.3. There appeared to be a temporal pattern in the gut pigment content (GPC) of *C. euxinus* females sampled in April and September, synchronised with diel vertical migration of the individuals (see Figure 4a,b). In April, GPC was analysed over 30 hours. The highest amount of GPC was observed during the night time (at 22:00, 03:30, 20:00 and 24:00h) when they were at the uppermost two layers (Fig. 1.3). The GPC ranged from 4 to 22 ng pigment female^{-1} in the uppermost two layers. During the daytime, *C. euxinus* females were absent from the upper two layers (Fig. 1.4a). At 17:00 and 18:00, females were captured from the 3rd and 4th depth layers, indicating that they began their upward migration around 2-3 h before sunset (sunset= 19.52h). During the daytime (at 07:00, 11:30, 17:00 and 18:00h) they were found within the OMZ. They contained a considerable amount of GPC, which increased towards evening, within the range of 0.8-16.2 ng pigment female^{-1} (Fig. 1.3). This indicates that feeding occurred in the OMZ.

In September, females were captured from the upper two layers during nighttimes (19:00, 23:30 and 03:30h). Around 2 h before the sunset (16:00, sunset was at 17:43h) they appeared in the second layer. In the first layer, the average GPC ranged from 9.4 to 20.5 ng pigment female^{-1} (Fig. 1.3). During the day (07:30 and 12:00h), females were absent from the upper layers (Fig. 1.4b). At all sampling times of the day, comparatively large number of females were found in the OMZ. In each of the hauls made at night (19:00, 23:30 and 03:30h), not enough individuals were captured for replicate analysis. In these samples, individuals had lower gut pigment content. During the daytime, females from the 5th layer (OMZ) contained considerable amounts of gut pigment (ranging from 0.9 ng pigment female^{-1} at 03:30h to 4.2 ng pigment female^{-1} at 07:30h) (Fig. 1.3).

The level of gut pigment content appeared to be associated with depth. Gut pigment levels in the upper layers were high throughout the night. In September, although the depth-integrated chl-*a* concentration (30.6 mg chl m^{-3}) in the upper 50 m was almost twice that observed in April (16.3 mg chl m^{-2}), the average GPC level from upper two layers was almost the same as in April. Thus, GPC is not always proportional to ambient chl-*a* concentrations. Most published reports on phytoplankton-zooplankton relationships do not show an expected direct relationship between GPC and chl-*a* concentrations in seawater (Boyd and Smith, 1980; Dagg and Wyman, 1983; Kleppel *et al*., 1988; Bautista *et al*., 1988; Wang and Conover, 1986; Turner *et al*., 1993). However, phytoplankton composition might play a role in such a relationship. Phytoplankton composition in April could better suit *Calanus* feeding. Petipa (1964) observed that Peridinea was the dominant group and Diatomea was the second most abundant group in the gut of *Calanus*. Arashkevich *et al*. (1998) observed parts of crustacean exoskeletons and small dinoflagellates (besides unidentifiable debris) in the gut of *Calanus*. Coccolithophorids were rarely observed as a food item. Whereas, in both seasons coccolithophorids were more important than other groups of phytoplankton in water: in April Peridinea abundance was 19% while that of Diatomea contributed only 1.3% of the total phytoplankton count. In September the percentages of these two groups of phytoplankton were equal (3%) (Eker *et al*., 1999).

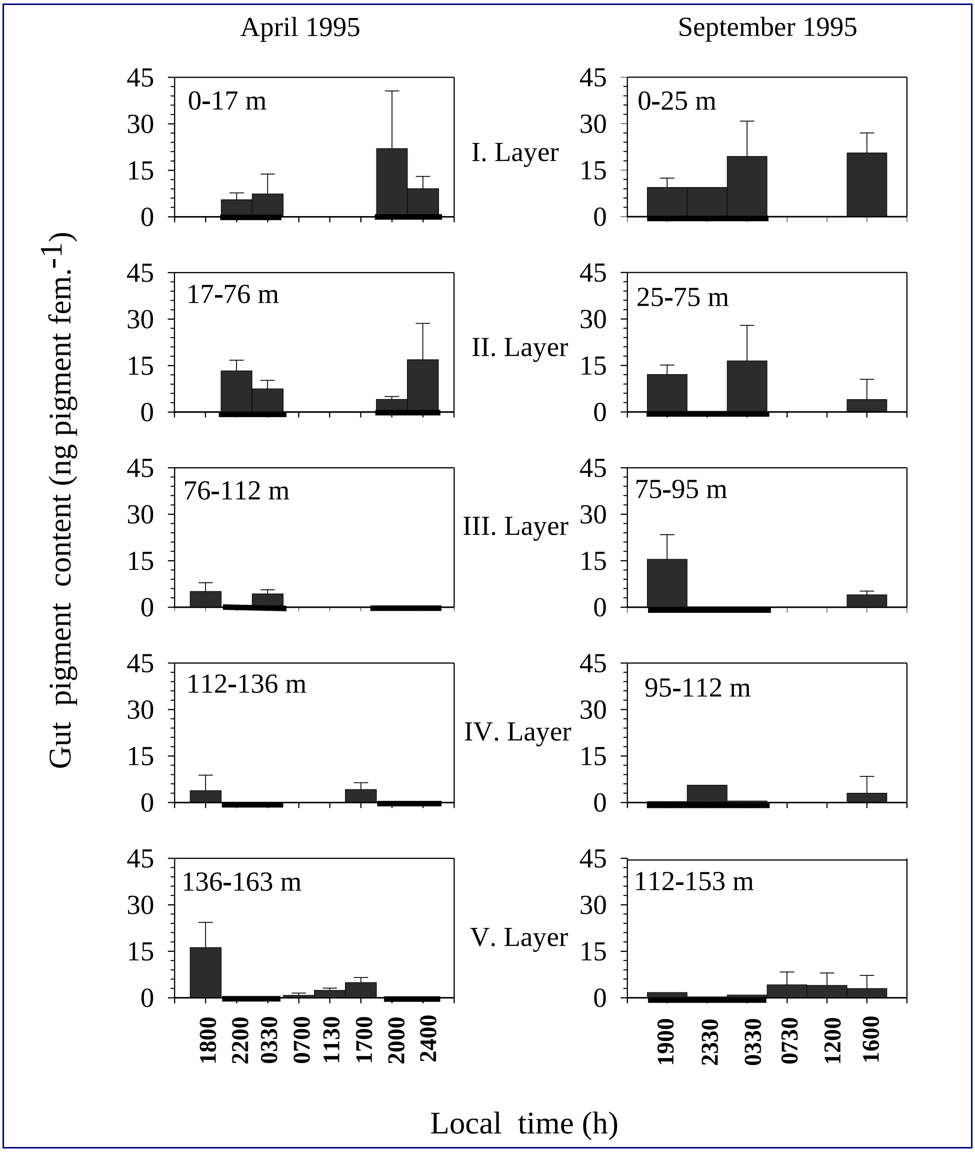

Figure 1.3. **Gut pigment contents of female *Calanus euxinus* from different depth layers collected during 26-28 April 1995 (sunset= 17:43h, sunrise= 06:06h) and 27-28 September 1995 (sunset= 17:43h, sunrise= 05:47h). The depth interval of each layer is indicated in each figure**

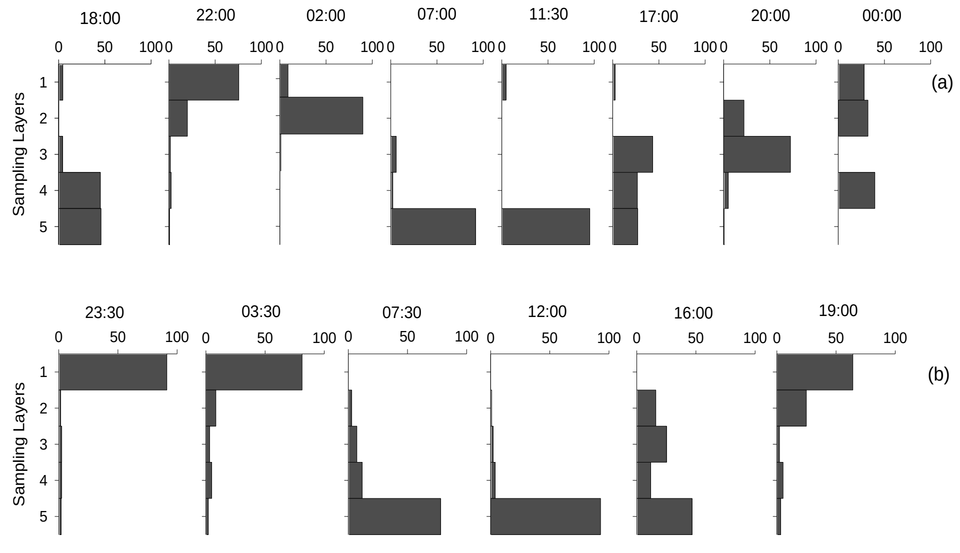

Figure 1.4. Vertical distribution of female *Calanus euxinus* at each sampling time during 26-28 April (a) and 27-28 September (b) 1995. Abundance is expressed as percent of the individuals m^{-3} for the entire profile at 5 depth strata (for more details about sampling layers see Fig. 1.1)

A considerable amount of GPC was present in females sampled from the OMZ during daytime, from 07:00 to 18:00h in April and from 07:30 to 16:00h in September. The average GPC of females from the OMZ was 6.1 and 2.4 ng pigment female^{-1} in April and September, respectively. These values are higher than the background pigment fluorescence of starved female *C. euxinus* (average value of 0.68 ng pigment female^{-1}). Increases in GPC towards evening within the OMZ, particularly in April, imply that feeding occurred in the OMZ. Dagg *et al*. (1998) observed low but continuous gut pigment levels in *Metridia lucens* from deep layers (50-108 m) where low chl-*a* concentrations were observed. Unlike *M. lucens*, they observed high gut pigment levels in *Calanus pacificus* in the deep layer at the onset of the descent phase, suggesting that copepods fed on chl-*a* rich surface waters then descended (containing a remarkable amount of gut pigment) to deeper layers. There may also have been different chl-a containing food sources for *C. euxinus* in lower layer. For instance, Yilmaz *et al*. (1998) reported variations in particulate organic carbon (POC) concentrations with depth in the Black Sea. They observed high POC concentrations (10-15 µM) in the euphotic zone, declining to 1-4 µM with greater depth, and then a small increase in POC in the suboxic/anoxic interface. The chl-*a* containing particles sinking to deeper layers included phytoplankton (especially diatoms) and faecal pellets. These contribute to the POC and may provide a food source for *C. euxinus* within the OMZ during daytime.

Repackaging of faecal pellets through coprophagy by copepods has been reported in the literature (Paffenhofer and Knowles, 1979; Gowing and Silver, 1985; Green *et al*., 1992). Karl and Knauer (1991) have previously observed numerous faecal pellets in their sediment traps deployed at 80m depth, almost at the upper boundary of the sulphide zone in the Black Sea. Additionally, aggregates of some chl-*a* containing small cells within the OMZ may serve as a food for the *Calanus*: For example chroococcoid (cyanobacterial) cells have been reported in the deeper layer of the Black Sea, with around 145 *Synechococcus* spp. cells ml^{-1} at 108 m depth where dissolved oxygen concentrations fell below 0.5 ml L^{-1} (Uysal, 2001). Besides these, Coble *et al*. (1991) observed two chl-*a* fluorescence maxima in the Black Sea water column during the 1988 R.V.Knorr cruise. A primary maximum was at the bottom of the euphotic zone, and a weaker secondary peak was present at the depth of sulphide interface. This secondary peak was associated with the photosynthetic sulphur bacteria population containing bacteriochlorophyll-e (Bchl-e). The absorption spectrum of Bchl-e is similar to that of chl-a (Coble *et al*., 1991). The sizes of both bacteria and *Synechococcus* spp. cells are less than 1 µm, and this size is not appropriate (too small) for copepods to remove individually from suspension. However, these cells might concentrate in aggregates or clusters to be eaten. Kosobokova *et al*., (2002) found 17:0 fatty acids as a biomarker of sulphate-reducing and other bacteria in the copepod *Spinocalanus antarchicus*. So, particle aggregates, their contents and sizes need to be studied in the Black Sea, especially in the suboxic zone, to understand the daytime feeding behaviour of *C. euxinus*.

Gut pigment content levels of female *C. euxinus* throughout the water column illustrate that maximum feeding occurred in the uppermost two layers (corresponding to the euphotic zone - euphotic zone depth was 38 m in April and 17 m in September 1995 - during nighttimes. The background fluorescence for starved individuals was measured as 0.68 ±0.39 ng pigment female^{-1}. After this value was accounted for, the overall average gut pigment concentration of female *Calanus* from the uppermost two layers was calculated to be 10.1 ng pigment female^{-1} in April and 14.0 ng pigment female^{-1} in September (Table 1.1). Using the linear equation of Dam and Peterson (1988), the estimated gut evacuation rate constant was 1.86 h^{-1} in April and 3.0 h^{-1} in September. Then, average ingestion rates were calculated from the uppermost two layers by multiplying the GPC and gut evacuation rate constant: it was 18.7 and 42 ng pigment ind.$^{-1}$ h^{-1} in April and in September, respectively. Daily consumption by the female *Calanus* was estimated by taking into account the feeding duration and gut pigment concentrations in the uppermost two layers. The GPC: Chl-*a* ratios were 76 in April and 65 in September (Eker, 1998). These ratios were used to convert the consumed gut pigment to carbon. Based on these rough estimations, the mean daily carbon rations derived from herbivorous feeding by female *C. euxinus* was 10.65 μgC ind.$^{-1}$ d^{-1}, equal to 9.3% of female body carbon weight (~115 μgC female^{-1}, Telli unpublished data) in April and it was 28.7 μgC ind.$^{-1}$ d^{-1}, representing 25% of the body carbon in September (Table 1.2). Daily assimilated carbon was calculated as 7.46 μgC ind.$^{-1}$ d^{-1} in April and 20.1 μgC ind.$^{-1}$ d^{-1} in September, assuming an assimilation efficiency of 70% (Conover, 1978), and around 8% of this assimilated carbon was devoted to respiration (Table 1.2) and rest, about 90% was dedicated to production (Besiktepe *et al*., 2005).

Diel gut pigment levels and grazing of three size fractions of copepods were measured in the Black Sea during September 1995. Copepods were collected from 50 m to the surface, at 4-h intervals, for a 26 h period. Copepod assemblages were grouped into three size categories: small (300-500 μm); medium (500-1000 μm); and large (1000-2000 μm).

Table 1.1. Gut pigment content (G, ng pigment copepod^{-1}) and ingestion rate (ng pigment copepod^{-1} h^{-1}) of female *Calanus euxinus* in the layers encompassing the euphotic zone during April and September 1995. Gut evacuation rate constants (k) were 1.86 h^{-1} in April and 3 h^{-1} in September. - = insufficient female copepods for analysis

Time of day	Gut Pigment Content in the layers from the thermocline to the surface	from the depth of σ_θ=14.6 to the thermocline	Ingestion Rate
	April 1995		
18:00	-	-	-
22:00	4.77	12.63	16.18
02:00	6.76	6.86	12.67
07:00	-	-	-
11:30	-	-	-
17:00	-	-	-
20:00	21.28	3.42	22.97
24:00	8.46	16.2	22.93
Average =	10.1±5.8		18.7±4.4
	September 1995		
19:00	8.7		26.2
23:30	8.7		26.1
03:30	18.8		56.3
07:30	-		-
12:00	-		-
16:00	19.9		59.6
Average=	14.0±5.3		42.0±15.9

Within the large fraction, there was a trend of increasing gut pigment content during night feeding period (Fig. 1.5). The highest value was reached towards the end of darkness (at 03:30). In this fraction, around 86% of species abundance was dominated by vertically migrating organisms (i.e. female, copepodite V and copepodite IV stages of *C.*

euxinus, see Table 1.3). During daytime (07:30, 12:00, 16:00) insufficient number of individuals could be found for GPC analysis.

Within the medium size fraction, variation in gut fluorescence values (Fig. 1.5) was high especially at night time. The average pigment ranged from 0.1 to 9.1 ng pigment ind.$^{-1}$ and the amount of explained variance was 78.5 % among the GPC data. However, there was evidence of diel patterns in gut fluorescence for the medium size fractions. The medium size fraction was dominated by female *P. elongatus* and female *A. clausi* throughout the study (Table 1.3). Daytime abundances were generally as high as those at night, suggesting that these copepods were either not migrating or they migrated within the uppermost 50 m layer.

Within the small size fraction, there was no trend in gut pigment content within the daily timeframe; the coefficient of variation was 32% among the GPC data. Small copepod species contained very low levels of pigment in their guts, ranging from 0.9 to 2.0 ng pigment ind^{-1} (Fig. 1.5). In the small size fraction, the copepodite stages dominated, as well as females of *P. elongatus* and *O. similis* (Table 1.3).

The average daily ingestion rate obtained by the medium size fraction was equivalent to 16.7% of primary production, whereas the large fraction grazed the equivalent of 8.1% of primary production. The lowest grazing value was found in the small size fraction, with a value equivalent to 6.7% of primary production (Table 1.4). Daily removal of the primary production by total mesozooplankton was 32%, corresponding to 128 mg C m^{-2} d^{-1} of 405 mg C m^{-2} d^{-1} in September. A limited impact of grazing by mesozooplankton on primary production has been reported in several studies for the other regions. Morales *et al.* (1991) estimated removal rates of 1-2% of primary production in the north-east Atlantic, while Dagg, (1993) estimated removal rates of 6-15% of primary production in the subarctic Pacific Ocean. Other estimates of rates of removal of primary production by large copepods during spring bloom range from 4-100 % day^{-1} in shelf waters off the New York Bight (Smith and Lane, 1988).

Table 1.2. Gut pigment content (GPC, ng pigment female^{-1}), Ingestion (I_1*; ng pig. female^{-1} h^{-1}, I_2; μgC female^{-1}d^{-1}), herbivorous daily carbon ration as percentage of body carbon (C), daily assimilated carbon (A*) and percentage of daily basic metabolic carbon requirements of *Calanus euxinus* from the euphotic zone during the sampling times in April and September 1995

Time	GPC	I_1	I_2	C	A	R
			April 1995			
18:00	-	-	-	-	-	-
22:30	8.7	16.18	9.22	8.02	6.45	8.96
02:30	6.8	12.67	7.22	6.28	5.05	11.44
06:30	-	-	-	-	-	-
11:30	-	-	-	-	-	-
16:00	-	-	-	-	-	-
20:00	12.35	22.97	13.09	11.38	9.16	6.31
24:00	12.33	22.93	13.07	11.36	9.15	6.32
Average	10.05	18.69	10.65	9.26	7.46	8.26
			September 1995			
19:00	8.7	26.2	17.88	15.55	12.51	12.29
23:30	8.7	26.1	17.81	15.49	12.47	12.34
03:30	18.8	56.3	38.42	33.41	26.89	5.72
07:30	-	-	-	-	-	-
12:00	-	-	-	-	-	-
16:00	19.9	59.6	40.68	35.37	28.47	5.40
Average	14.03	42.05	28.7	25.0	20.1	8.94

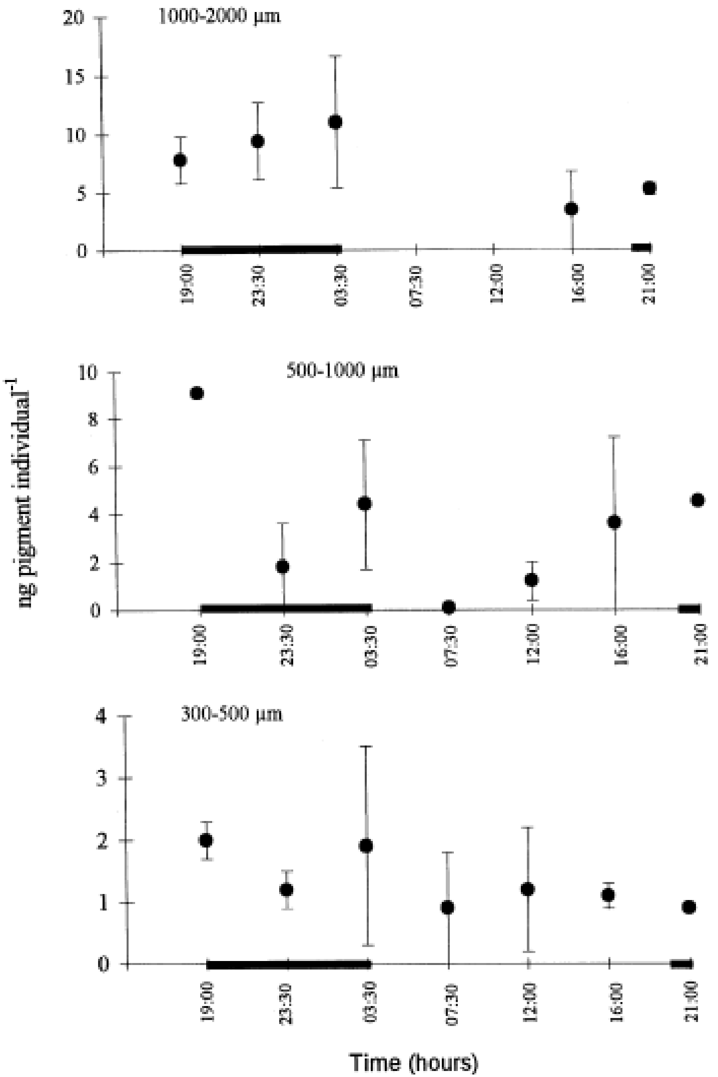

Figure 1.5. Diel gut fluorescence for three size fractions of copepods sampled from 50 m to the surface in September 1995. Each point represents mean ±SD. Shaded bar along the x-axis indicates the dark hours.

The copepod community grazing impact on primary production was estimated by employing measurements of ingestion rates, copepod abundances and primary production. The variability in grazing impact was mainly a result of differences in both copepod abundance and gut pigment content. Although large copepods showed the highest daily ingestion rates on an individual basis, the overall highest grazing on primary production was performed by medium sized copepods, due to their numerical abundance. It is calculated that 53% of the community grazing was due to the medium size fraction. In contrast to Morales *et al.* (1991) and Dam *et al.* (1993), who discussed the relative importance of small size fraction grazing on primary production in the North Atlantic, medium sized copepods grazing was the most important (among the three size classes) in September 1995 in the Black Sea.

The small size copepod fraction only included those larger than 300 µm. Many copepodites and *Oithona similis* individuals are likely to have passed through the 300 µm mesh filter and thus have not been considered in calculating grazing pressure. The estimated percentage abundance of loss from 300 µm mesh filter was 15% of the total abundance of small sized copepods. When considering this loss, the ingestion rate of small size fraction was still lower than that large size fraction.

The grazing pressure estimated from the gut pigment content was relatively high. For example, the daily carbon ration ranged from 20 to 70% of the body carbon for the large size fraction, from 10 to 360% for the medium size fraction and from 4 to 310% of the body carbon content for the small size fraction. In general, this data show that the phytoplankton carbon ingested apparently met all of the basic metabolic requirements when respiration rates were estimated using the Dag *et al.* (1982)'s equation (Table 1.5). These results imply that phytoplankton were the major food source of mesozooplankton during September 1995 in the Black Sea.

Table 1.3. Percentage abundance of three size fractions of copepods sampled from 50m to the surface in September 1995. -- = no individuals observed

Species	2000-1000 µm	1000-500 µm	500-300 µm
Calanus euxinus			
female	28.3	--	--
male	3.3	--	--
copepodite V	43.7	0.7	--
copepodite IV	14.1	2.1	--
copepodite III	2.5	7.5	0.4
copepodite II	0.8	4	4.7
copepodite I	0.2	1.9	3.3
Pseudocalanus elongatus			
female	3.9	44.8	28
male	--	1.3	0.7
copepodite stages	1.5	13.2	36.9
Acartia clausi			
female	1.1	13.6	2.2
male	--	5.9	1.8
copepodite stages	0.3	4	5.2
Paracalanus parvus			
female	0.2	--	1.3
male	--	--	0.5
copepodite stages	--	--	0.9
Oithona similis			
female	--	1.0	10.9
male	--	--	--
copepodite stages	--	--	3.2

Table 1.4. The gut pigment content (G; ng pigment individulas^{-1}), ingestion rates (I; ng pigment individulas^{-1} hour^{-1}) and the percentage grazing rate of each size fraction of copepod assemblages during the sampling times in September 1995. Gut evacuation rate = 2.4 h^{-1}; Phytoplankton carbon/chlorophyll-a ratio = 65; Primary production = 405 mg C m^{-2} d^{-1}. Values in parenthesis are standard deviations.

	Large Size				**Medium Size**				**Small Size**			
Time of Day	G	I	İnd. m^{-2}	% Grazing	G	I	Ind. m^{-2}	% Grazing	G	I	Ind. m^{-2}	% Grazing
19:00	7.8 (2.0)	18.6	1873.7	13.4	9.1 (0.0)	21.8	7242.1	60.6	2.0 (3.0)	4.8	3410.6	6.3
23:30	9.4 (3.0)	22.6	2863.2	24.9	1.7 (1.8)	4.1	3536.8	5.6	1.2 (0.3)	2.9	17852.6	19.6
03:30	11.0 (6.0)	26.4	926.3	9.4	4.4 (2.8)	10.5	1937.2	7.8	1.9 (1.6)	4.6	3536.8	6.3
07:30	0.0	0.0	176.3	0.0	0.1 (0.1)	0.3	1221.1	0.1	0.9 (1.1)	2.2	3368.4	2.9
12:00	0.0	0.0	305.3	0.0	1.2 (0.8)	2.8	2863.2	3.1	1.2 (1.0)	2.8	3368.4	3.7
16:00	3.4 (3.0)	8.2	1557.9	4.9	3.6 (3.8)	8.6	7409.5	24.1	1.1 (0.2)	2.6	5894.7	5.8
21:00	5.2 (0.5)	12.6	863.5	4.2	4.5 (6.0)	10.8	3789.5	15.7	0.9	2.2	2610.5	2.2
Average	5.6 (4.4)	12.6 (10.5)	1223.7 (947)	8.1 (8.8)	3.5 (3.0)	8.4 (7.1)	3999.9 (2438.0)	16.7 (21.0)	1.3 (0.5)	3.1 (1.1)	5720.3 (5447.6)	6.7 (5.9)

Table 1.5. Grazing pressure, biomass and basic metabolic requirements of copepods from the first 50 m in September 1995.

GP = Grazing pressure (mg C m^{-2} d^{-1}), B*=Biomass (mg C m^{-2}), R§ = Respiration (mg C m^{-2} d^{-1})

	Large Size			**Medium Size**			**Small Size**		
Time of Day	GP	B	R	GP	B	R	GP	B	R
19:00	54.3	116.2	7.3	245.7	24.2	2.2	25.5	4.9	0.5
23:30	100.9	169.8	10.7	22.7	22.1	1.8	79.5	21.8	2.1
03:30	38.1	63.6	4.0	31.6	9.0	0.8	25.5	7.9	0.7
07:30	0.0	0.0	0.0	0.4	5.0	0.4	11.8	7.4	0.7
12:00	0.0	0.0	0.0	12.6	16.5	1.4	15.0	8.6	0.8
16:00	18.9	94.1	5.9	97.7	31.4	2.7	23.5	15.0	1.4
21:00	17.0	52.6	3.3	63.6	17.8	1.5	8.9	7.5	0.7

*The wet weight of copepods for each size fraction was calculated by using constant value estimated by Ukrainian scientists for each stage of the copepod species in the Black Sea (Niermann *et al*. 1995). The dry weight was estimated from the assumption of 20% of wet weight, and converted to carbon assuming that 40% of the dry weight is due to carbon (Parson *et al*. 1990).

§ Respiration rates were estimated using Dagg *et al*.'s (1982) equation: $R = 0.101\ W_c^{0.884}$; where R is the respiration rate as µgC $animal^{-1}$ day^{-1}, W_c is the animal size as µgC

To estimate the grazing rate of copepod assemblages, the pigment consumption per individual was converted into pigment consumption per biomass (mg C m^{-2}). The biomass as wet weight was calculated by using constant values for each stage of copepod species in the Black Sea (Niermann *et al*., 1995). Wet weights were converted to dry weight assuming that 20% of the wet weight and carbon weight is 40% of the dry weight. The grazing rate was calculated according to following formula;

Grazing Pressure (mg C m^{-2} day^{-1}) = Grazing rate x Biomass (mg C m^{-2})

The grazing rate of copepod assemblages was calculated as 0.76±0.26 day^{-1} in September 1995 in the Black Sea.

References

Alldredge, A.L., Robison, B.H., Flaminger, A., Torres, J.J., King, J.M., Hammer, W.H., 1984. Direct sampling and *in situ* observation of a persistent copepod aggregation in the mesopelagic zone of the Santa Barbara Basin. *Mar. Biol*. 80: 75-81.

Araschkevich, E., Svetlichny, L., Gubareva, E., Besiktepe, S., Gucu, A.C., Kideys, A.E., 1997. Physiological and ecological studies of *Calanus euxinus* (Hulsemann) in the Black Sea including its life cycle. In*:* Ecosystem Modeling as a Management Tool for the Black Sea. Ivanov, L.I., Oguz, T. (Eds.), NATO ASI Series, Vol. 47 (1). Kluwer Academic Publishers, Dordrecht, pp. 327-349.

Ask, J., Reinikainen, M., Bamstedt, U., 2006. Variation in hatching success and egg production of *Eurytemora affinis* (Calanoida, Copepoda) from the Gulf of Bothnia, Baltic Sea, in relation to abundance and clonal differences of diatoms. *J. Plankton Res*., 28(7): 683-694.

Ayukai, T., 1987. Predation by *Acartia clausi* (Copepoda: Calanoida) on two species of tintinnids. *Mar. Microb. Food Webs,* 2: 45-52.

Bamsedt U., D.J. Gifford, X. Irigoien, A. Atkinson, M. Roman, 2000. Feeding. In ICES Zooplankton Methodology Manual, R.P.Harris, P.H. Wiebe, J. Lenz, H.R. Skjoldal, M. Huntley (Eds.). Academic Press, New York, pp.297-399.

Båmsteadt, U., Nejstgaard, J.C., Solberg, P.T., 1999. Utilisation of small-sized food algae by *Calanus finmarchicus* (Copepoda, Calanoida) and the significance of feeding history. *Sarsia* 84: 19-38.

Bargu, S., Koray, T., Lundholm, N., 2002. First report of *Pseudo-nitzschia calliantha* Lundholm, Moestrup & Hasle 2003, a new potentially toxic species from Turkish coasts. E.U. *Journal of Fisheries & Aquatic Sciences*, 19 (3-4), 479-483.

Bautista, B., Rodriguez, V., Jimenez, F., 1988. Short-Term Feeding Rates Of *Acartia grani* In Natural Conditions: Diurnal Variation. *J. Plankton Res*. 10:5, 907-920.

Benli, H.A., 1987. Investigation of phytoplankton distribution in the southern Black Sea and its effect on particle flux. In: Degens, E.T., Izdar, E. (Eds.) Particle Flux in the Ocean, Mitteilungen des Geologish-Palaontologischen Institutes, vol. 62. Universitat Hamburg, pp. 77-87.

Berggreen, U., Hansen, B., Kiorboe, T., 1988. Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. *Mar. Biol*. 99:341-352.

Besiktepe, S., Kideys, A.E., Unsal, M., 1998. *In situ* grazing pressure and diel vertical migration of female *Calanus euxinus* in the Black Sea. *Hydrobiologia*, 363, 323-332.

Besiktepe, S., Unsal, M. 2000. Population structure, vertical distribution and diel migration of *Sagitta setosa* (Chaetognatha) in the south-western part of the Black Sea. *J. Plankton Res*., 22, 669-683.

Besiktepe, S. 2001. Diel vertical distribution, and herbivory of copepods in the south-western part of the Black Sea. *J. Mar. System*, 28(3-4), 281-301.

Besiktepe, S., Dam, H.G., 2002. Coupling of ingestion and defecation as a function of diet in the calanoid copepod *Acartia tonsa*. *Mar. Ecol. Prog. Ser*., 229: 151-164.

Besiktepe, S., Telli, M., 2004. Egg production and growth rates of *Calanus euxinus* (Copepoda) in the Black Sea. *J. Plankton Research* 26:571-578.

Besiktepe, S., Svetlichny, L., Yuneva, T., Romanova, Z., Shulman, G., 2005. Diurnal gut pigment rhythm and metabolic rate of *Calanus euxinus* in the Black Sea. *Mar. Biol*. 146: 1189-1198.

Besiktepe, S., Ryabushko, L., Ediger, D., Yilmaz, D., Zenginer, A., Ryabushko, V., Lee, R., 2008. Domoic acid production by *Pseudo-*

nitzschia calliantha Lundholm, Moestrup et Hasle (Bacillariophyta) isolated from the Black Sea. Harmful Algae, in press.

Bird, D.F., Karl, D.M., 1991. Microbial Biomass and Population Diversity in the Upper Water Column of the Black Sea. *Deep-Sea Res. II*, 38: S1069-S1082.

Bodeanu, N., Moncheva, S., Ryta, G., Popa, L., 1998. Long-term evolution of the algal blooms in Romanian and Bulgarian Black Sea waters. *Cercetari Marine*. IRCM Constanta, 31: 37-55.

Bologa, A.S., 1986. Planktonic primary productivity of the Black Sea: a review. *Thalassia Yugoslavia* 21/22: 1-22.

Bouvier, T., Becquevort, S., Lancelot, C., 1997. Biomass and feeding activity of phagotrophic mixotrophs in the northwestern Black Sea during the summer 1995. *Hydrobiologia*, 363: 289-301.

Boyd, C.M., Smith, S.L., 1980. Grazing patterns of copepods in the upwelling system off Peru. *Limnol. Oceanogr*. 25:4, 583-596.

Bundy, M.H., Vanderploeg, H.A., 2002. Detection and capture of inert particles by calanoid copepods: the role of the feeding current. *J. Plankton Res*., 24: 215-223.

Coble, P.G., Gagosian, R.B., Codispoti, L.A., Friederich, G.E., Christensen, J.P., 1991. Vertical distribution of dissolved and particulate fluorescence in the Black Sea. *Deep Sea Research*. 38 (2): S985-S1002.

Cokacar, T., Oguz, T., Kubilay, N., 2004. Interannual variability of the early summer coccolithophore blooms in the Black Sea: impacts of anthropogenic and climatic factors. *Deep-Sea Res. I*, 51: 1017-1031.

Colin, S.P., Dam, H.G., 2002. Testing for toxic effects of prey on zooplankton using sole versus mixed diets. *Limnol. Oceanogr*. 47(5): 1430-1437.

Conover RC (1978) Transformation of organic matter. In: Kinne O (Ed.) Marine Ecology. Vol. 4, John Wiley and Sons, New York, pp 221-499

Dagg, M.J., Wyman, K.D., 1983. Natural ingestion rates of the copepods *Neocalanus plumchrus* and *N. cristatus* calculated from gut contents. *Mar. Ecol. Progr. Ser.* 13: 37-46.

Dagg, M.J., 1993. Sinking particles as a possible source of nutrition for the large calanoid copepod *Neocalanus cristatus* in the subarctic Pacific Ocean. *Deep-Sea Res I* 40(7): 1445-1993.

Dagg, M.J., Frost, B.W., Newton, J., 1998. Diel vertical migration and feeding in adult female *Calanus pacificus*, *Metridia lucens* and *Pseudocalanus newmani* during a spring bloom in Dabob Bay, a fjord in Washington USA. *Limnol. Oceanogr.* 42(5): 974-980.

Dam, H.G., Peterson, W.T., 1988. The effect of temperature on the gut clearance rate constant of planktonic copepods. *J. Exp. Mar. Biol. Ecol.*, 123, 1-14.

Dam, H.G., Peterson, W.T., 1993. Seasonal contrast in the diel vertical distribution, feeding behavior, and grazing impact of the copepod *Temora longicornis* in Long Island Sound. *J. Mar. Res.*, 51: 561-594.

Dam, H.G., Miller, C.A., Jonasdottir, S.H., 1993. The trophic role of mesozooplankton at 47°N, 20°W during the north Atlantic bloom experiment, *Deep-Sea Res. II*, 40: 197-212.

Davidovich, N.A., Bates, S.S., 1998. Sexual reproduction in the pennate diatoms *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima* (Bacillariophyceae). *J. Phycol*. 34, 126-137.

DeMott, W.R., 1988. Discrimination between algae and artificial particles by freshwater and marine copepods. *Limnol. Oceanogr.*, 33: 397-408.

Eker, E., 1998. Abundance and biomass of micro- nanophytoplankton in the Northwestern and Southwestern Black Sea in 1995. MS.

Thesis in Marine Sciences, Middle East Technical University, Turkey, 206 pp.

Eker, E., Georgieva, L., Senichkina, L., Kideys, A.E., 1999. Phytoplankton distribution in the western and eastern Black Sea in spring and autumn 1995. *ICES J. Mar. Sci*., 56: 15-22.

Eker-Develi, E., Kideys, A.E., 2003. Distribution of phytoplankton in the southern Black Sea in summer 1996, spring and autumn 1998. *J. Mar. Syst*., 39: 203-21.

Enright, J.T., 1977. Diurnal vertical migration: Adaptive significance and timing. Part I. Selective advantage: A metabolic model. *Limnol. Oceanogr*. 22: 856-872.

Erkan, F., Gucu, A.C., Zagorodynyaya, J., 2000. The diel vertical distribution of zooplankton in the Southeast Black Sea. *Turk. J. Zool*., 24: 417-428.

Frangoulos, M., Guisande, C., Maneiro, I., Riveiro, I., Franco, J., 2000. Short-term and long-term effects of the toxic dinoflagellate *Alexandrium minimum* on the copepod *Acartia clausi*. *Mar. Ecol. Prog. Ser*., 203: 161-169.

Frost B.W., 1972. Effects of size and concentration of food particles on the feeding behaviour of the marine copepd *Calanus pasificus*. *Limnol. Oceanogr*., 17 (6), 805-815.

Frost, B.W., 2005. Diatom blooms and copepod responses: Paradigm or paradox? *Progr. Oceanogr*., 67: 283-285.

Gavrilova, N., Dolan, J.R., 2008. A note on species lists and ecosystem shifts: Black Sea tintinnids, ciliates of the microzooplankton. *Acta Protozoologica*, 46: in press.

Gowing, M.M., Silver, M.W., 1986. Trophic relationships of deepsea calanoid copepods from the benthic boundary layer of the Santa Catalina Basin, California. *Deep-Sea Res* 33:939–964.

Green, E.P., Harris, R.P., Duncan, A., 1992. The production and ingestion of faecal pellets by nauplii of marine calanoid copepods. *J. Plankton Res*. 14: 1631-1643.

Harris, R.P., 1996. Feeding ecology of Calanus. *Ophelia*, 44: 85-109.

Head, E.J.H., Harris, R.L., 1992. Chlorophyll and carotenoid transformation and destruction by *Calanus* spp. grazing on diatoms. *Mar. Ecol. Prog. Ser*. 86: 229-238.

Houde, S.E., Roman, M.R., 1987. Effects of food quality on the functional ingestion response of the copepod *Acartia tonsa*. *Mar Ecol Prog Ser* 86: 229-238.

Humborg, C., Ittekkot, V., Cociasu, A., Bodungen, B.V., 1997. Effect of Danube River dam on Black Sea biogeochemistry and ecosystem structure. *Nature*, 386: 385-388.

Huntley, M.E., Ciminiello, P., Lopez, M.D.G., 1987. Importance of food quality in determining development and survival of *Calanus pacificus* (Copepoda: Calanoida). *Mar. Biol*., 95: 103-113.

Ikeda, T., 1970. Relationship between respiration rate and body size in marine plankton animals as a function of temperature of habitat. *Bull. Fac. Fish*. Hokkaido Univ., 21: 91-112.

Irigoien, X., Harris, R.P, Verheye, H.M., Joly, P., Runge, J., Starr, M., Pond, D., Campbell, R., Shreeve, R., Ward, P., Smith, A.N., Dam, H.G., Peterson, W., Tirelli, V., Koski, M., Smith, T., Harbour, D., Davidson, R., 2002. Copepod hatching success in marine ecosystems with high diatom concentrations – the paradox of diatom-copepod interactions revisited. *Nature* 419: 387-389.

Karl, D.M., Knauer, G.A., 1991. Microbial production and particle flux in the upper 350 m of the Black Sea. *Deep-Sea Res II* 38 (2): 921-942.

Kerfoot, W.C., 1985. Adaptive value of vertical migration: Comments on the predation hypothesis and some alternatives. *Contr. Mar. Sci*., 27: 91-113.

Kerfoot, W.C., Kirk, K.L., 1991. Degree of taste discrimination among suspension-feeding cladocerans and copepods: implications for detritivory and herbivory. *Limnol. Oceanogr*., 36: 1107-1123.

Kiorboe, T., Saiz, E., 1995. Planktivorous feeding in calm and turbulent environments, with emphasis on copepods. *Mar. Ecol. Prog. Ser.*, 122: 135-145.

Kleppel, G.S., Pieper, R.E., Trager, G., 1988. Variability In The Gut Contents On Individual *Acartia tonsa* From Waters Off Southern California, *Mar. Biol*., 97, 185-190.

Kosobokova, K., Hirche, H.J., Scherzinger, T., 2002. Feeding ecology of *Spinocalanus antarcticus*, a mesopelagic copepod with a looped gut. *Mar. Biol.*, 141(3): 503-511.

Lampert, W., 1993. Ultimate causes of diel vertical migration of zooplankton: New evidence for the predator-avoidance hypothesis. *Arch. Hydrobiol. Beih*., 39:79-88.

Lincoln, J.A., Turner, J.T., Bates, S.S., Leger, C., Gaythier, D.A., 2001. Feeding, egg production, and egg hatching success of the copepods *Acartia tonsa* and *Temora longicornis* on diets of the toxic diatom *Pseudo-nitzschia multiseries* and the non-toxic diatom *Pseudo-nitzschia pungens*. *Hydrobiologia*, 453/454: 107-120.

Lundholm, N., Moestrup, O., Hasle, G.R., Hoef-Emden, K., 2003. A study of the *Pseudo-nitzschia pseudodelicatissima/cuspidata* complex (Bacillariophyceae): What is *P-pseudodelicatissima*? *J of Phycol*., 39 (4), 797-813.

Mackas, D., Bohrer, R., 1976. Fluorescence analyses of zooplankton gut contents and an investigation of diel feeding patterns. *J. Exp. Mar. Biol. Ecol*. 25: 77-85.

Maneiro, I., Iglesias, P., Guisande, C., Riveiro, I., Barreiro, A., Zervoudaki, S., Granelli, E., 2005. Fate of domoic acid ingested by the copepod *Acartia clausi*. *Mar. Biol*. 148: 123-130.

Mayzaud, P., Tirelli, V., Bernard, J.M., Roche-Mayzaud, O., 1998. The influence of food quality on the nutritional acclimation of the copepod *Acartia clausi*. *Journal of Marine Systems*, 15: 483-493.

McLaren, I.A., 1963. Effects on temperature on growth of zooplankton and the adaptive value of vertical migration. *J. Fish. Res. Bd. Can.*, 20: 685-727.

McLaren, I.A., 1966. Predicting development rate of copepod eggs. *Biol. Bull.*, 131: 457-469.

Mikaelyan, A.S., Pautova, L.A., Pogosyan, S.I., Sukhanova, I.N., 2005. Summer bloom of coccolithophorids in the northeastern Black Sea. *Oceanology* 45: 127-138.

Miralto, A., Barone, G., Romano, G., Poulet, S.A., Ianora, A., Russo, G.L., Buttino, I., Mazzarella, G., Laabir, M., Cabrini, M., Giacobbe, M.G., 1999. The insidious effect of diatoms on copepod reproduction. *Nature*, 403:173-176.

Moncheva, S., Krastev, A., 1997. Some aspects of phytoplankton long-term alterations off Bulgarian Black Sea shelf. In: Sensitivity to Change: Black Sea, Baltic Sea and North Sea. Ozsoy, E., Mikaelyan, A. (Eds.) NATO ASI Series, Vol. 27. Kluwer Academic Publishers, Dordrecht, pp. 79-94.

Moncheva, S., Shtereva, G., Krastev, A., Bodeanu, N., Kideys, A.E., Bayraktar, S., 1998. Vertical distribution of summer phytoplankton in the western Black Sea during 1991-1995 with respect to some environmental factors. In: Ecosystem Modeling as a Modelling Tool for the Black Sea, Ivanov, L.I., Oguz, T. (Eds.), NATO ASI Series, Vol. 47 (1). Kluwer Academic Publishers, Dordrecht, pp. 327-349.

Moncheva, S., Gotsis-Skretas, O., Pagou, K., Krashev, A., 2001. Phytoplankton blooms in the Black Sea and Mediterranean coastal ecosystem subjected to anthropogenic eutrophication: similarities and differences. *Estuar. Coast. Shelf Sci.*, 53: 281-295.

Moncheva, S., Gorinstein, S., Shtereva, G., Toledo, F., Aranbicia-Avila, P., Goshev, I., Trakhtenberg, S., 2003. Biomass, protein- and carbohydrate-composition of phytoplankton in Varna Bay, Black Sea. *Hydrobiologia*, 501: 23-28.

Morales, C.E., Bedo, A., Harris, R.P., Tranter, P.R.G., 1991. Grazing of copepod assemblages in the north-east Atlantic: the importance of the small size fraction. *J. Plankton Res*. 13 (2): 455-472.

Morales, C. E., Harris, A.R.P., Head, R. N., Tranter, P.R.G., 1993. Copepod grazing in the oceanic northeast Atlantic during 6 week drifting station: the contribution of size classes and vertical migrants. *J. Plankton Res.* 15 (2): 185-211.

Mutlu, E., Bingel, F., 1999. Distribution and abundance of ctenophores and their zooplankton food in the Black Sea. I. *Pleurobrachia pileus*. *Mar. Biol*., 135 (4): 589-601.

Mutlu, E., 1999. Distribution and abundance of ctenophores and their zooplankton food in the Black Sea. II. *Mnemiopsis leidyi*. *Mar. Biol.*, 135 (4): 603-613.

Mutlu, E., 2003. Acoustical identification of the concentration layer of a copepod species, *Calanus euxinus*. *Mar. Biol*. 142 (3): 517-523.

Nival, P., Nival, S., 1976. Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): effects on grazing. *Limnol. Oceanogr*., 21: 24-38.

Olson, B.M., Lessard, E.J., Wong, C.H.J., Bernhardt, M.J., 2006. Copepod feeding selectivity on microplankton including the toxigenic diatoms Pseudo-nitzschia spp., in the coastal Pacific Northwest. *Mar. Ecol. Prog. Ser.*, 326: 207-220.

Paffenhofer, G.A., Knowles, S.C., 1979. Ecological implications of faecal pellet size, production and consumption by copepods. *J. Mar. Res*., 37: 35-49.

Paffenhofer, G.A., Van-Sant, K.B., 1985. The feeding response of a marine planktonic copepods to quantity and quality of particles. *Mar. Ecol. Prog. Ser*., 27:55-65.

Parsons, T.R., Takahashi, M., Hargrave, B., 1990. Biological Oceanographic processes. 3rd ed. Pergamon Press. 330pp.

Petipa, T.S., 1964. Diurnal rhythm of food and diurnal diet of *Calanus helgolandicus* in the Black Sea. Works of Sevastopol Biological Station. Academy of Sciences, USSR, (In Russian).

Petruanu, M.A., Bodeanu, N., Bologa, A.S., Dumitrache, C., Moldoveanu, M., Radu, G., Tiganus, V., 1999. Status and evaluation of the Romanian Black Sea coastal ecosystem. In: Proceedings of the NATO Advanced Research Workshop on Environmental Degradation of the Black Sea: Challenges and Remedies. Besiktepe, S.T., Unluata, U., Bologa, A.S. (Eds.)., Kluwer Academic Publishers, Netherlands, pp.175-196.

Pohnert, G., 2005. Diatom/Copepod interactions in plankton: The indirect chemical defence of unicellular algae. *Chem. Bio. Chem*., 6: 946-959.

Roman, M.R., Gauzens, A.L., Rhinehart, W.K., White, J.R., 1993. Effects of low oxygen waters on Chesapeake Bay zooplankton. *Limnol. Oceanogr*., 38 (8): 1603-1614.

Saiz, E., Alcaraz, M., Paffenhofer, G.A., 1992. Effects of small scale turbulence on feeding and gross growth efficiency of three *Acartia* species (Copepoda: Calanoida). J. *Plankton Res*., 14: 1085-1097.

Schmidt, K., Koski, M., Engström-Öst, J., Atkinson, A., 2002. Development of Baltic Sea zooplankton in the presence of a toxic cyanobacterium: a mesocosm approach. *J. Plankton Res*., 24: 979-992.

Smith, S.L., Lane, P.V.Z., 1988. Grazing of the spring diatom bloom in the New York Bight by the calanoid copepods *Calanus finmarchicus*, *Metridia lucens* and *Centropages typicus*. *Cont. Shelf Res*., 8: 485-509.

Stelmakh, L.V., Yunev, O.A., Finenko, Z.Z., Vedernikov, V.I., Bologa, A.S., Churilova, Yu.T., 1998. Peculiarities of seasonal variability of primary production in the Black Sea. In: Ecosystem Modeling as a Modelling Tool for the Black Sea, Ivanov, L.I., Oguz, T. (Eds.), NATO ASI Series, Vol. 47 (1). Kluwer Academic Publishers, Dordrecht, pp. 93-104.

Svetlichny, L.S., Hubareva, E.S., Erkan, F., Gucu, A.C., 2000. Physiological and behavioural aspects of *Calanus euxinus* females (Copepoda: Calanoida) during vertical migration across temperature and oxygen gradients. *Mar. Biol.*, 137 (5/6): 963-971.

StØttrup, J.G., Jensen, J., 1990. Influence of algal diet on feeding and egg production of the calanoid copepod *Acartia tonsa* Dana. *J. Exp. Mar. Biol. Ecol.* 141: 87-105.

Teegarden, G.J., Cembella, A.D., 1996. Grazing of toxic dinoflagellates, *Alexandrium spp,* by adult copepods of coastal Maine: Implications for the fate of paralytic shellfish toxins in marine food webs. *J. Exp. Mar. Biol. Ecol.*, 196, 145-176.

Turner, J.T., Tester, P.A., Strickler, J.R., 1993. Zooplankton feeding ecology: A cinematographic study of animal-to-animal variability of feeding behavior of *Calanus finmarchcus. Limnol. Oceanogr.*, 38, 255-264.

Turner, J.T., Tester, P.A., 1997. Toxic marine phytoplankton, zooplankton grazers and pelagic food webs. *Limnol Oceanogr.*, 42, 1203-1214.

Turner, J.T., Lincoln, J.A., Cembella, A.D., 1998. Effects of toxic and non-toxic dinoflagellates on copepod grazing, egg production and egg hatching success. In: Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T., (Eds.) Harmful algae. Xunta de Galicia and Intergovermental Oceanographic Commission of UNESCO, Paris.

Uysal, Z., 2001. Chroococcoid Cyanobacteria *Synechococcu*s spp. in the Black Sea: pigments, size, distribution, growth and diurnal variability. *J. Plankton. Res*., 23(2): 175-189.

Yilmaz, A., Yunev, O.A., Vladimir, I., Vedernikov, V.I., Moncheva, S., Bologa, A.S., Cociasu, A., Ediger, D., 1998. Unusual temporal variations in the spatial distribution of chlorophyll-*a* in the Black Sea during 1990-1996. In: Ecosystem Modeling as a Modelling Tool for the Black Sea, Ivanov, L.I., Oguz, T. (Eds.), NATO ASI Series, Vol. 47 (1). Kluwer Academic Publishers, Dordrecht, pp. 105-120.

Yunev, O.A., Carstensen, J., Moncheva, S., Khaliulin, A., Ertebjerg, G., Nixon, S., 2007. Nutrient and phytoplankton trends on the western Black Sea shelf in response to cultural eutrophication and climate changes. *Estuar. Coast. Shelf Sci*., 74: 63-76.

Valiella, 1995. Marine Ecological processes, Springer-Verlag, New York, 686 pp.

Vanderploeg, H.A., 1990. Feeding mechanisms and particle selection in suspension feeding-zooplankton. In: Wotton RS (Ed.) The biology of particles in aquatic systems. CRC Press, Boca Raton, p. 183-211.

Vershinin, A., Morton, S., Leighfield, T., Pankov, S., Smith, L., Quilliam, M., Ramsdell, J., 2006. Alexandrium in the Black Sea - identity, ecology and PSP toxicity. *African Journal of Marine Science* 28(2): 209-213.

Vinogradov, M.Ye, Flint, M.V., Shushkina, E.A., 1985. Vertical distribution of mesoplankton in the open area of the Black Sea. *Mar. Biol*. 89:1. 95-107.

Vinogradov, M.Ye., Shushkina, E.A., Flint, M.V., Tumantsev, N. I., 1986. Plankton in the lower layers of the oxygen zone in the Black Sea. *Oceanology*. 26:2. 222-228.

Vinogradov, M.Ye., Musayeva, E. I., Semeneva, T.N., 1990. Factor determining the position of the lower of mesoplankton concentration in the Black Sea. *Oceanology*. 30:2. 217-224

Vinogradov, M.Ye, Sapozhnikov, V.V., Shushkina, E.A., 1992a. The Black Sea Ecosystem. Moskva Russia Nauka Dumka, 112pp.

Vinogradov M.Ye., Arashkevich, E.G., Ilchenko, S.V., 1992b. The ecology of the *Calanus ponticus* population in the deeper layer of its concentration in the Black Sea. *J. Plankton Res*. 14 (3): 447-458.

Wang, R., Conover, R.J., 1986. Dynamics in the gut pigment in the copepod *Temora longicornis* and the determination of in situ grazing rates. *Limnol. Oceanogr*., 31, 867-877.

Zagorodnyaya, Yu.A., 1970. Feeding habits and migration of the Black Sea copepod *Pseudocalanus elongatus* (Boeck) in winter. *Oceanology*., 10, 35-41.

Zenkevitch, L., 1963. Biology of the Seas of the U.S.S.R. George Allen and Unwin ltd. Rushin House Museum Street, London. Pp.403-426.

Zubkov, M.V., Sazhin, A.F., Flint, M.V., 1992. The microplankton organisms at the oxic-anoxic interface in the pelagial of the Black Sea. *FEMS Microbial Ecology*, 101: 245-250.

2. ENERGY ALLOCATION AND DEVELOPMENT PATTERNS IN *CALANUS EUXINUS* (COPEPODA)

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Calanus euxinus Hulsemann (Hulsemann, 1991) is a key component of the Black Sea pelagic ecosystem. Due to its high abundance and ability to accumulate large amount of lipids in the body, *Calanus euxinus* is of great importance for a diet of many species of fish.

Calanus in the Black Sea was first identified by Karavaev (1894) and named as *Calanus finmarchicus*. Jaschnov (1955) pointed out the separate status of *C. finmarchicus* and *C. helgolandicus*, and distinguished the Black Sea population to be a subspecies, namely *C. helgolandicus ponticus*. Later, Fleminger and Hulsemann (1987) proposed to give the Black Sea population the rank of species and the name of *C. ponticus*. Finally, Hulsemann (1991) gave a new name, *Calanus euxinus*, to this species. However, Papadopoulos *et al*. (2005) and Unal *et al*. (2006) showed that genetic differences between *C. euxinus* and *C. helgolandicus* are exceedingly subtle and typical for conspecific populations.

Fleminger and Hulsemann suggested that Black Sea *Calanus* penetrated into the Black Sea from the Mediterranean Sea some 7,000 years ago, after the formation of the Dardanelles and Bosphorus straits. According to another hypothesis, *Calanus*, as the case with other “boreal relicts”, could have entered the Black Sea when it was connected with the North Sea across Europe during the period of retreating glaciers (Zaitsev, 2006). This is in agreement with the results of Papadopoulos *et al*. (2005), suggesting that the divergences between the North East Atlantic, Mediterranean and Black Sea *C. helgolandicus* populations are much older than the estimated dates of colonisation of the Mediterranean and Black Seas. However, according to Polischuk (1984), the last connection between the North and Black Seas could have been in the Late Holocene.

The ecology of Black Sea *C. euxinus* differs significantly from that of *C. helgolandicus. Calanus helgolandicus* mainly inhabits neritic waters, having an extremely wide geographical range from the eastern North Atlantic and the North Sea, across the Atlantic Ocean to the seas of the Mediterranean basin. This species inhabits saline waters (32-39‰) with a temperature range of 9-20°C, but for separate populations the temperature range is not so wide and is mainly due to seasonal variations. According to Bonnet *et al.* (2005), in European waters the size of *C. helgolandicus* female prosome length changes from 1.94 mm in the Aegean Sea to 2.6 mm in the North Sea, with larger females occurring at high latitudes and lower temperatures. Seasonal changes in abundance of *C. helgolandicus* are positively correlated with temperature (Bonnet *et al*., 2005) and food availability (Rey-Rassat *et al*., 2004). Over-wintering *C. helgolandicus* populations which descend to deep water are separated spatially by hundreds of metres from the part of population developing in the surface layers.

In contrast, *C. euxinus* is adapted to low salinity of the Black Sea waters (18-20‰). Varying from 7 to 25°C in the surface layers due to seasonal fluctuations, the temperature of the deeper zone is relatively constant throughout the year at 6-8.5ºC. The Black Sea upper aerobic zone is separated from lower anaerobic layers (containing hydrogen sulphide) by the main pycnocline lying at depth of 70-200 m (Vinogradov *et al*., 1992 b). The pycnocline is accompanied by the oxycline where the dissolved oxygen concentration decreases from 7.9-9.8 mg l^{-1} to 0.47-0.84 mg l^{-1}, with a specific density (σ_t) range of 15.3-15.9. Below this, the oxygen concentration decreases rapidly to <0.16 mg O_2 l^{-1} at σ_t = 15.9-16.0, and can no longer be detected at σ_t = 16.15-16.20.

During the period of seasonal temperature stratification in the deep regions of the Black Sea, pre-adult and adult *C. euxinus* form two ecological groups: migrating and non-migrating individuals. In one group of copepodites, V (CV) all males and females perform active diel vertical migrations from warm oxygen-saturated surface layers to the cold (~8°C) oxygen minimum zone (OMZ, 0.5-0.8 mg O_2 l^{-1}). The other group of copepodites, consisting of diapausing CV, predominantly inhabits underlying water layers with more pronounced oxygen deficiency (as low as 0.3 mg O_2 l^{-1}) (Vinogradov *et al.*, 1992a). However, there is no strict border between the habitats of migrating and diapausing CV.

Whilst *C. helgolandicus* produce 5 generations per year in the Western English Channel (Rey-Rassat *et al.*, 2004), in the Black Sea *C. euxinus* undergoes 6-8 generations per year (Sazhina, 1987) and can be found all year round. The abundance in *C. euxinus* does not differ significantly in warm and cold seasons (Sazhina, 1987; Vinogradov *et al.*, 1992b). Even during the summer phytoplankton depression, *C. euxinus* is able to accumulate large amounts of lipids (mainly wax esters) in the oil sac (Yuneva *et al.*, 1997a). Oil sac volume in *C. euxinus* may reach 30-40% of body volume (Svetlichny *et al.*, 1998), a value which is close to lipid content of *Calanus* from the North-East Atlantic (Miller *et al.*, 2000) where phytoplankton production is significantly higher than that in the Black Sea (Joint and Pomroy, 1993; Chua *et al.*, 2005). Prosome length in females from the Black Sea population (2.6 mm) is similar to (or may even exceed) the maximum length of *C. helgolandicus*.

Trying to explain the large body size of adult *C. euxinus*, Fleminger and Hulsemann (1987) suggested that low temperature and hypoxia were the main factors determining the development of the Black Sea population (in contrast to the Mediterranean and Atlantic *Calanus* populations).

It is known that under variable physical and chemical conditions the development of planktonic organisms accelerates and their adaptability increases (Galkovskaya and Suschenya, 1978). Svetlichny *et al.* (1998, 2006) and Yuneva *et al.* (1999) hypothesised that during the summer-autumn season, temperature stratification and low phytoplankton concentration in the water column result in a higher frequency of later development stages of *C. euxinus*. Energy efficiency is reduced by the diel vertical migration from warm upper layers of the Sea to the cold OMZ allowing the energy from a more plentiful food supply consumed at night near the surface to drive the process. In daytime under hypoxic conditions, copepods decrease the catabolism of lipids and accelerate their synthesis (in the form of wax esters) from dietary non-lipid components.

The phenomenon of *C. euxinus* migrations to oxygen-deficient water layers raises a series of important questions:

- Do *Calanus* actively choose their habitat depth due to an oxygen regime preference (Flint, 1989), or CV descend passively and concentrate in the water layers with the density being equal to the body density (Rudyakov, 1986; Vinogradov, 1990)?
- How do vertical migrations affect energy budget and its different components?
- What metabolic substrates are used in catabolic processes under aerobic and hypoxic conditions?
- What is the role of reserve lipids and how they are formed and used?
- How do the vertical profiles of temperature and oxygen concentration influence individual growth and population structure?

The answers to these questions would greatly contribute to understanding the energy strategy of *C. euxinus* development in the Black Sea.

2.1 Metabolic rates in oxygenated water

In aerobic organisms oxygen consumed during respiration is used in oxidation of metabolic substrates. Due to these processes, animals obtain energy to perform essential vital functions. Total respired energy (total metabolism) in Copepoda (Q_t) can be expressed as $Q_t = Q_b + Q_l + Q_f + Q_{sda}$, where: Q_b is the basal metabolism (respiration rate of the individual during rest); Q_l and Q_f are energy losses for locomotion and filtration of food, respectively; and Q_{sda} is the energy expenditure for food digestion and formation of new body tissues, or "specific dynamic action of food" (SDA). The difference between Q_t of the animals with maximum enforced activity and Q_b is defined as the scope of activity.

Ivlev (1959) considered the ability to increase the scope of activity as a progressive feature in evolutionary process. In crustaceans, the scope of activity averages 6 (Torres and Childress, 1983; Cowles and Childress, 1988), whilst the Q_t of fishes may be 20-fold higher than their Q_b (Brett, 1964).

Petipa (1966) was the first to estimate the energy expenditure for swimming in *Calanus helgolandicus* (*euxinus*), taking into account the losses of reserve lipids during vertical migrations. The values obtained were 12.4-34.3 times higher than standard metabolism under laboratory conditions. Nevertheless, these and other data showing extremely high energy expenditure for locomotion in copepods (Petipa, 1981; Pavlova, 1987; Ostrovskaya, 1976; Minkina, 1981) disagree with the results of calculations on the base of numerical models (Klyashtorin and Yarzhombek, 1973; Klyashtorin, 1984; Morris *et al*., 1985; Morris *et al*., 1990) and theoretical analyses of energy balance in copepods (Winberg, 1982), suggesting that swimming might account for 40-60% of total metabolic activity.

Vlymen (1970), on the basis of the hydrodynamic studies, estimated the cost of escaping predation to be less than 0.3% of the standard metabolic rate *of Labidocera trispinosa*. However, Strickler (1977) showed that during escape, reaction energy expenditure may be 400 times greater than during undisturbed swimming. Later, Alcaraz and Strickler (1988) measured the drag force in *Cyclops scutifer* attached to a spring, and calculated the work done to constitute only 0.069% of total metabolism. Even lower values (0.01-0.06% of total energy budget) of viscous dissipation of energy into the water, during grazing and escape swimming of copepods, have been obtained by other workers (Yen *et al*., 1991; Jiang *et al*., 2002; van Duren and Videler, 1995, 2003).

Basing on studies which had shown very low energy expenditure for filtering in crustaceans (Bohrer and Lampert, 1988; Kiorboe *et al*., 1985), Thor (2000, 2002, 2003) considered Q_{sda} as the basic component of the energy budget in *Calanus finmarchicus* and *Acartia tonsa*. Reasons underlying the widely varying energy cost values of swimming in copepods (Mauchline, 1998) are still not fully understood.

To verify conflicting estimates of swimming cost in copepods, direct measurements of energy expenditure during different moving activities are required (as for fish by Brett, 1964).

2.1.1 Metabolic scope of activity

After studying respiration rate and locomotion in 6 copepod species from the Black and Mediterranean Seas, Svetlichny (1992a) found that for *Euchaeta marina* weight-specific respiration rate increased 6.3-fold, from 0.3 μL O_2 mg^{-1} h^{-1} during the rest period to 1.9 μL O_2 mg^{-1} h^{-1} at high moving activity. In food-deprived *Calanus euxinus* tethered to a force sensor at 20 ºC, carbon-specific respiration rate (R) changes proportionally with mechanical energy (E, J) according to the equation $R=85.9xE^{0.32}$ (Svetlichny and Hubareva, 2005; Fig. 2. 1).

At continuous locomotion by mouth appendages with a frequency of 40 Hz, total mechanical energy output accounted for 0.0031 J h^{-1} and the carbon-specific respiration rate was 14.4 nL O_2 mgC^{-1} h^{-1}.

Escape locomotion is extremely irregular. In our experiments the most active enforced female undertook 226 escape reactions during one hour including 2130 elementary jumps, with a total duration of about 28 s, and developed mechanical energy of 0.0034 J h^{-1}. In addition, the cost of cruising swimming by mouth appendages in this female accounted for 0.001 J h^{-1}. As a result, the carbon-specific respiration rate was equal to 12.5 nL O_2 mgC^{-1} h^{-1}. Since the average carbon-specific respiration rate corresponding to mouth appendage mechanical work of 0.001 J h^{-1} should be equal to 9.4 nL O_2 mgC^{-1} h^{-1}, it is possible to assume that the maximal energy expenses at escape reaction amount to 3.1 nL O_2 mgC^{-1}

h^{-1}. Therefore, at maximum rate of locomotion by mouth appendages and escape reactions, the carbon-specific respiration rate should reach 17.5 nL O_2 mgC^{-1} h^{-1}, i.e. 5.8-fold higher than the metabolic rate at low activity.

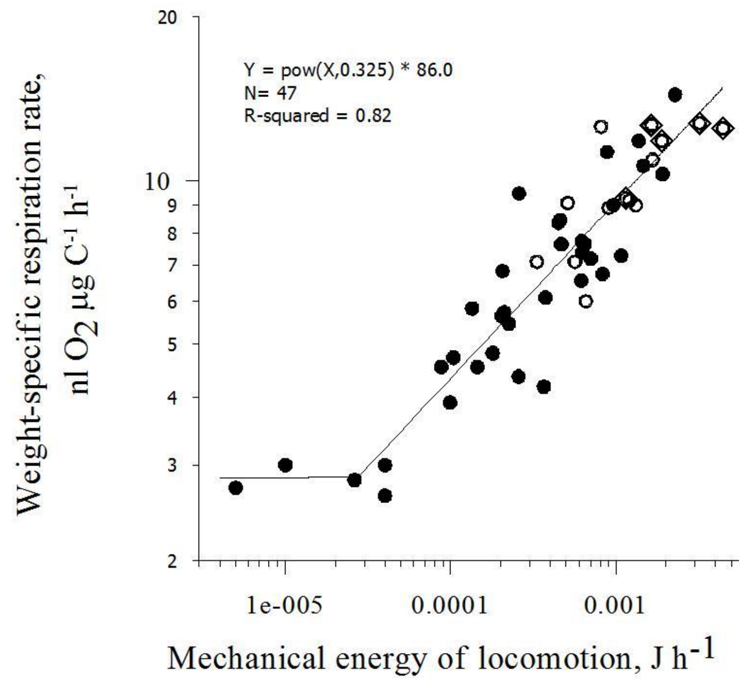

Figure 2.1. Weight-specific respiration rate versus mechanical energy of locomotion (E, Jh^{-1}) in *Calanus euxinus* attached to force sensor in the respirometric chamber. Closed circles indicate swimming by mouth appendages only and escape motion, respectively. Compound locomotion (energy losses for cruising mode and escape response are nearly equal) is shown as open circles

According to Buskey (1998), in *Dioithona oculata* active metabolism was 6 times greater than standard metabolism. He revealed a linear relationship between respiration rate and swimming speed of the copepods. The y-intercept of this regression line was used to estimate the respiration rate expected in the absence of any activity. However, such an approach may underestimate basal metabolism because at low moving activity energy losses for locomotion do not significantly affect the metabolic rate (see Fig. 2.1).

In our experiments, at E lower than 4.0 10^{-5} J h^{-1} the respiration rate in *C. euxinus* did not change above a value of 3.0 nL O_2 μgC^{-1} h^{-1}, or 0.5 μg O_2 ind^{-1} h^{-1}. Such a metabolic level in slow-moving animals is close to the respiration rate of narcotised copepods (0.45 ± 0.026 μg O_2 ind^{-1} h^{-1}) at 22 ºC (Svetlichny *et al.*, 2000) and can be considered as basal metabolism. The maximum carbon-specific respiration rate of *Calanus* females constantly moving by mouth appendages amounted to 14.4 nL O_2 μgC^{-1} h^{-1}. Consequently, the scope of activity can be estimated as 11.4 nL O_2 μgC^{-1} h^{-1}. Taking into account that an oxycalorific value of 1 mL O_2 is equal to 20.1 J (Omori and Ikeda, 1984), we quantified the metabolic energy of mouth appendage locomotion as 2.6 10^{-2} J h^{-1}. The maximum mechanical energy during mouth appendage locomotion was equal to 3.1 10^{-3} J h^{-1}, therefore, the coefficient of mechanical-chemical conjugation constituted 11.9%. This value is about half the maximum theoretical efficiency of muscle contraction (25%) under aerobic conditions (Bagshaw, 1982).

2.1.2 Energy required for food filtration and swimming using mouth appendages

Filter-feeding copepods use numerous mouth appendages in order to provide simultaneously body propulsion and food filtration. We can estimate mechanical efficiency of the rowing locomotion as the ratio between the energy necessary for body propulsion and the total mechanical energy expenditure for limbs movement.

The mechanical energy required for body propulsion (E_b, J) during cruising can be calculated as $E_b = 0.5\rho C_D v^3 S$, where: ρ is the water density, g cm^{-3}; C_D is the hydrodynamic drag coefficient; v is the swimming speed, cm s^{-1}; and S is the cross-sectional area of the body (Vlymen, 1970).

The flow field around a foraging copepod is purely laminar. At laminar flow C_D depends upon Reynolds number Re = vd ν^{-1}, where: d is maximum body width, cm; and ν is the coefficient of kinematic viscosity, cm^2 s^{-1}. The relationship C_D = f(Re) can be expressed by the equation C_D=CRe^{-n} = $C\nu^n$ $v^{-n}d^{-n}$, where C and n are empirical coefficients (Stepanov, Svetlichny, 1981). Finally, assuming that $S=0.25\pi d^2$, $E_b=0.125\ \pi\ 10^{-7}\ C\ \nu^n\ \rho\ d^{2-n}\ v^{3-n}$.

Svetlichny (1983b) found that for *Calanus helgolandicus* (*euxinus*) moving with spread antennae at $0.3\leq Re\leq 2.3$, C=126±2.6 and n=0.85. In the range of Reynolds numbers from 2.9 to 23 C=143±3.0 and n=0.75. The results of our videotaping showed that at 20 ºC the swimming speed of horizontal continuous gliding amounted to 3.2 cm s^{-1} in *C. euxinus* females with a total body length and width of 0.35 and 0.08 cm, respectively. At this swimming speed, the net energy to overcome hydrodynamic drag of the body amounted to 4.0 10^{-4} J h^{-1}, equivalent to over 10% of the total mechanical energy for mouth appendage motion (3.1 10^{-3} Jh^{-1}) and 1.5% of the metabolic energy required for routine swimming.

According to Alexander (1968), the hydrodynamic efficiency of rowing locomotion ($ή_g$) does not exceed 50%. Morris *et al.* (1985) calculated a value for $ή_g$ of 0.34 for escape swimming by thoracic legs in *Pleuromamma xiphias*. In *C. helgolandicus* during routine swimming by mouth appendages, $ή_g$ amounted to 0.31 (Svetlichny, 1992b). Consequently, mechanical energy losses for body propulsion using mouth appendages would be equal to 4.0 10^{-4}/0.31 = 1.3 10^{-3} Jh^{-1}. Probably, the residual 1.8 10^{-3} Jh^{-1}, or 58% of gross energy expenditure, is used to generate feeding currents.

Specific dynamic action of food

The determination of SDA from the difference between respiration rates of fed and unfed animals is the most available experimental method. However, in this case it is necessary to exclude those factors influencing the metabolic rates of individuals, especially if long-term experiments are conducted. The presence of food may stimulate moving activity of copepods (Yule and Crisp, 1983; Mackas and Burns, 1986; Price and Paffenhofer, 1986a,b; Poulet and Gill, 1988; van Duren and Videler, 1995) and cause an "apparent SDA". It is necessary to take into account the phenomena of short-term and diel endogenous rhythms in the activity of copepods (Champalbert, 1978; Svetlichny and Yarkina, 1989).

In our experiments, the respiration rate of feeding females of *C. euxinus* (placed into respiration chambers with the alga *Prorocentrum micans*) was significantly higher than that of unfed females only in those cases when the values of time spent swimming were identical during pre-feeding and feeding periods. The mean SDA was 1.2±0.44 nL O_2 mgC^{-1} h^{-1}, regardless of the activity level. Therefore, the contribution of SDA to total metabolic rates varied from 23 to 85% in *Calanus* with a low activity level, and constituted only 10% in animals showing maximum activity (Fig. 2.2).

Thor (2000, 2002) reported that at 14°C during an 8-h feeding period, the respiration rate of *C. finmarchicus* copepodite V and females increased gradually from 3.5-5.0 nL O_2 mgC^{-1} h^{-1} to a maximum of 9.8 nL O_2 mgC^{-1} h^{-1} but reduced to the preceding level after removing the food. These values were used to estimate SDA without taking into account the effect of locomotor activity on respiration rate of *C. finmarchicus*, because Thor (2000, 2002) considered energy losses for swimming in copepods to be insignificant.

According to our calculations, carbon-specific respiration rates of starved *C. finmarchicus* reported by Thor (2000, 2002) and normalised to 20°C (5.5-7.8 nL O_2 mgC^{-1} h^{-1}, Q_{10}=2.1) are close to values for *C. euxinus* with low locomotor activity (Fig. 2.1). However, in starved slow-acting copepods, feeding events may stimulate the increase in both frequency and total duration of locomotion (Svetlichny and Hubareva, 2005) resulting in a many-fold increase in respiration rate.

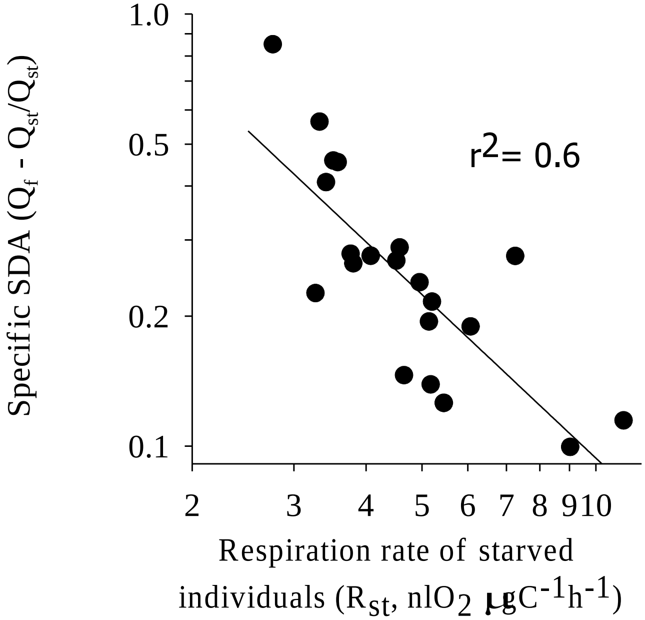

Figure 2.2. Relative cost of food digestion at different prefeeding motor activity levels in copepodites V and females of *Calanus euxinus*

2.2 Ontogenetic changes in metabolic rates

Studies on respiration rate changes with age in *Calanus* genus are very scarce and concerned largely with late copepodite stages and adults of *C. finmarchicus* (Marshall and Orr, 1972). Only Vidal (1980b) reported respiration rates for all copepodite stages and females of *C. pacificus*. In *C. euxinus* metabolic rates of CV and females have been examined (Pavlova, 2007; Pavlova and Minkina, 1987).

In our experiments, respiration rate (R, μgO_2 ind^{-1} h^{-1}) was determined in *C. euxinus* from CI to adult females (collected in the south-eastern Black Sea in April 2003) during shipboard experiments (R/V "Knorr") at 20°C and in CV and females (collected near Sevastopol) in the laboratory at 8 °C. Also we studied respiration rate in *C. euxinus* copepodites and females sampled in October 2002 and in September-

October 2003 near Sevastopol at 20 °C. Before starting the experiments, individuals were kept for one day in seawater containing a mixture of algae *Prorocentrum micans* and *Thalassiosira weisflogii* at the stated temperatures for acclimation.

Respiration rates were determined using experimental and control chambers (2.5 ml-capacity all-glass syringes). One active copepodite V (CV), adult female and male or ten copepodites I (CI) were placed in an experimental syringe filled with filtered seawater. Each pair of experimental and control syringes were connected with a fine plastic tube and then the water was pumped several times through a pair of syringes to achieve equal initial oxygen concentration in experimental and control chambers. Afterwards, the syringes were separated, closed by stoppers and incubated at 20 ºC for 1-2 h.

Oxygen concentration was determined by a polarographic membrane oxygen sensor joined with the measuring chamber (all-glass syringe) of 0.5 ml volume, with a magnetic stirrer inside (Svetlichny and Umanskaya, 1991). The water sample from the experimental syringe was transferred to the measuring chamber through the needle without contacting the surrounding air.

According to the results obtained (Fig. 2.3A), the relationship between respiration rate during ontogeny from CI to adults and body size at 20 °C is described by the allometric equation $Q=1.1WW^{0.98}$, where: WW is the wet weight, mg. No significant differences were found between the weight-specific respiration rates (at 20 °C) of females collected in April at at a surface water temperature of 8 °C (1.34±0.4 μg O_2 $ind^{-1}h^{-1}$) or those measured in females captured in September at a surface water temperature of 20 °C (1.32±0.17 μg O_2 $ind^{-1}h^{-1}$). This indicates the absence of seasonal temperature metabolic compensation in *C. euxinus* in the Black Sea.

The data given above concern the respiration rates of *C. euxinus* with an undifferentiated level of moving activity. However, it is important to consider ontogenic changes in respiration rate of the individuals with maximum locomotor activity (Q_{max}), using only the results of those experiments, in which copepods move constantly in the respirometers. Within body wet weight (WW) range from 0.03 to 1.27 mg, at 20 °C respiration rate increased from 0.052 in CI to 2.13 μg O_2 ind^{-1} h^{-1} in adult females (Fig. 2.3B) according to the equation: $Q_{max} = 1.61WW^{1.01}$. Changes in *C. euxinus* respiration rate during ontogeny from CV to females at 8 °C in April 2003 were described by the equation $Q=0.69WW^{1.05}$. The slopes in these regressions are significantly higher than the exponent values for dry body weight of about 0.8 reported for oxygen consumption rates of planktonic copepods (Vidal, 1980b; Ikeda *et al.*, 2001). However, if the body weight units are changed from WW to defatted dry weight (DFDW) representing the amount of metabolically active matter in the body, the above equations will be $Q=6.69DFDW^{0.8}$ for 20 °C and $Q=2.84DFDW^{0.8}$ for 8 °C, respectively. This is due to the low contribution of DFDW to body volume in the early copepodite stages of *C. euxinus* (see Section 4.4). In April 2003, the mean Q_{10} in the temperature range of 8-20 °C for *C. euxinus* individuals with the same body size was 2.04±0.02.

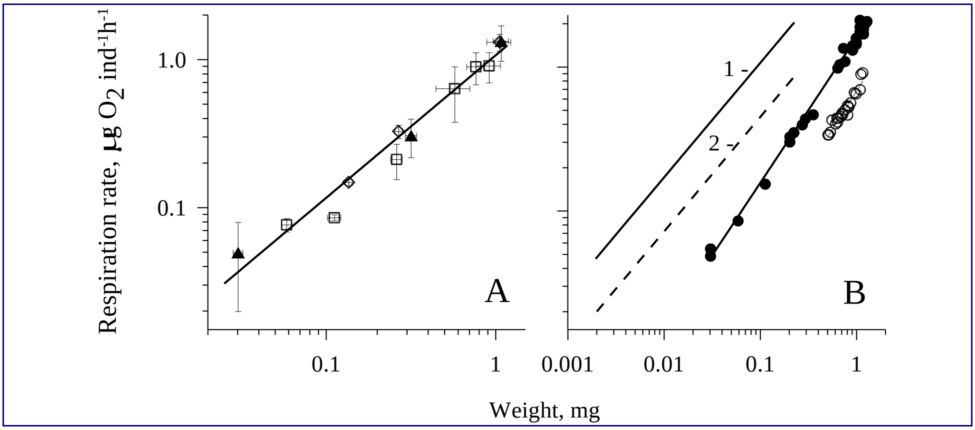

Figure 2.3. Relationships between mean values of respiration rate and wet weight of CI to adults of *Calanus euxinus* at 20 °C in October 2002 (◊), April 2003 (▲) and September - October 2003 (□) (A); respiration rate of CI to adults of *Calanus euxinus* with maximum swimming and feeding activity versus wet weight at 20 °C (●) and 8 °C (○), and versus defatted dry weight at 20 °C (1) and 8 °C (2) (B)

In our experiments with free-swimming *Calanus,* maximum weight-specific respiration rate in females with a dry weight (DW) of 0.267 mg reached 7.9 µg O_2 mg DW^{-1} h^{-1}, or 13.8 µg O_2 µgC^{-1} h^{-1}, close to the maximum energy expenditure in *C. euxinus* females during permanent swimming in large bottles reported by Pavlova and Minkina (1987). According to the equation of Ikeda *et al.* (2001), in epipelagic copepods with the same body weight at 20 °C weight-specific respiration rate accounted for 4.96 µg O_2 mg DW^{-1} h^{-1}. After using the equation of Vidal (1980 b) for *C. pacificus* at 20 °C, we estimated the weight-specific respiration rate in *C. euxinus* females as 8.86 µg O_2 mg DW^{-1} h^{-1}, being 11% higher than the corresponding value obtained in our experiments. This difference may be due to the lower salinity of the Black Sea. For example, Anraku (1964) found more than a 2-fold decrease in the respiration rate of *C. finmarchicus* at a salinity reduction from 31.5 to 21.5‰. In our experiments, the weight-specific respiration rate of *C. euxinus* reared at 39‰ was 1.3 times higher than that at the Black Sea salinity of 18-20‰.

2.3 Influence of temperature and oxygen concentration

In studies concerning the metabolism of marine animals, investigators have chosen to focus their attention on long-term acclimation to constant environmental parameters, since during short-term experiments the animals may show unstable physiological responses to changes in studied factors (Suschenya 1972; Ivleva 1981) due to the ability to under- or overcompensate for changes in environmental conditions (Vilenkin and Vilenkina, 1979). However, the migration of *Calanus* between oxygen-saturated, warm surface water layers and cold hypoxic zones takes a maximum of several hours (Petipa 1981; Besiktepe *et al.* 1998). Under these conditions, the complete acclimation to any environmental parameter seems unlikely. Despite the fact that temperature is considered to be the main factor affecting the metabolism of zooplankton (Ivleva, 1981) including copepods (Ikeda *et al.*, 2001), there is paucity of data on the temperature dependence of respiration rates in *C. euxinus*.

The effect of hypoxia on metabolism and behavior of *C. euxinus* is also poorly investigated. Vinogradov *et al.* (1992a), using the Winkler technique, reported that the respiration rate of diapausing CV (CV_d) decreased from 0.13 to 0.043 $\mu l O_2$ mg^{-1} h^{-1} at oxygen concentrations changing from 9.2 to 0.2 mg l^{-1}. They also showed that CV_d were able to survive for some days under oxygen deficiency (down to 0.08 mg O_2 l^{-1}). However, an oxygen concentration of 0.06 mg l^{-1} was considered to be lethal.

2.3.1 Effect of temperature and oxygen concentration on respiration rate and behavior of migrating *C. euxinus*

Laboratory and shipboard experiments were carried out on *C. euxinus* CV and females collected from offshore regions of the Black Sea during the cruises of the R/V "Bilim" and from offshore of Sevastopol in 1995-2003. Before the experiments, copepods were maintained to acclimatise for about 0.5 h in 1-µm filtered sea water with the appropriate temperature and oxygen concentration. Controlled temperatures were maintained by refrigeration and the oxygen concentration was decreased by bubbling nitrogen through the water. The effects of these changes on the metabolism and behavior of *C. euxinus* were investigated in separate experiments: a) the influence of temperature in the range from 24 to 7.5 ºC in oxygen-saturated water; b) the influence of oxygen concentration in the range from 10.0 to 0.4-0.5 mg O_2 l^{-1} at a constant temperature of about 8 ºC.

These experiments, designed to reproduce the conditions experienced during vertical migration, demonstrated the relationship between metabolic rates, parameters of locomotory activity of *C. euxinus*, temperature and oxygen concentration. When temperature decreased from 22.1±0.71 ºC to 8.0±0.34 ºC in oxygen-saturated water, the mean value of weight-specific respiration rate in migrating CV and females decreased 2.76 times from 1.38±0.41 to 0.5±0.18 µg O_2 mgW^{-1} h^{-1}. The weight-specific respiration rate of narcotised CV and females (Q_b) decreased 2.26 times from 0.456±0.026 to 0.20±0.015 µg O_2 $mgW^{-1}h^{-1}$.

Consequently, the scope of activity (calculated from the difference between Q_t and Q_b) diminished 3-fold (Fig. 2.4B,D).

The average value of Q_{10} in females is equal to 2.05 for total metabolism, 1.87 for basal metabolism and 2.23 for scope of activity. Our results are close to Q_{10} mean value of 2.25 which is typical for crustaceans acclimated to a temperature range from 10 to 25 ºC (Ivleva, 1981; Winberg, 1983). Vidal (1980b) studied weight-specific respiration rates of *C. pacificus* at different temperatures (8, 12, 15.5 ºC). The mean value of Q_{10} was 1.8. Ikeda *et al*. (2001) reported that in epipelagic marine copepods from the Antarctic, Arctic, boreal, subtropical and tropical waters, Q_{10} for oxygen consumption values varied only to a small extent according to body mass in the range of 1.8-2.1.

The frequency of mouth appendage movements (F) in *C. euxinus* is due to their individual neurally-controlled rhythms. In oxygen-saturated water at 22.1±0.7 ºC, the frequency of mouth appendage oscillations in different individuals varied from 27 to 45 Hz, with a mean value of 34.3±3.6 Hz. The frequencies of mouth appendage locomotion in *C. euxinus* and *C. helgolandicus* from the English Channel (Poulet, Gill, 1988) at 15 ºC are similar: 29-33 Hz and 28.7-29.9 Hz, respectively. When the temperature was decreased to 8 ºC, F (of *C. euxinus*) diminished to 21.7±3.8 Hz (Fig. 2.5B).

The integral drag force R (Fig. 2.5D) within the same range of temperature under normal oxic conditions changed from 0.21±0.029 to 0.15±0.03 dyne, and maximal mechanical energy (E_{max}) of mouthpart constant locomotion decreased from 0.11±0.023 to 0.05±0.017 μgO_2 $mgW^{-1}h^{-1}$ (Fig. 2.5F).

The temperature coefficients Q_{10} of frequency, drag force and mechanical energy of locomotion (1.38, 1.26 and 1.7, respectively) are close to the temperature coefficients of locomotion of *C. euxinus* acclimated to constant values of temperature (Svetlichny, 1989). Lenz *et al*. (2005) also found that in contrast to biological processes, kinematical and mechanical aspects of the escape locomotion in *C. finmarchicus* showed its weaker dependence on temperature, with Q_{10} values of 1.28-1.86 for kick frequency and 1.2-1.9 for maximum force production.

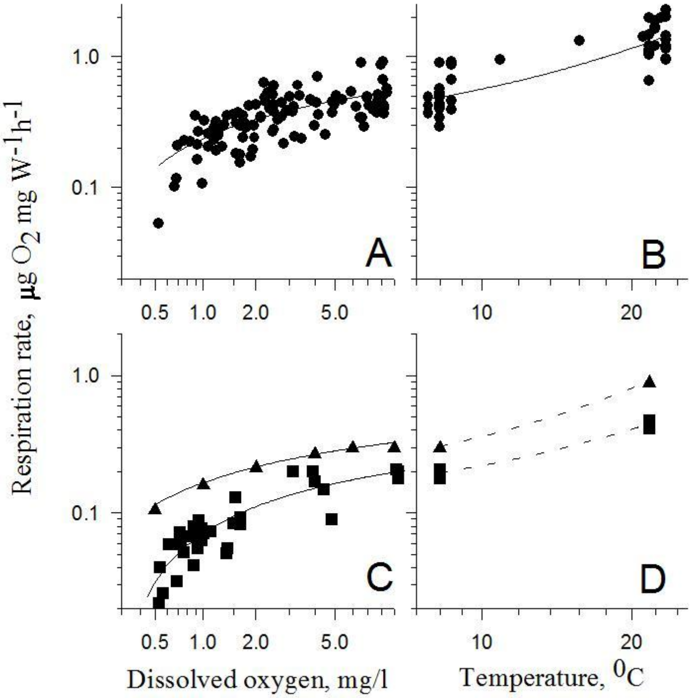

Figure 2.4. The effect of oxygen concentration (A, C) and temperature (B, D) on total (●), basal metabolism (■) and scope of activity (▲) of *C. euxinus* CV and females

At a constant temperature of 8 ºC, oxygen concentration changes from 7.6±0.68 to 0.78±0.16 mg O_2 l^{-1} cause an additional significant decrease in total metabolism from 0.5±0.18 to 0.193±0.087 µg O_2 mgW^{-1} h^{-1}. The decrease in metabolism is more pronounced at oxygen concentration below 2-3 mg l^{-1} (consistent with the results of Marshall and Orr, 1972). Due to a sequential decrease in temperature and oxygen concentration, the level of total metabolism, basal metabolism and scope of activity in *C. euxinus* fell by 7.2, 7.82 and 6.72 times, respectively (Fig. 2.4).

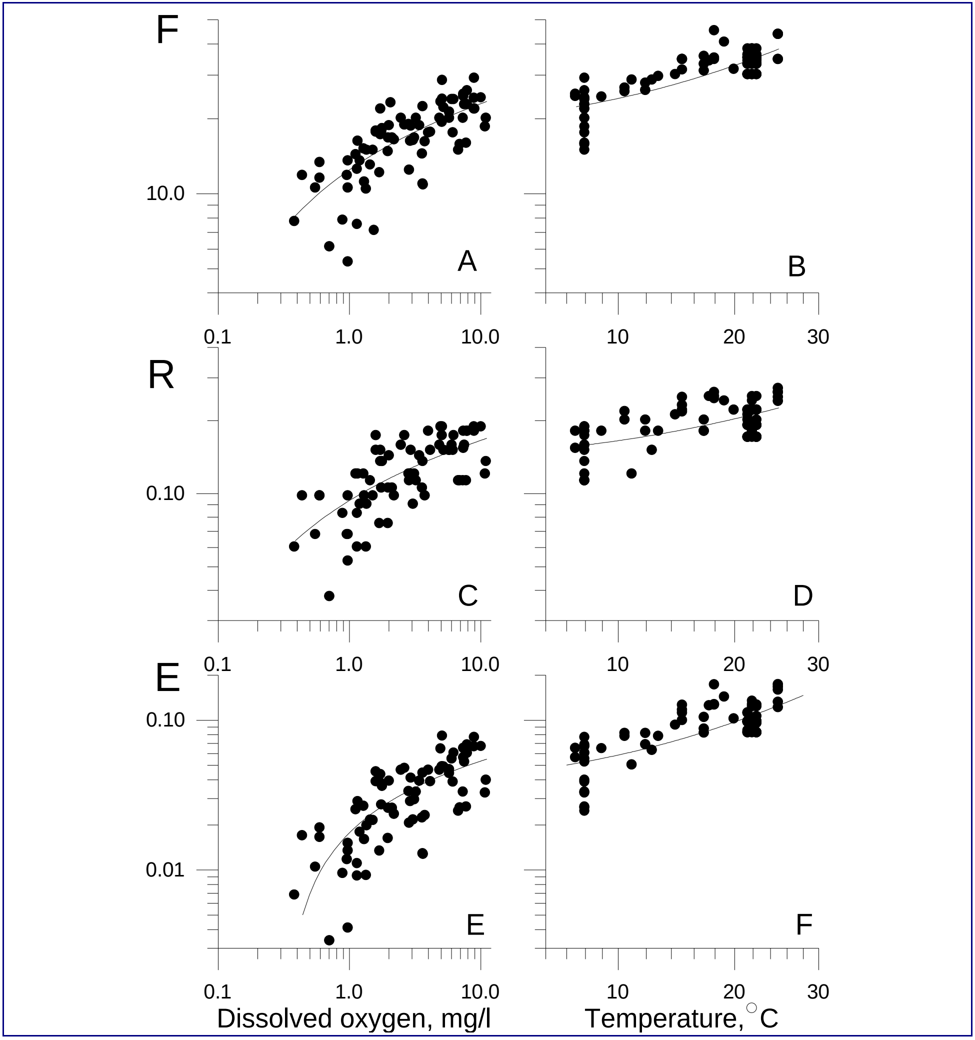

Figure 2.5. The relationship between frequency (F, Hz), drag force (R, dyne) and mechanical power (E, μg O_2 mg $W^{-1}h^{-1}$) of the mouth appendage locomotion in *C. euxinus* and oxygen concentration (A, C, E) and water temperature (B, D, F)

When oxygen concentration diminishes, the frequency (F), drag force (R) and maximum mechanical energy (E) of mouth appendage locomotion significantly decline (2.0-4.5 times, Fig. 2.5A,C,E). In summary, due to the combined effects of temperature and oxygen concentration during vertical migration F, R and E in *C. euxinus* decrease 3.4, 2.8 and 9.55 times, respectively.

Due to the absence of pronounced behavioural compensatory reactions to temperature and oxygen concentration, *C. euxinus* should be considered as an animal acclimated to a variable environment (Galkovskaya and Suschenya, 1978).

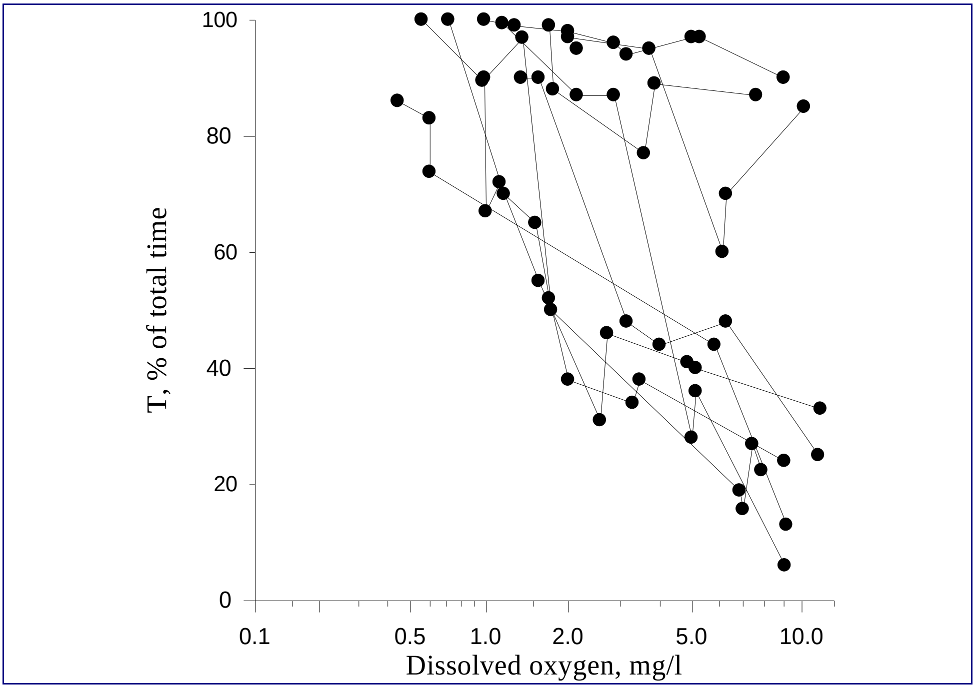

Figure 2.6. The relationship between time spent for swimming (% of total time) and oxygen concentration

In contrast to the parameters of locomotion determined by the characteristics of muscle contraction, the time spent swimming (T, %) does not depend on water temperature. Under normal oxic conditions, T varied widely from 15-20 to 90-95%. However, when oxygen concentration diminished to values of *Calanus* daytime habitat conditions at depth (0.8-1.15 mg O_2 l^{-1}), T of all investigated animals increased to 80-100%, independently of the duration of locomotion at the beginning of the experiment (Fig. 2.6). Copepods can maintain such nearly continuous mouth appendage locomotion for up to an hour. A similar relationship between time spent swimming in *C. euxinus* and oxygen concentration at the habitat depth was revealed by Mutlu (2003) on the basis of acoustic data obtained using an echosounder at 120 and 200 kHz. However, under oxygen concentration of below 0.5 mg O_2 l^{-1}, migrating *C. euxinus* became torpid.

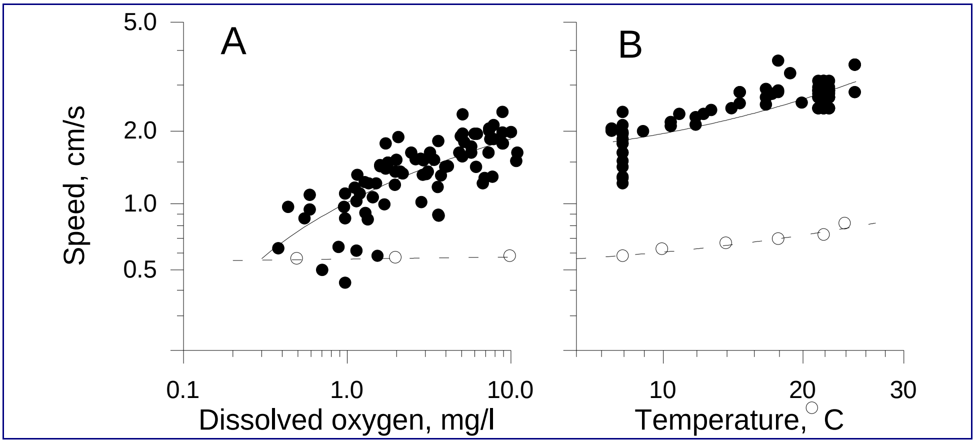

Figure 2.7. The effect of oxygen concentration (A) and temperature (B) on maximum speed of routine swimming (•) and gravity sinking speed (o) in *Calanus euxinus* females

In the Black Sea, the layers where *Calanus* aggregate during daytime lie quite near the anoxic and sulphide zones. Therefore, a dramatic decrease in the ability to swim within cold hypoxic layers is dangerous for *Calanus* females, especially since they have a low lipid content (not exceeding 7% of body weight, see Yuneva *et al*. 1997a), and a body density of about 1.06 g cm^{-3} (Stepanov and Svetlichny, 1981) which is much higher than the surrounding water density (1.015 g cm^{-3}). Fig. 2.7 shows the relationships between the calculated maximum speed of routine swimming, the speed of passive sinking of animals with a body length of 0.35 cm, temperature and oxygen concentration. In aerated water at 22±2 °C the speed of constant swimming of females is equal to

2.8 cm s^{-1}, being 2.5 times higher than the speed of passive sinking. Close results (2.7-2.8 cm s^{-1}) were obtained by filming of *Calanus* routine swimming under similar laboratory conditions (Svetlichny, 1987; Minkina, 1983).

At low temperature and under hypoxia, the speeds of active swimming (0.7 cm s^{-1}) and passive sinking (0.54 cm s^{-1}) are close. Consequently, the increase in time spent swimming at oxygen concentration of about 1 mg O_2 l^{-1} is a behavioural compensative reaction preventing the animals from descending into anoxic layers. Copepods aggregating near the aerobic zone boundary have to maintain a low level of constant locomotor activity. In one experiment lasting up to 90 min, under hypoxia (1.4 mg O_2 l^{-1}) at 8°C, *Calanus* females exhibited constant oscillations of mouthpart appendages with the frequency of 12.9 Hz (cv = 2.7%). During this time, there were only 5 locomotory thrusts of thoracic legs with a mean duration of 0.017 s. Similar behaviour of *C. euxinus* aggregating during daytime at depth was observed from a submersible by Flint (1989). The energy cost of such an active mode of staying beneath the oxycline is close to a half of the *Calanus* basal metabolic rate in surface layers.

2.3.2 Weight-specific ammonia excretion rate in migrating *C. euxinus* and the role of proteins as metabolic substrates under hypoxic conditions

In contrast to total energy metabolism, the energy of the protein catabolism in *C. euxinus* did not depend upon oxygen concentration. In active CV the ammonia excretion rate varied little between 0.04±0.022 μg N mg^{-1} h^{-1} in saturated water and 0.027±0.005 μg N mg^{-1} h^{-1} under hypoxic conditions. These values are consistent with the results of Harris and Malej (1986) who found that ammonia excretion rate of fed *C. helgolandicus* ranged from 0.027 to 0.076 μg N mg^{-1} h^{-1} at 15^{o}C. Making up about 60% of total energy metabolism under normal oxygen conditions, at 0.8 mg O_2 l^{-1} energy protein catabolism exceeded the respired energy by 1.5 times (Fig. 2.8). These data indicate not only that protein is utilised under hypoxic conditios, but also the great contribution of anaerobic protein catabolism to total metabolism.

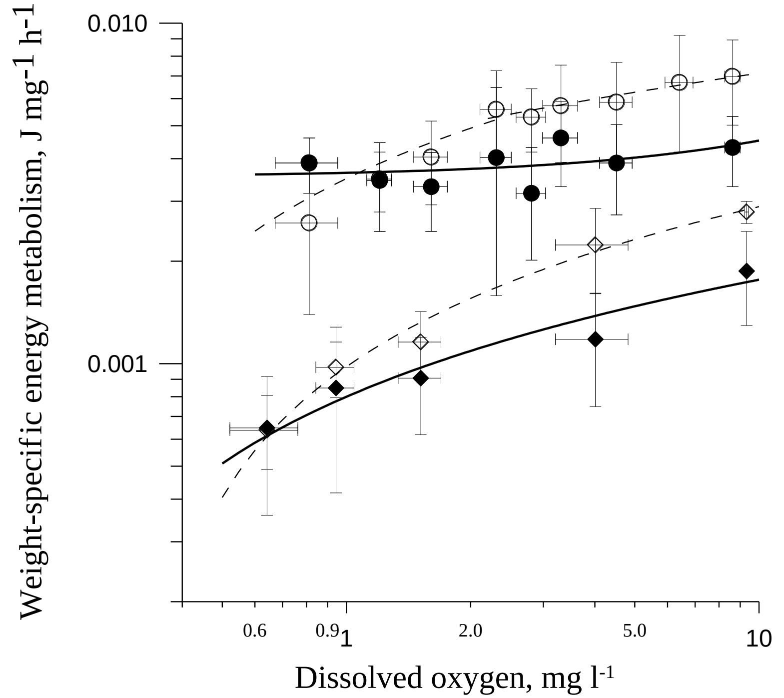

Figure 2.8. The effect of oxygen concentration on the total (○) and basal (◊) energy metabolism in migrating *C. euxinus* calculated from the weight-specific respiration rate and total energy of protein catabolism of active (●) and narcotized (♦) individuals calculated from the weight-specific ammonia excretion rate

In narcotised individuals from migrating group, the protein catabolism reduced proportionally to oxygen concentration. The energy generated in this course of catabolic process increased from 68 to 100% of respired energy (Fig. 2.8). At a minimum oxygen concentration of 0.6-0.8 mg O_2 l^{-1} the ratio between the total (including energy of anoxic protein catabolism) and basal metabolism amounted to 6.5, reaching a maximum metabolic range for this species (see Section 2.1.).

2.3.3 Metabolism and behavior of diapausing CV

The rates of metabolism in diapausing and active copepods of the *Calanus* genus have been compared in numerous studies (Hirche, 1983, 1996; Bamstedt and Tande, 1985; Ingvarsdóttir *et al*., 1988; Smith, 1988; Drits *et al*., 1993, 1994; Arashkevich *et al*., 1996; Svetlichny *et al*., 1998). It was shown that the entry of copepods into diapause is accompanied by a pronounced decrease (by a factor of 2-4) in their respiration and excretion rates. However, these data concern mainly copepods living under oxygenated conditions throughout their life cycle.

In the Black Sea *C. euxinus* diapausing copepodites undergo long-term hypoxia which results in specific changes in their metabolism. A two-fold decrease (approximately) in total metabolism (in comparison with migrating CV) was observed in diapausing CV (CV_d) at different oxygen concentrations, accounting for 42-45% of total metabolism; basal metabolism in CV_d varied in the same way. In oxygen-deficient water (about 1mg O_2 l^{-1}), values of basal metabolism in migrating CV (CV_m) and CV_d were close, whereas under oxygenated conditions the metabolism of narcotised CV_d was 1.5-fold lower than that of CV_m. However, more pronounced differences between these ecological groups were found after studying their protein catabolism. At oxygen concentration of 0.8 mg l^{-1} the ammonia excretion rate in untreated CV_d was equal to 0.0066 μg N mg^{-1} h^{-1} (4.1 times lower than in CV_m), and decreased to 0.0026 μg N mg^{-1} h^{-1} at *ca*. 0.3 mg O_2 l^{-1}. The average O:N ratio in CV_d at hypoxia is equal to 26.4 (Svetlichny *et al*., 1998), indicating a mixed protein-lipid type of catabolism. The amount of oxygen available in the water is probably sufficient for aerobic utilisation of these metabolic substrates due to the lower energy requirements of *Calanus* under hypoxic conditions.

The measured respiration rate of CV_d *C. euxinus* at low oxygen concentration and 8 °C (0.08 μgO_2 $mgWW^{-1}$ h^{-1}, or 0.28 μlO_2 $mgDW^{-1}$ $h^{-1)}$ is similar to that reported by Hirche (1983) for non-active *C. finmarchicus* (0.1- 0.4 μlO_2 $mgDW^{-1}$ h^{-1}), and fits into the range (0.08-

0.35μlO_2 mgDW^{-1} h^{-1}) reported by Ingvarsdottir *et al*. (1999) for *C. finmarchicus* overwintering at a very low temperature (~0 °C).

The ammonia excretion rate in CV_d of *C. euxinus* can be compared with that of *Calanus* starved for a long time at low temperature. In *C. plumchrus* fasting at 10 °C the ammonia excretion rate decreased after 49 days to 0.006 μg N mgWW^{-1} h^{-1} (Ikeda, 1977). In copepodites V of *C. finmarchicus* starved for 34 days at 5 °C the ammonia excretion rate diminished from 0.23 to 0.07 μg N ind^{-1} day^{-1} (Mayzaud, 1976), or approximately from 0.013 to 0.006 μg N mgWW^{-1} h^{-1}.

Low levels of metabolism in CV_d correspond with low parameters of locomotion activity. The experiments above demonstrate a significant relationship between the time spent swimming (T%) and oxygen concentration. The frequency of mouth appendage beat patterns (F) in CV_d was 1.5-1.7 times lower than that of CV_a at similar oxygen concentrations. At oxygen concentration of 0.4 mg O_2 l^{-1} the mean values of F and T in CV_d amounted to 7.6 Hz and ~50%, respectively. However, this level of activity is enough to regulate habitat depth (Svetlichny *et al*., 1998). Direct observations (Flint, 1989) have shown that in the Black Sea *C. euxinus* copepodites in such a “resting phase” can, in fact, maintain significant activity.

2.3.4 Cost of diel vertical migrations

Questions concerning the energetic cost, mechanism and speed of vertical migrations in copepods were widely discussed in the literature (Vlymen, 1970; Enright, 1977; Corkett, McLaren, 1978; Vinberg, 1982; Raymont, 1983; Mauchline, 1998). Petipa (1981) estimated that the cost of *Calanus* vertical migration with an average velocity of 12 cm s^{-1} increased daily energy losses by a factor of 6 to 13. Klyashtorin (1984) calculated that energy expenditure during migration constituted 40-60 % of daily total metabolism. Rudyakov (1986) and Geptner (1996) concluded that copepods might passively descend without energy losses.

Swimming trajectories of *C. euxinus* during vertical migration were studied by a ship-borne acoustic Doppler current profiler with a 150 kHz transducer (Erkan and Gucu, 1998). Using these data, Svetlichny *et al.* (2000) showed that in this species the speed of active swimming was 2.8 cm s^{-1} in aerated water and the speed of passive sinking amounted to 0.54 cm s^{-1} under hypoxia. Using an echosounder at 120 and 200 kHz, Mutlu (2003) showed that downward migration of *C. euxinus* took 2 h, starting ~1 h before and ending ~1 h after sunrise. According to his observations, upward migration was completed in 3.5 h, starting 2.5 h before and ending 1 h after sunset. The swimming speed of *C. euxinus* during migration ranged from passive sinking speed of 0.57 cm s^{-1} within the suboxic zone to active swimming speed of 2–3 cm s^{-1} (upwards) and 2.7 cm s^{-1} (downward) through well-oxygenated water. Similar migration speeds (0.94 and 2.8 cm s^{-1}) for this species were obtained by a series of vertical tows (Besiktepe, 2001).

By our calculations, confirmed by the observation of descending of narcotised individuals (Stepanov and Svetlichny, 1981), the speed of passive sinking in *Calanus* females with a body density of 1.06 g cm^{-3} decreased from 0.75 cm s^{-1} at 24 ºC to 0.5 cm s^{-1} at 7.5 ºC. In view of the temperature and water density changes that occur during migration, an average value would be equal to 0.55 cm s^{-1}. Therefore, during 2.0 h of morning downward movement, animals can passively descend only for 30 m whilst the depth of *Calanus* daytime habitat reaches 180 or even 240 m (Vinogradov *et al.*, 1992b). Consequently, animals seem to migrate actively to deep layers. Taking into account sharp decrease in jerk locomotion with the reductions in oxygen concentration and temperature, copepods appear to reach such depths using mainly mouth appendages for locomotion.

According to the results of Erkan and Gucu (1998), in July 1997 the lower boundary of the acoustic density maximum indicating the presence of migrating *C. euxinus* aggregations was found at a depth of about 142 m, where the oxygen concentration amounted to 0.6 mg l^{-1}. Since at night the core of the acoustic density maximum relates to the upper boundary of thermocline (about 25 m), the average distance of migration may be estimated as 117 m. The migration downwards takes

2.0 h and the movement upwards lasts for 3.5 h, therefore, the mean speeds of descent and ascent are equal to 1.63 and 1.08 cm s^{-1}, respectively.

We may calculate the energy cost of diel vertical migration of *C. euxinus* based on the observed experimental relationship between metabolic rate, locomotory activity, water temperature and oxygen concentration (see Section 2.3.1), in accordance with their vertical profiles in the investigated regions (Fig. 2.9).

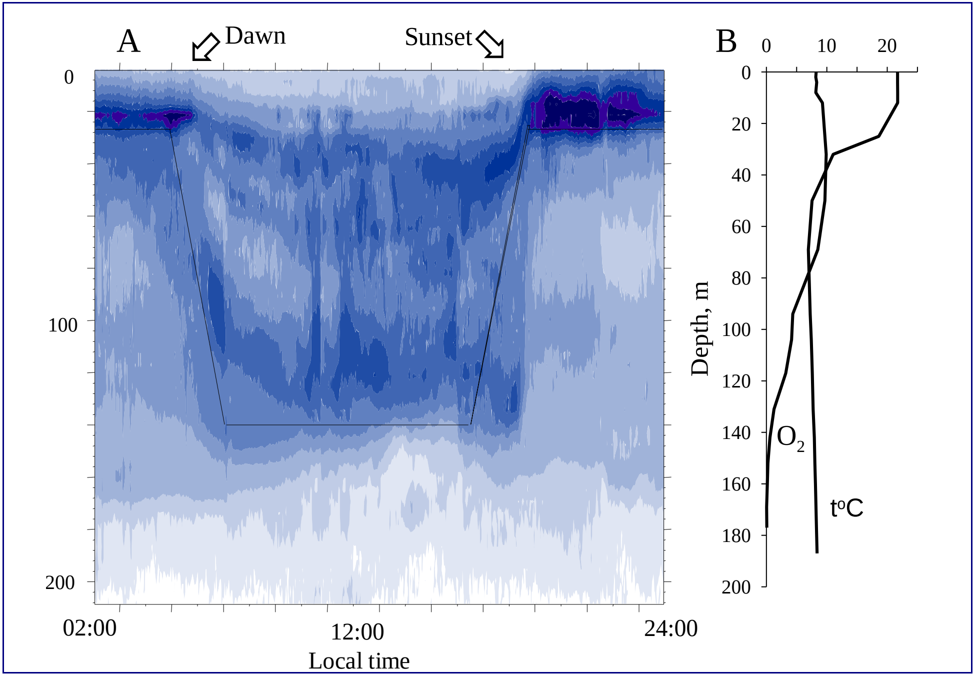

Figure 2.9. Diel dynamics of the scattering layer (A) and vertical profiles (B) of temperature (^{0}C) and oxygen concentration (O_2) in the southern Black Sea in September 1996. The solid line in (A) shows the migration path of *C. euxinus* (After Svetlichny *et al*., 2000)

To simplify the calculation, we have made the following assumptions:

The speed of *Calanus* descent is expressed as $v_d = v_a + v_g$, the speed of the ascent is defined as $v_u = v_a - v_g$, where v_a is the maximum swimming speed by mouth appendages; and v_g is the speed of passive (gravity) sinking (cm s^{-1}). We can assume that $v_a = FH$, where F is the frequency of locomotor acts depending upon temperature and oxygen concentration; H is the empirical value of the distance of a single locomotor "step" of *Calanus* being equal to 0.26 L_{pr} (Svetlichny, 1993).

1. The total duration of migration is equal to the sum of time intervals necessary for copepods to pass through the sequence of 10-m layers with corresponding mean values of temperature, oxygen concentration and conditional water density.

2. The total metabolism (Q_{tot}) during migration is a combination of basal metabolism (Q_b, see Section 2.3.1) and scope of activity (Q_a) in accordance with temperature, oxygen concentration and the duration of *Calanus* residence in the above mentioned 10-m layers. This can be expressed as $Q_{tot} = Q_b + Q_a$. Q_a can be calculated as $Q_a = E\ k^{-1}$, where: E is the mechanical energy of locomotion (see Section 2.1.1); and k is the coefficient of muscle contraction efficiency, 0.1 (Svetlichny, 1996).

3. The copepods start migrating downward at 5:00 h and begin to ascend at 15:30 h. Our calculation shows that copepods could take 1.6 h to descend from 25 m to 142 m, with a mean speed of 2.03 cm s^{-1}. This is close to the value of 1.67 cm s^{-1} recorded using acoustic profiling equipment. The duration of ascent (3.47 h) and its mean speed (0.94 cm s^{-1}) are also in agreement with those calculated from acoustic observations data (for more details see Svetlichny *et al.*, 2000).

Our calculation shows that net energy required for locomotion during downward and upward migration is equal to 2.07 μg O_2 ind^{-1} day^{-1}, or 11.6% of diel energy losses (17.87 μg O_2 ind^{-1} day^{-1}). *C. euxinus* loses the main part of its energy (78.6%) in the surface oxygen-saturated stratum, at a temperature of 18 ºC for 10.03 h, while they expend only 5.4% of diel energy losses while staying in hypoxic layers at a temperature of 8 ºC for 8.9 h. This phenomenon is due to a considerable (7.7 times) decrease in the metabolic rate of copepods living in deep layers. Therefore, vertical migrations of *C. euxinus* to cold oxygen-deficient zone can provide a substantial benefit, allowing the animals to conserve the energy reserved from feeding in the surface sea layers.

2.4 Life cycle strategies of *Calanus euxinus* development and lipid accumulation

Experiments described above have shown that *C. euxinus* migrating daily from weel-oxygenated surface layers to the cold oxygen-minimum zone (OMZ) significantly decrease their metabolic rate (Svetlichny *et al.*, 2000). Therefore, we hypothesise that due to diel migrations *Calanus* is able to reduce its energy expenditure and increase the proportion of energy from food consumed near the sea surface for growth and lipid accumulation.

To test this hypothesis, we conducted comparative studies of *C. euxinus* from deep regions within the OMZ and from shallow zones of the Black Sea (with a depth of less than 100 m where the oxygen concentration is high even near the bottom).

Zooplankton samples were collected with a closing Nansen net (opening diameter 71 cm, mesh size 112 μm) during cruises of the R/V “Bilim”, “Knorr” and “Vladimir Parshin” in 1996 - 2005 in the deep south and south-western regions of the Black Sea. During daytime, zooplankton was sampled by vertical hauls from a depth with σ_t=16.2 to the surface. At night depth-stratified sampling was performed from the depth below the oxycline with $15.8<\sigma_t<16.2$ to collect diapausing (non-migrating) CV, and in the upper layers to catch migrating *C. euxinus*.

The samples were immediately preserved with 4% borax-buffered formaldehyde. In the laboratory eac sample was sub-divided and the number of specimens of each copepodite stage of *C. euxinus* counted in every sub-sample in a Bogorov chamber under a dissecting microscope. When possible, 30-40 (up to 190) individuals of every copepodites stage or adults were selected for the measurements of body size and oil sac volume, and not less than 8 (up to 67) individuals of each jaw phase were identified in CIII – CV according to tooth formation inside the gnathobases of mandibles. Morphological examination of mandibular gnathobases was performed under a light microscope. The left mandible was dissected with needles, transferred to a drop of glycerine on a microscope slide, covered with a cover slip and examined at a magnification of x150. The jaw phase (postmoult, late post-moult, intermoult, early premoult and premoult) was determined under a microscope at a magnification of x150 using morphological characteristics defined by Miller and Nielsen (1988), Miller *et al*. (1991) modified by Marker *et al*. (2003) and Arashkevich *et al*. (2004) for *C. finmarchicus*, and Johnson (2004) for *C. pacificus*. The examination of gonads in adults females was undertaken according to Niehoff and Hirche (1996) and Niehoff and Runge (2003).

Body volume (V_b, mm^3) was calculated as $V_b = kL_{pr}\, d_{pr}^2$, where k is the empirical coefficient of 0.64 in males, and 0.58 in females and copepodites (Svetlichny, 1983 a). The oil sac volume (V_s, mm^3) was determined as the ellipsoid volume: $V_s=\pi/6L_s\, d_s^2$.

Wet weight of *C. euxinus* copepodites and females was calculated as $WW=V_b\, \rho$, where ρ (g cm^{-3})' the average body density $=1.06-0.0016\, SV_s$ (Svetlichny *et al*., 1998), in which SV_s (%) is the proportion of body volume occupied by the oil sac.

2.4.1 Seasonality in abundance, size/age structure, moulting patterns and lipid accumulation in *Calanus euxinus* in the deep zones of the Black Sea

Very few studies have been undertaken so far on the seasonal dynamics of abundance and size characteristics in developmental stages of *C. euxinus*. Sazhina (1987) analysed the abundance of *C. euxinus* developmental stages near Sevastopol and suggested that this species could produce up to 8 generations per year. Nevertheless, one can distinguish two main developmental patterns for this species (see Sazhina, 1987, Fig. 34).

According to Vinogradov *et al*. (1992b), the biomass of *C. euxinus*, amounting to about 5.0 g m^{-2} in winter and spring 1988, was significantly higher in summer 1978 (7.9 g m^{-2}). In August 1993 CV and adults dominated in net zooplankton and their biomass ranged from 0.6 to 5.5 g m^{-2} (Vinogradov *et al*., 1995).

Current studies (Fig. 2.10) showed minimum total abundance (5500 ind m^{-2}), and especially in numbers of early copepodite stages of *C. euxinus*, to occur during the summer phytoplankton depression period. During other periods the abundance of copepodites and adults varied between 12100 and 23990 ind m^{-2} (with the maximum value in February 1999), whilst biomass values ranged from 6.6 to 12.6 g m^{-2}.

During the winter-spring period, average prosome length increased from 0.69±0.05 in CI to 2.67±0.06 mm in females and 2.52±0.08 mm in males (Table 2.1). During summer-autumn period in CIII, CIV and females prosome length was significantly ($p < 0.001$) 3.7-5.1% higher. In other stages, prosome length during warm and cold periods did not differ significantly. This may be the result of *C. euxinus* development in the Black Sea Rim Current zone, in layers with low temperature throughout the year.

The content of lipids in the oil sac varied greatly with the developmental stage of *Calanus*. Analysis of oil sac volume of CIII - CVI showed that large lipid reserves were formed in CV (Table 2.1**.** In comparison with CIII and CIV, the mean oil sac volume (V_s, % of body volume) of CV increased dramatically - 8-17 times - amounting to 15.7-17.4% of body volume on average. In females using lipids for gonad formation V_s is occupied 6.8-7.9% of body volume, whereas in males the proportion reached 12.2-15.8%. Though seasonal differences in average oil sac volume were not significant, the upper limit of V_s estimated using 10 extremely lipid-rich CV collected during winter-spring phytoplankton-rich period in the Black Sea made up 31.2±4.6% of body volume. This was significantly higher ($p<0.001$) than the average V_s value in samples collected during the summer phytoplankton depression period (26.3±3.1%).

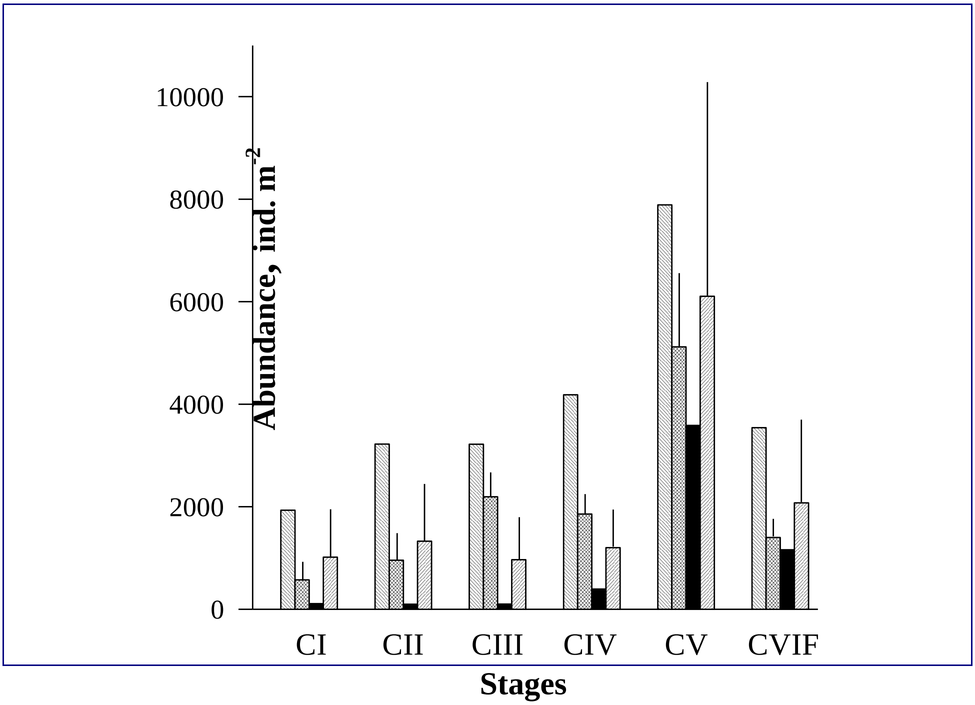

Figure 2.10. Copepodite stage abundance in *Calanus euxinus* population in the southern and south-western Black Sea during winter (▧, February 1999), spring (▩ , April 2003), summer (▨, June 2000) and autumn (■ , October 2005)

According to the results presented, *C. euxinus* CV constituted a major part of the population, doubling from 33% in February to 66% in July (Fig. 2.10). The CV population inhabiting the main Black Sea body consisted predominantly (40-70%) of postmoults throughout the year (Fig. 2.11A). Among CV staying at depth during night, postmoults constituted 93±6% of the population.

Table 2.1. Prosome length (L_{pr}) from copepodites I (CI) to adult females (CVIF) and males (CVIM) and oil sac volume (V_s, % of body volume) in *Calanus euxinus* collected in the south-western part of the Black Sea during 1996 – 2005

Season	CI	CII	CIII		CIV		CV		CVIF		CVIM	
	L_{pr}, mm	L_{pr}, mm	L_{pr}, mm	V_s, %	L_{pr}, mm	V_s, %	L_{pr}, mm	V_s, %	L_{pr}, mm	V_s, %	L_{pr}, mm	V_s, %
Winter-Spring	0.69 ±0.05	0.94 ±0.05	1.36 ±0.04	1.1 ±1.0	1.78 ±0.06	2.1 ±2.0	2.28 ±0.09	17.4 ±7.2	2.67 ±0.06	6.8 ±4.1	2.52 ±0.08	12.2 ±5.2
Summer-Autumn	0.71 ±0.01	0.97 ±0.03	1.29 ±0.04	1.3 ±1.0	1.7 ±0.07	2.1 ±1.0	2.20 ±0.10	15.7 ±5.3	2.57 ±0.09	7.9 ±3.5	2.51 ±0.06	15.8 ±7.4

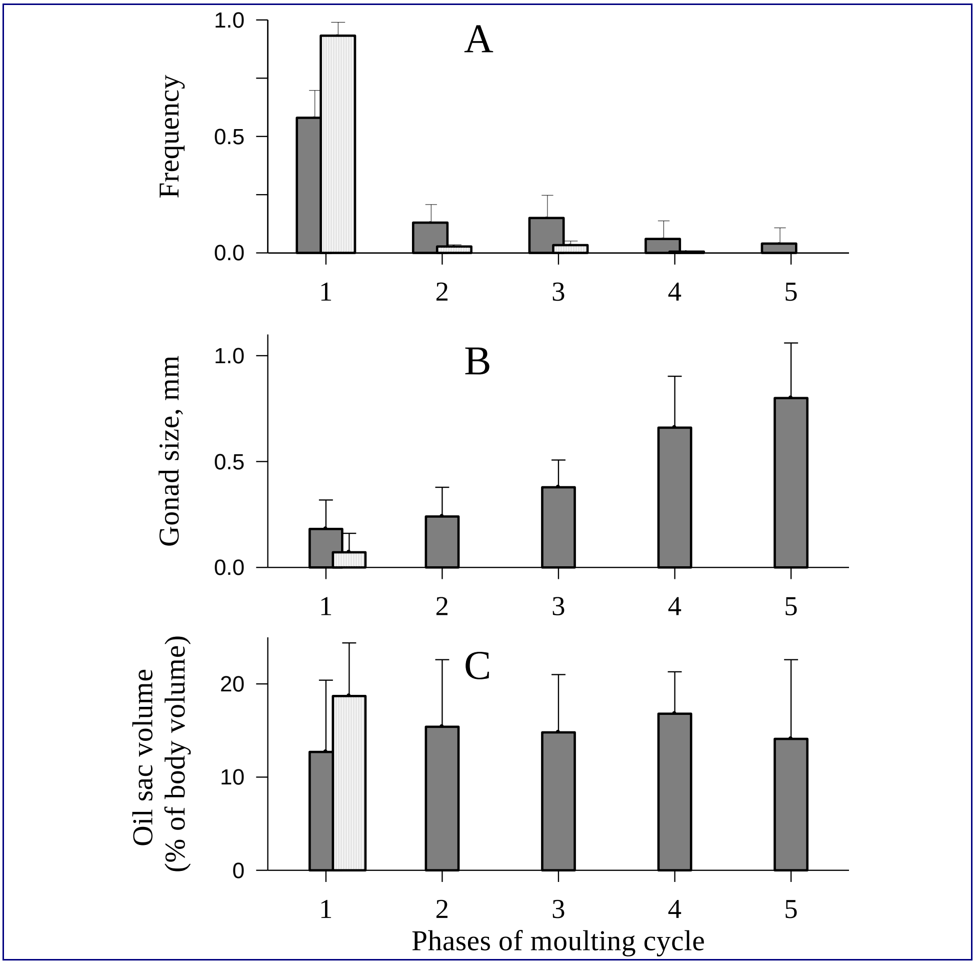

Figure 2.11. Frequency distribution of moulting cycle phases (A), gonad size (B) and oil sac volume (C) in deep-living migrating (■) and diapausing (□) V copepodites of *Calanus euxinus* from deep stations in the south-western region of the Black Sea (x±SD)

Gonads of CV postmoults were extremely small (Fig. 2.11B) or absent (especially in diapausing animals). There is a strong correlation (r = 0.78, n = 278) between the state of gonads and new teeth formation phase of CV in Black Sea *C. euxinus*, just as in *C. finmarchicus* from the Norwegian shelf (Arashkevich *et al*., 2004), Mean oil sac volume (Fig.

2.11C) reached a maximum value (18.7±5.7%) in diapausing CV. Consequently, vigorous lipid accumulation in the oil sac took place in postmoult CV before entering diapause. In July 2000 V_s was less than 0.08 mm^3 in about a half of postmoult CV, whilst in diapausing CV it accounted for 0.06-0.24 mm^3 (Fig. 2.12).

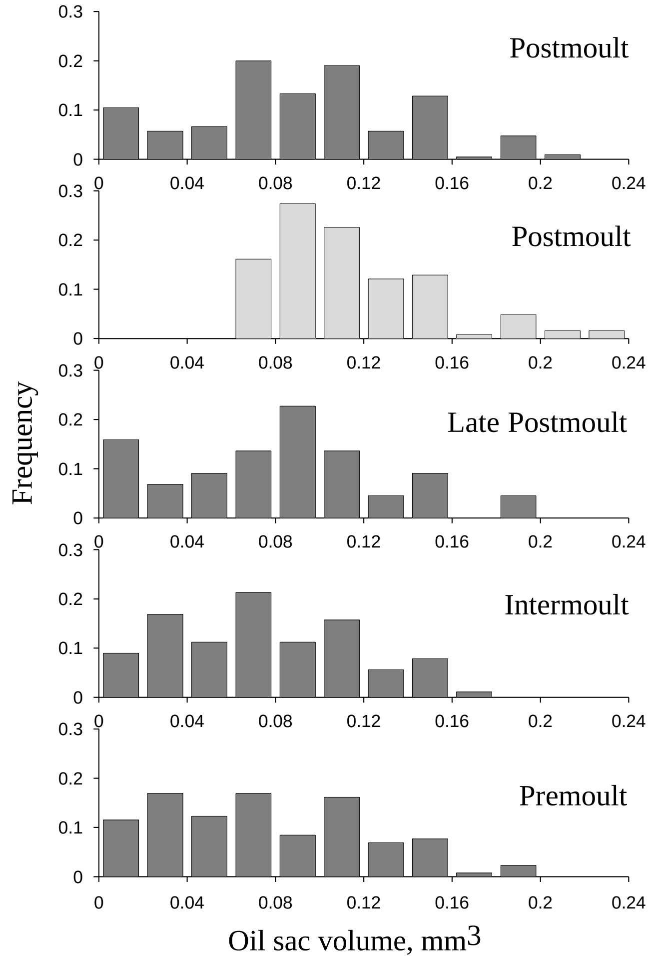

Figure 2.12. Frequency distribution of oil sac volume in diapausing (□) and migrating (■) CV of *Calanus euxinus* at different moulting cycle phases

After accumulation of large lipid amounts, postmoult CV stop migrating, enter diapause and aggregate near the lower boundary of the OMZ. In April 2003 the roportion of CV remaining at depth during night was about 9.3%, but in July 2000 this varied from 13 to 25% of the total. According to Vinogradov *et al.* (1992b), the proportion of diapausing CV in *C. euxinus* increased from spring to autumn constituting 30-70%. Arashkevich *et al.* (2004) reported that *C. finmarchicus* from depths below 500 m were in the postmoult phase, with undeveloped gonads, during the long overwintering period (from October to March).

The termination of diapause in *Calanus* is related to progression of the moulting process and gonadogenesis. According to Tande and Hopkins (1981), from February to April, 90-100% of CV from the overwintering stock of *C. finmarchicus* possessed large advanced gonads.

Irigoien (2004) suggested that the amounts of lipid reserves determine the overwintering depth when the buoyancy of *C. finmarchicus* is neutral. At 8 °C neutral buoyancy is likely to occur in *C. euxinus* CV with lipid reserves of 27% of body volume, or 23% of body weight (Svetlichny *et al.*, 1998). These values are close to the results of the experiments with *C. finmarchicus* (Vesser and Jónasdóttir, 1999). However, Campbell (2004) reported that “something approximating neutral buoyancy is only attainable in a very narrow window of lipid compositions”. In fact, in September - October 1996 (Svetlichny *at al.*, 1998) and in July 2000 there was a significant number of individuals among diapausing CV of *C. euxinus* with lower (about 10%) and higher (up to 40%) lipid content. This variability transferred into corresponding speeds of passive sinking (0.3 cm s^{-1}) and ascending (0.25 cm s^{-1}). Consequently, in order to stay at constant depth, these copepodites have to make swimming movements. Ship-board experiments (Svetlichny *et al.*, 1998) showed that diapausing CV at an oxygen concentration of ~0.2 mg l^{-1} and a temperature of 8 °C (at the lower boundary of their aggregation layer) can swim enough for constantly using their mouth appendages with the speed of 0.55 cm s^{-1}. This is sufficient to allow them to maintain their position at a depth with optimal habitat conditions for diapausing

However, *C. euxinus* CV in the Black Sea may develop without diapause. A similar developmental pattern (without diapause) was observed in *C. finmarchicus* on the Georges Bank in summer (Durbin *et al.*, 2000). It can be suggested that the preponderance in the Black Sea during April and June of migrating late postmoults, intermoults and premoults (developed from postmoults which had not entered diapause) is unlikely, because diapause in *C. euxinus* CV in late spring and the middle of summer is terminated. Our data indicate that in CV developing without diapause, V_s size increases from postmoults to intermoults, with maximum values being close to those of diapausing postmoults. For example, during April 2003 the upper limit of V_s size in intermoults (0.226 ± 0.04 mm^3) did not differ significantly from that of diapausing CV (0.225 ± 0.026 mm^3). A near-maximum size of V_s (0.208 ± 0.05 mm^3) was found in February 1999 also in intermoults. In autumn 1998 and during the summer phytoplankton depression period in 2000 maxima, V_s sizes in CV intermoults amounted to 0.139 ± 0.016 mm^3 and 0.119 ± 0.026 mm^3, respectively, lower than those occurring in winter and spring.

2.4.2 Lipid accumulation and moulting patterns in *C. euxinus* from shallow zones of the Black Sea

Our previous long-term studies concerning *C. euxinus* regional distribution in the Black Sea showed that late developmental stages rarely occurred during the summer in shallow zones at depths less than 100 m. However, in July 2000, at a 75 m-deep station near Sinop Harbor, large aggregation of *C. euxinus* were found (Fig. 2.13A) where females were more abundant (2073.6 ind m^{-2}) than in the deep-sea (300 m) station (1160.5 ind m^{-2}). Probably, this *Calanus* aggregation in Sinop Harbor formed due to an anticyclonic gyre branching off from the main Black Sea Current. Vertical profiles of relative fluorescence obtained at these stations (Fig. 2.13B) indicated that phytoplankton concentration near the coast was very similar to that in the offshore region. Nevertheless, the oil sac volume of CV and females from the coastal zone was 3.0 times lower ($p<0.001$) than *C. euxinus* from the deep sea station (Fig. 2.13C). The frequency distribution spectra of moulting phases in CIII from the two regions were similar, but intermoults and premoults dominated in CIV and especially in CV from shallow water (Fig. 2.13D). The same tendency, i.e. the increase in the share of intermoults and premoults of CV and reduction of their lipid content while approaching the coast, was observed near Sevastopol in September 2002 (Svetlichny and Kideys, 2005). We suggest that hypoxia may inhibit the development of copepodites during postmoult period, thereby allowing CV from deep regions to accumulate large amounts of lipids.

Energy model of *C. euxinus* growth during summer - autumn temperature stratification

To estimate quantitatively the effect of temperature and oxygen concentration on lipid accumulation in *C. euxinus*, a model of their diel growth (G, % of body energy content) was developed, using the equation:

$$G = (I_f\text{-}Q\text{-}U_f)100/CW,$$

where: I_f (cal ind^{-1}day^{-1}) is the ingestion rate according to the results of feeding experiments with *C. helgolandicus* (Huskin *et al.*, 2000), and assuming that both deep- and shallow-living copepods consume food only at night during an 11 h period (see Section 2.3.4) with identical ingestion rates;

Q is mean daily energy losses (Svetlichny *et al.*, 2000) calculated for deep-living *Calanus* (Q_1= 0.044 cal ind^{-1} day^{-1}) which pass through temperature (18-8°C) and oxygen (10-0.8 mgO_2 l^{-1}) gradients during a 2 h period, and spend 11 h under hypoxic conditions;

Shallow-living *Calanus* (Q_2=0.066 cal ind^{-1} day^{-1}) are assumed to undergo temperature changes only;

U_f is the energy of undigested food being equal to 0.3I_f (Conover, 1966);

CW is the body energy content, assuming that carbon content in both CV and females of *C. helgolandicus* (with prosome lengths similar to those in *C. euxinus*) constituted about 0.11 mg (Williams and Robins, 1982) and caloric value of 1 mg carbon is 9.4 cal.

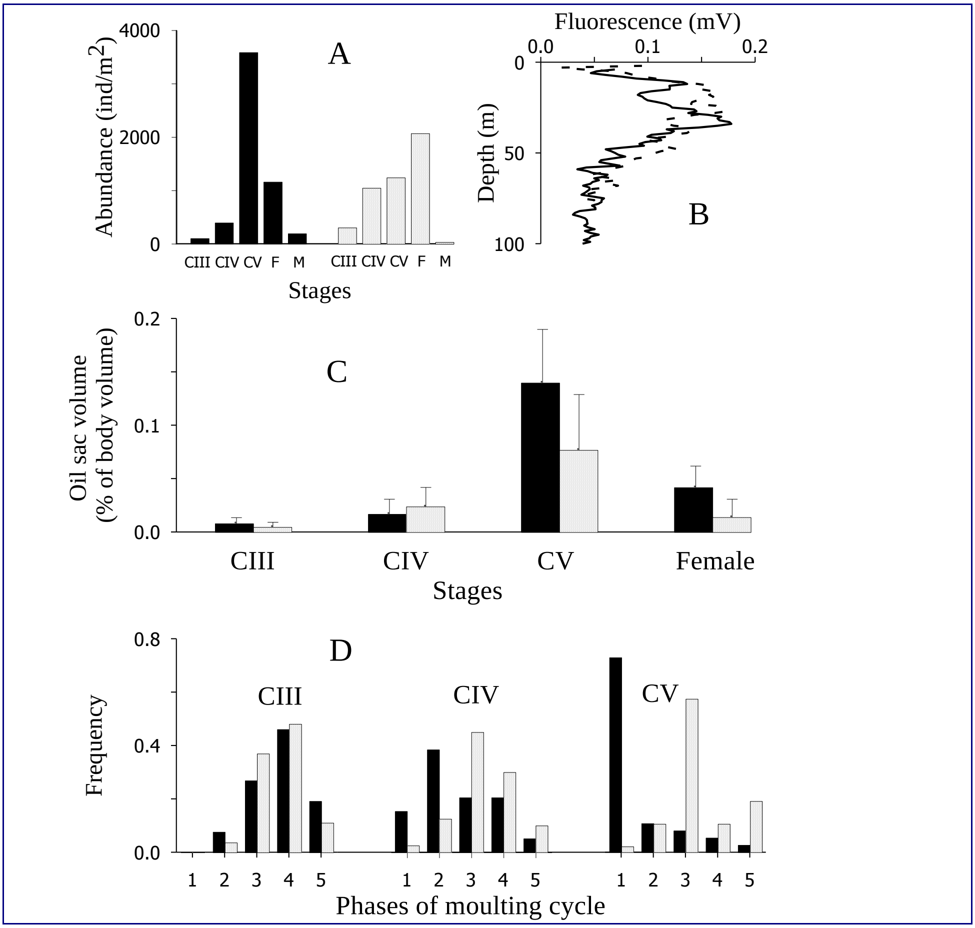

Figure 2.13. Comparative characteristics of *Calanus euxinus* from deep (■) and shallow (□) zones in Sinop region. A: Abundance of III-V copepodites (CIII-CV), females (F) and males (M); B: Vertical profiles of relative fluorescence at deep (—) and shallow (---) stations; C: Oil sac volume; D: Frequency distribution of moulting cycle phases in III-V copepodites (x±SD)

Our calculation showed (Fig. 2. 14) that the benefit of *C. euxinus* staying in hypoxic layers during daytime is insignificant when I_f exceeds 2 cal mgC^{-1} day^{-1}, or 21.3 μgC ind^{-1} day^{-1}. Such ingestion rates were determined experimentally in *C. helgolandicus* females at phytoplankton concentration more than ~300 μgC l^{-1} (Huskin *et al.*, 2000). However, at scarce rations, this advantage is more pronounced. For example, at daily ration of 1.0 cal mgC^{-1} day^{-1}, or 12 μgC ind^{-1} day^{-1}, which corresponds to food concentration of about 30 μgC l^{-1} (Fig. 2.14), shallow-living *C. euxinus* cannot grow whilst those copepods migrating to an oxygen-deficient lower layer are able to accumulate lipids up to 5% of body energy content per day. Similar growth rates (7.6%) were obtained for *C. helgolandicus* maintained in the laboratory at 15 °C and food concentration of 77.5 μgC l^{-1} (Rey-Rassat *et al.*, 2002). Only at high food concentrations (about 300 μgC l^{-1}) did the growth rate of late copepodite stages increase up to 13% (Mauchline, 1998) and even 18% per day (Rey-Rassat *et al.*, 2002).

Thus, the results of our field observations and application of our model support our hypothesis that diel vertical migrations to hypoxic layers are essential for lipid reserve formation in *C. euxinus* in the Black Sea.

However, the effect of hypoxia results not only in energy expenditure decrease, but also in the changing of metabolic pathways. According to data presented by Svetlichny *et al.* (1998), under conditions of short-term acute hypoxia, the stoichiometric O:N ratio of *C. euxinus* reduces from 20 to 8.4, indicating the transition from mixed protein-lipid catabolism to pure protein utilisation. At low oxygen concentrations, lipid catabolism is limited and protein becomes the main metabolic substrate. This may suppress somatic growth and increase the efficiency of wax ester accumulation. The biochemical aspects concerning wax ester formation under hypoxic conditions were analysed by Sargent *et al.* (1978) and Sargent and Henderson (1986).

The question we address here is how *Calanus* species living in well-oxygenated waters of the North Atlantic can accumulate lipids?

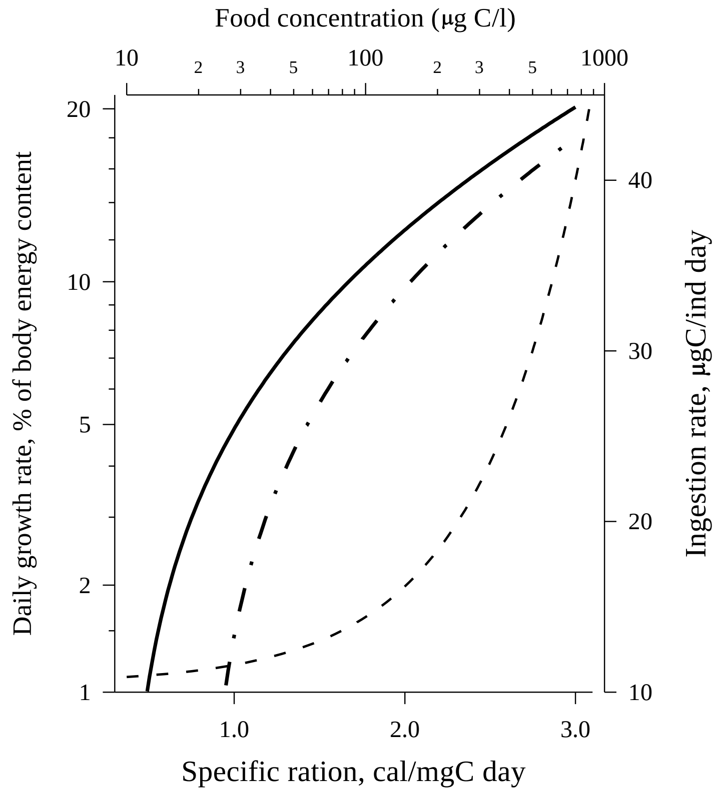

Figure 2.14. Energy model of growth for preadult and adult *Calanus euxinus* from deep (—) and shallow (— • —) regions. The relationship between daily ration and food concentration for *Calanus helgolandicus* (- - -) is based on Huskin *et al*. (2000) (see Table 3, ingestion rates at *Prorocentrum micans* concentrations of 854 and 220 µg C l^{-1})

Lipid reserve formation in *Calanus* species is a highly specialised adaptation for life at high latitudes with short and sporadic periods of food availability (Marshall and Orr, 1972). The four generations of *C. helgolandicus* off Plymouth described by Rey-Rassat *et al.* (2004) were produced either during or following a chlorophyll peak of 4-6 µg l^{-1}, corresponding to food concentration of about 200-300 µgC l^{-1}. *C. helgolandicus* off NW Spain showed a strong link between spring phytoplankton blooms (with maximum chlorophyll-*a* concentrations of up to 7.6 μg l^{-1}) and reproduction time (Ceballos *et al.*, 2004). According to Harris *et al.* (2000), as food concentrations changed from 61 to 238 μgC l^{-1} the ration of *C. helgolandicus* CV increased from 16 to 27 μgC ind^{-1} d^{-1}. At chlorophyll-*a* concentration of <3 μg l^{-1}, *C. helgolandicus* and *C. finmarchicus* sharply decreased their rates of egg production.

Campbell *et al.* (2001) observed a strong effect of food limitation on lipid storage; the critical concentrations for development and growth in *C. finmarchicus* were about 70 and 100 μgC l^{-1}, respectively. Minimum food concentration when there was little visual evidence of substantial lipid storage in *C. finmarchicus* CV was found to be 24 μgC l^{-1}, or ~0.5 μg chl *a* l^{-1}. Rey-Rassat *et al.* (2002) reported an approximate two-fold difference in V_s of *C. helgolandicus* from mesocosms with food concentrations of 77.5 and 278 μgC l^{-1}. Hygum *et al.* (2000) studied the patterns of structural growth with the production of lipid for storage by *C. finmarchicus* copepodites reared in mesocosms at different natural phytoplankton concentrations in a Norwegian fjord. The total storage lipid content in CV from mesocosms with low (0.62-0.88 μg chl *a* l^{-1}) and high (1.59-2.68 μg chl *a* l^{-1}) food resources amounted to 28.9-60.2 and 69.9-77.0 μg, respectively. Hygum *et al.* (2000) considered this maximum lipid content to be an upper threshold for the potential amount of reserve lipid. However, in the Black Sea, even during the warm period which is characterised by low phytoplankton concentrations (0.29-0.68 μg chl *a* l^{-1}), the migrating and diapausing CV of *C. euxinus* possessed total lipid contents of 36.3-115 μg and 112.6-196.6 μg, respectively (Yuneva *et al.*, 1997b).

2.4.4 Energy strategy of *C. euxinus* development during spring cold homothermy

During the period of seasonal homothermy when the temperature of aerobic layers in the Black Sea varies between 6.5 and 8 °C, hypoxia is considered to be the main factor allowing *C. euxinus* to gain energy advantage from diel vertical migrations. The ratio between daily energy losses in copepods undergoing oxygen deficiency and those in oxygenated water amounts to 1.5 under variable temperature conditions, and even 2.0 at constant temperature. Vinogradov *et al*. (1992b) reported that in February-March nearly all *C. euxinus* copepodite Vs undertake diel vertical migrations.

Nevertheless, during the phytoplankton bloom period, diel vertical migration appears not to be obligatory for *C. euxinus*. During a cruise of the R/V "Knorr" in April 2003 in the slope regions of south-western Black Sea (at maximum depths of about 900 m), the phytoplankter *Proboscia alata* was found to be abundant in samples collected from deep water. During this period 97% of CIV, 86% of CV and 89% of females and males had guts filled with green paste during daytime, above the OMZ. Probably, *P. alata* descending to deep layers allowed *Calanus* to feed all day long, without vertical migrations. Eker-Develi and Kideys (2003) also reported that in March-April 1998 *P. alata* aggregated far below the euphotic zone (56-86 m). The presence of substantial amounts of large diatoms below the euphotic zone may be due to their sinking after the winter-spring phytoplankton bloom.

The guts of the majority of CV (61%) collected within the OMZ at night also contained food. The rest of CV sampled within the OMZ, or 9.3% of total CV number had empty guts and large oil sac volumes, suggesting that the diapausing stock of this present population started to form during this period.

Crain and Miller (2001) found that food deficiency could depress moulting processes; postmoults and premoults appeared in *C. finmarchicus* population only at high phytoplankton concentrations. The great number of CIV premoults (especially in the OMZ) provides evidence of favourable nutritional conditions for *C. euxinus* in April 2003. However, within this period postmoults dominated CV with a small percentage of intermoults and premoults. Thus, despite food abundance, CV postmoults delay moulting in order to accumulate significant lipid amounts. The predominance of postmoult jaw phase could be found not only in CV preparing to diapause (Johnson, 2004), but in CV developing without diapause.

In April 2003, the mean V_s size increased from 0.008±0.007 mm^3 in postmoults to 0.109±0.066 mm^3 in late postmoults, and reached 0.165±0.054 mm^3 in CV intermoults. Consequently, during postmoult period V_s in *C. euxinus* increased nearly 14-fold. In contrast, in CIII and CIV V_s, size increased during all intermoult period (Fig. 2.15). In April 2003 reserved lipids (RL) in the oil sac and defatted dry weight (DFDW) of diapausing postmolts was 14.7 and 1.7 times higher, respectively, than those in CV just after the ecdysis (Table 2.2), indicating that lipid accumulation in postmoults was accompanied by somatic growth.

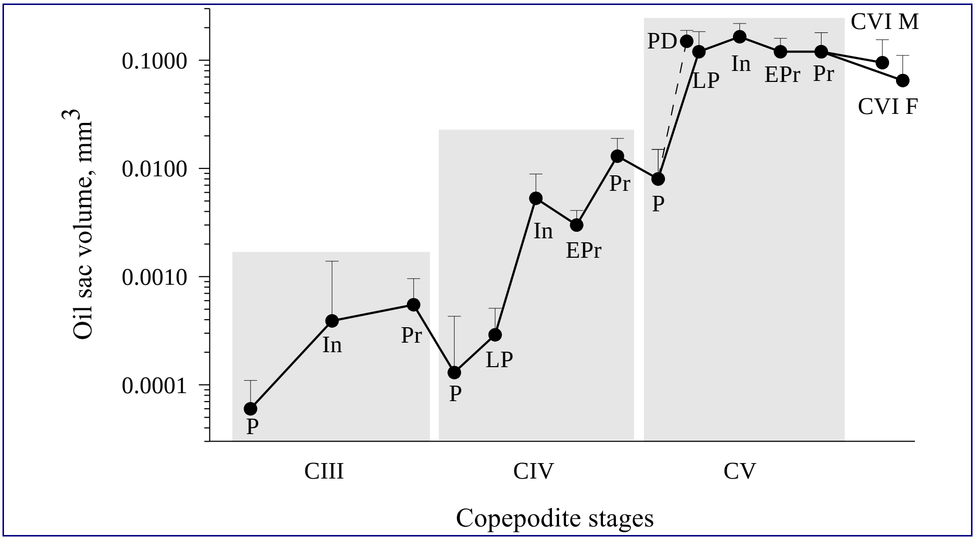

Figure 2.15. Changes in oil storage during the development of *Calanus euxinus* from CIII to female (CVIF) and males (CVIM) in April 2003. P: postmoults; PD: diapausing postmoults; LP: late postmoults; in: intermoults; EPr: early premoults; Pr: premoults

During the period of development from CI to adults in *C. euxinus* collected on 16-22 April 2003, dry weight determined as the sum of DFDW and total lipids (TL) increased (Fig. 2.16) proportionally with prosome length (L_{pr}, mm), according to the equation $DW=0.0082L_{pr}^{3.45}$. Besiktepe and Telli (2004) described close-DW relationships of in *C. euxinus* in October 2000 ($DW=0.0069L_{pr}^{3.41}$) and May 2001 ($DW=0.0062L_{pr}^{3.34}$). The regression lines for L_{pr} against DW have stronger slopes ($DW \sim L_{pr}^{3.45}$) than that for L_{pr} against V_b ($V_b=0.057L_{pr}^{2.83}$) indicating that during *C. euxinus* ontogeny DW increases with higher speed than V_b, due to the reduced water content (% of body volume) of preadults and adults.

Table 2.2. Mean values of defatted dry weight (DFDW), reserved lipids in the oil sac (RL), total lipid content (TL) and total dry weight (DW, calculated as the sum of DFDW and TL) in April 2003 expressed as carbon content (mgC ind^{-1}) in *Calanus euxinus*

Stage	Jaw phase	DFDW	RL	TL	DW
CI	Total	0.7	0	0.6	1.3
CII	Total	2.2	0	1.9	4.1
CIII	1+2	3.5	0.2	0.9	4.4
	3	3.9	0.9	2.4	6.3
	4+5	4.6	1.9	2.9	7.5
CIV	1	10.1	0.4	1.3	11.4
	2	10.3	0.7	2.9	12.2
	3	12.9	5.3	10.3	23.2
	4	14.9	8.2	14.5	29.4
	5	13.7	7.9	14.2	27.9
CV	1	31.5	7.1	12.9	44.4
	1D	48.3	104.1	137.6	185.9
	2	40.2	82.9	106.7	146.9
	3	46.2	119.1	159.2	205.4
	4	41.2	87.0	112.0	153.2
	5	41.8	82.9	106.7	148.5
CVIF	Total	66.6	35.3	48.7	115.3
CVIM	Total	48.3	104.0	137.6	185.9

1- postmoults, 1D - diapausing postmoults, 2 - late postmoults, 3 - intermoults, 4 - early premoults, 5 - premoults

The development time of *C. euxinus* is poorly studied in comparison with other species of the *C. helgolandicus* group. Sazhina (1987) found that in a laboratory experiment at 18 °C, the development time (from egg to adult) of the Black Sea *Calanus* amounted to 34 days. Stage durations were 3 days for CI and CII, 4 days for CIII, 5 days for CIV and CV.

However, there is no information available concerning growth of this species in the natural environment at 8 °C.

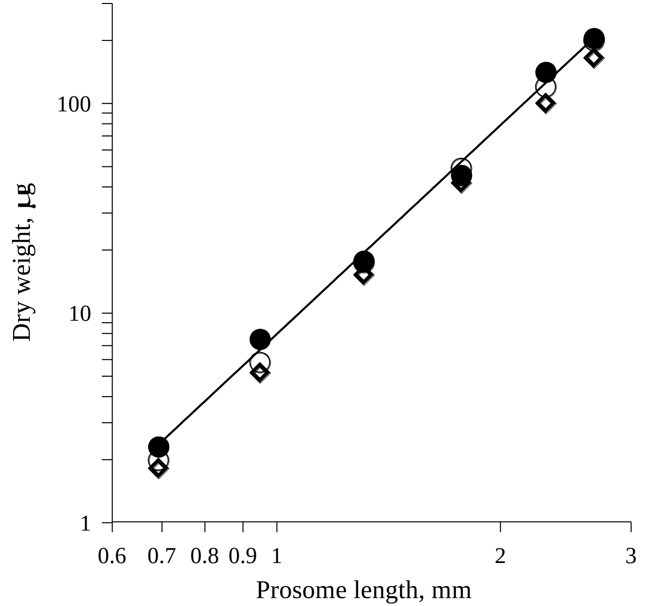

Figure 2.16. Dry weight versus prosome length in April 2003 (●) (our data), October 2000 (○) and May 2001 (◊) (Besiktepe & Telli, 2004)

Although the copepodites and adults collected in April 2003 do not represent a temporal progression of successive stages in cohort, we can estimate stage duration and total generation time of *C. euxinus.*

To assess the complete duration of copepodite development in *C. euxinus* during winter-spring homothermy at 8 °C, we calculated time dependent changes in carbon body weight (CW) of CI (1.3 µgC) through CV intermoult (205 µgC) according to the equation: $CW_n=CW_{n-1}+P_{n-1}$, where: n is the time (d^{-1}); and P is the absolute growth rate (mgC ind^{-1} d^{-1}).

P was calculated on the basis of age changes in specific growth rate (g, d^{-1}) of *C. finmarchicus* copepodites with close body size and carbon content, reared at 8 °C under a non-limiting food concentration (Campbell *et al.*, 2001, Table 5). For CI-CV of this species, specific growth rate decreased from 0.31 to 0.042 d^{-1}. Similar dynamics of specific growth rate were found in *C. sinicus* with smaller body length at 8 °C (Uye, 1988).

After plotting specific growth rate magnitudes reported by Campbell *et al*. (2001) against a scale of CW in *C. euxinus* copepodite stages, we found the correlation between g and CW, and calculated daily P as $P = CW(e^g - 1)$. Obtained absolute growth rates (Fig. 2.17) increased from 0.00047 mgC d^{-1} in CI to 0.0075 mgC d^{-1} in CV intermoults, following the equation $P = 0.017CW^{0.53}$.

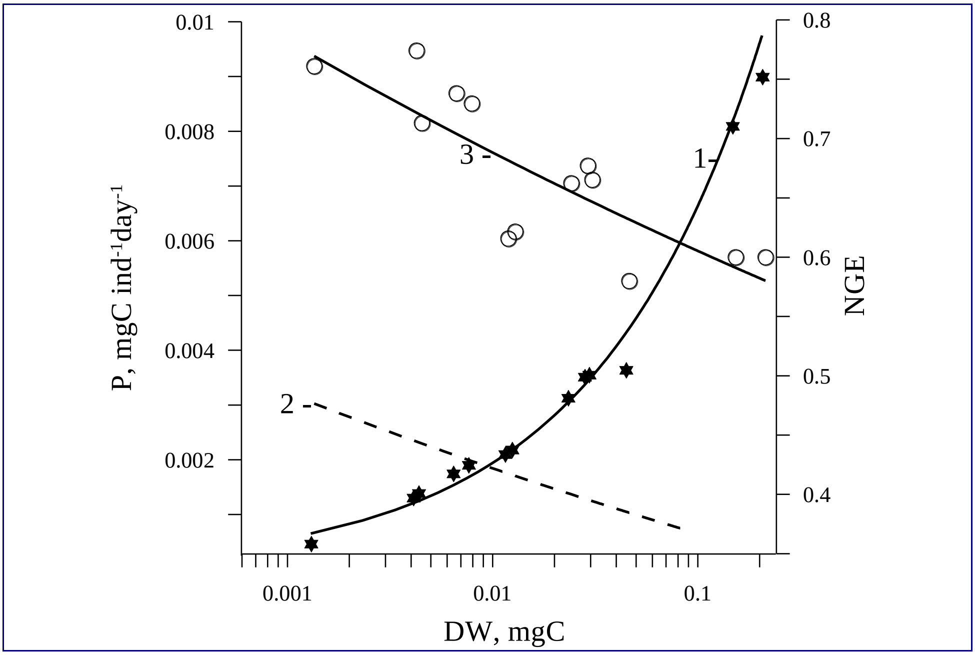

Figure 2.17. Absolute growth rate (mgC ind^{-1} day^{-1}) and net growth efficiency (NGE) in *Calanus euxinus* copepodites CI-CV. 1,✸ absolute growth rate (our results); 2, net growth efficiency (Ikeda *et al.*, 2001); 3, ○ net growth efficiency (calculated as NGE=P/P+R)

P depends upon the respiration rate and the coefficient of net growth efficiency (K_2) according to the equation $K_2 = P/P + R$. Ikeda *et al.* (2001) showed that K_2 in preadult and adult epipelagic copepods changed from 0.54 to 0.21 with increasing temperature and body weight. According to the equations of these authors, at 8 °C K_2 in *C. euxinus* CI through CV should decrease from 0.47 to 0.37 (Fig. 2.17). We estimated age dynamics of K_2 in *C. euxinus* from obtained P and maximum values of Q_t after converting respiration data in μg O_2 (Fig. 2.3B) to carbon units in μg C, using the coefficient of 0.363 at RQ=0.97 (Ikeda *et al.*, 2000). In our experiments at nearly maximum respiration rates (expressed as $R=2.84DFDW^{0.8}$ for 8°C) K_2 reduced from 0.77 in early copepodite stages to 0.6 in CV being close to maximum values for marine copepods (Ikeda *et al.*, 2001). Such a pattern is in accord with the well-known conception (Winberg, 1968) that in juvenile stages K_2 can be close to a theoretical maximum (about 0.8), and then decreases during growth. In the experiments of Petipa (1966) K_2 in *C. euxinus* diminished 10-fold from 0.55 in CI to 0.056 in CV. Consequently, P magnitudes obtained in our study using the values of g by Campbell *et al.* (2001) agree with maximum growth efficiency.

By our calculation, the period of the *C. euxinus* development at 8 °C from CI to CV postmoults (when structural growth predominates over lipid accumulation) amounts to 20 days (Fig. 2. 18). A median development time (MDT) in CI-CV of *Calanus* species at 7.6 °C (26.2 days) was obtained by Thompson (1982). In the growth experiments of Vidal (1980a), the development time for CII-CV of *C. pacificus* was equal to 16.7 days. Therefore, MDT for CI-CV should not be less than 20 days. The MDT of CI to CV in *C. finmarchicus* at 8 °C accounted for 25 and 17 days at medium and high food concentrations, respectively (Campbell *et al.*, 2001). Tande (1988) reported that in the same species at 6 °C, MDT was equal to 31.8 days.

The period of lipid reserve formation in CV postmoults to intermoults was estimated to be 26 days. Although the duration of CV is usually increased (in comparison with other copepodite stages), in copepods accumulating large amounts of lipid, we calculated this period to be substantially higher than the MDT (9.6 – 12.1 days) of CV in *Calanus* studied at ~8 °C (Thompson, 1982; Vidal, 1980 a; Corkett *et al*., 1986; Campbell *et al.*, 2001). However, one should take into account that in spring *C. euxinus* CV accumulates up to 90% of total lipid reserves (Table 2.2), and the weight increase of CIV to CV based on field observations was nearly 3 times higher than that for *C. finmarchicus* CV reared under high food conditions (Campbell *et al*., 2001, see Fig. 1, Table 2 therein).

Finally, complete generation time from CI to adults in *C. euxinus* during winter-spring homothermy should not be less than 46 days. If we add to this value the duration of development of eggs and nauplii accounting for about 16 days at 8 °C (Corkett *et al*., 1986; Campbell *et al*., 2001), the total development time for *C. euxinus* will be approximately 66 days, in contrast to the 43.6 and 32-46 days values reported for *C. finmarchicus* according to Corkett *et al*. (1986) and Campbell *et al*. (2001), respectively. One should take into account that the development time in *C. euxinus* may be underestimated because we have included in our calculation absolute growth rates which correspond to maximum net growth efficiency for preadult and adult copepods (Ikeda *et al*., 2001).

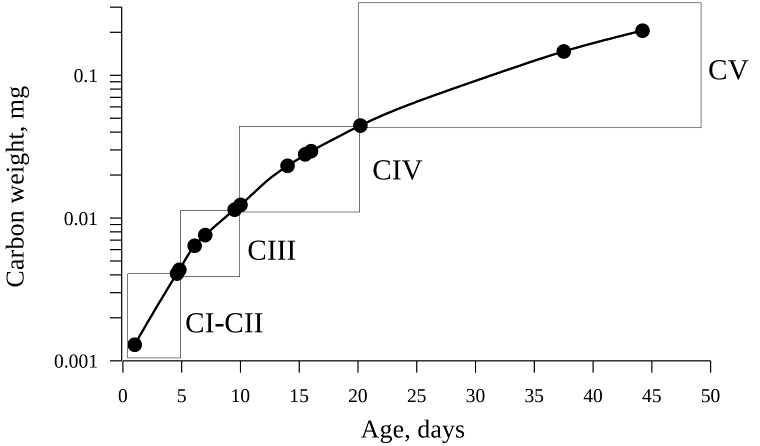

Figure 2.18. Increase in body carbon weight of *C. euxinus* from CI to intermoults of CV relative to age

Now, calculated development time can be used to estimate *C. euxinus* population dynamics. A great number of newly CV postmoults were present in the second week of April. Consequently, the generation of these CV should appear at the beginning of March (Fig. 2.18), whilst the previous generation was formed early in January. Hence, during the cold homothermy period in the Black Sea, *C. euxinus* cannot produce more than two generations.

References

Alcaraz, M., Strickler, J. R., 1988. Locomotion in copepods: pattern of movements and energetics of *Cyclops*. *Hydrobiologia*, 167/168, 404-414.

Alexander, R. MCN., 1968. Animal mechanics. Lectures in Zoology at the University College of North Wales Bangor. Sigwick and Jackson, London, 339 pp.

Anraku M., 1964. Influence of the Cape Cod Canal on the hydrography and on the copepods in Bussards Bay and Cape Cod Bay, Massachusetts. II. Respiration and feeding. *Limnol. Oceanogr*., 9,195-206.

Arashkevich, E.G., Drits, A.V., Timonin, A.G., 1996. Diapause in the life cycle of *Calanoides carinatus* (Kroyer), (Copepoda, Calanoida), *Hydrobiologia*, 320, 197--208.

Arashkevich, E.G., Tande, K.S., Pasternak, A.F., Ellertsen, B., 2004. Seasonal moulting patterns and the generation cycle of *Calanus finmarchicus* in the NE Norwegian Sea, as inferred from gnathobase structures, and the size of gonads and oil sacs. *Mar. Biol*., 146, 119-132.

Bagshaw, C. R., 1982. *Muscle contraction*. Lecturer. Department of Biochemistry. University of Leicester. Chapman and Hall. London, New York, 127 pp.

Bamstedt, U., Tande, K.S., 1985. Respiration and excretion rates of *Calanus glacialis* in the Arctic waters of the Barents Sea, *Mar. Biol*., 87 (3), 259-266.

Besiktepe, S., 2001. Diel vertical distribution, and herbivory of copepods in the south-eastern part of the Black Sea. *J. Mar. Syst*., 28, 281-301.

Besiktepe, S., Telli, M., 2004. Egg production and growth rates of *Calanus euxinus* (Copepoda) in the Black Sea. *J. Plankton Res*., 26, 571-578.

Besiktepe S., Kideys A.E., Unsal M., 1998. In situ grazing pressure and diel vertical migration of female *Calanus euxinus* in the Black Sea. *Hydrobiologia*, 363, 323-332

Bohrer, R. N., Lampert, D.W., 1988. Simultaneous measurement of the effect of food concentration on assimilation and respiration in *Daphnia magna* Straus. *Funct. Ecol*., 2, 463-47.

Bonnet, D., Richardson, A., Harris, R., Hirst., A., Beaugrand, G., Edwards, M., Ceballos, S., Diekman, R., Lopez-Urrutia, A., Valdes, L.., Carlotti, F., Molinero, J.C., Weikert, H., Greve, W., Lucic, D., Albaina, A., Yahia, N.D., Umani, S.F., Miranda, A., Dos Santos, A., Cook, K., Robinson, S., De Puelles, M.L.F., 2005. An overview of *Calanus helgolandicus* ecology in European waters. *Prog. Oceanogr.*, 65, 1–53.

Brett, J. R., 1964. The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Board Can*., 21, 1183-1226.

Buskey, E. J., 1998. Energetic costs of swarming behavior for the copepod *Dioithona oculata*. *Mar. Biol*., 130, 425 - 431.

Campbell, R.W., 2004. Reply to horizons article ‘Some ideas about the role of lipids in the life cycle of *Calanus finmarchicus*’ Irigoien (2004): I. *J. Plankton Res*., 26, 979-980.

Campbell, R.G., Wagner, M.M., Teegarden, G.J., Boudreau, C.A., Durbin, E.G., 2001. Growth and development rates of the copepod *Calanus finmarchicus* reared in the laboratory. *Mar. Ecol. Progr. Ser*., 221, 161-183.

Ceballos, S., Cabal, J.A., Alvarez-Marquės, F., 2004. Reproductive strategy of *Calanoides carinatus* and *Calanus helgolandicus* during a summer upwelling event off NW Spain. *Mar. Biol.*, 145, 739-750.

Champalbert, G. 1978. Rythmes d'activité natatoire de quelques copépodes hyponeustoniques (*Anomalocera patersonii* Templeton, *Pontelle mediterranea* Claus, *Labidocera wollastoni* Lubbock) en fonction des conditions d'éclairement et de pression. *J. Exp. Mar. Biol. Ecol.*, 35, 233-249.

Chua, P.C., Ivanov, L.M., Margolinab, T.M., 2005. Seasonal variability of the Black Sea chlorophyll-a concentration. *J. Mar. Sys.*, 56, 243– 261.

Conover, R.J., 1966. Factors affecting the assimilation of organic matter by zooplankton and the question of superfluous feeding. *Limnol. Oceanogr.*, 11, 346-354.

Corkett C.J., Mclaren I.A., 1978. The biology of *Pseudocalanus*. *Adv. Mar. Biol.*, 15, 231pp.

Corkett, C.J., McLaren, I.A., Sevigny, J-M., 1986. The rearing of the marine calanoid copepods *Calanus finmarchicus* (Gunnerus), *C. glacialis* Jaschnov and *C. hyperboreus* Krøyer with comment on the equiproportional rule. *Syllogeus*, 59, 539–546.

Cowles, D.L., Childress J.J., 1988. Swimming speed and oxygen consumption in the bathypelagic mysid *Gnathophausia ingens*. *Biol. Bull. Mar. Biol. Lab. Woods Hole*, 175, 11-121.

Crain, J.A., Miller, C.B., 2001. Effects of starvation on intermolt development in *Calanus finmarchicus* copepodites: a comparison between theoretical models and field studies. *Deep-Sea Res. II*, 48, 551-566.

Drits, A.V., Pasternak, A.F., Kosobokova, K.N., 1993. Metabolism, and body composition of the Antarctic copepod *Calanus propinquus* Brady with special reference to its life cycle. *Polar. Biol.*, 13, 1, 13-21.

Drits, A.V., Pasternak, A.F., Kosobokova, K.N., 1994. Physiological characteristics of the Antarctic copepod *Calanoides acutus* during late summer in the Weddell Sea. *Hydrobiologia*, 292/293, 201-207.

Durbin, E.G., Garrahan, P.R., Casas, M.C., 2000. Abundance and distribution of *Calanus finmarchicus* on the Georges Bank during 1995 and 1996. *ICES J. Mar. Sci*, 57, 1664-1685.

Eker-Develi, E., Kideys, A.E., 2003. Distribution of phytoplankton in the southern Black Sea in summer 1996, spring and autumn 1998. *J. Mar. Sys*., 39, 203-211.

Enright, J.T., 1977. Copepods in a hurry: sustained high-speed upward migration. *Limnol. Oceanogr*., 22, 118-125.

Erkan F., Gucu A.C., 1998. Analyzing shipborne ADCP measurements to estimate distribution of southern Black Sea zooplankton. 4th European Conference on Underwater Acoustics, Sep 21-25, Rome, Italy.

Fleminger, A., Hulsemann, K., 1987. Geographical variation in *Calanus helgolandicus* s.l. (Copepoda, Calanoida) and evidence of recent speciation of the Black Sea population. *Biol. Oceanogr*., 5, 43-81.

Flint, M.V., 1989. Vertical distribution of mass mesoplankton species in lower layers of aerobic zone in relation to the structure of oxygen field. In: Vinogradov M.E., Flint M.V. (eds) Structure and productional characteristics of planktonic populations in the Black Sea. Nauka, Moscow, 187-213 (in Russian).

Galkovskaya, G.A., Suschenya, L.M., 1978. Growth of aquatic animals at variable temperatures. Nauka i Technika, Minsk, 141 p (in Russian).

Geptner, M.V., 1996. The typology of vertical distribution of oceanic zooplankton. *J. General Biol*., 27, 44-66 (in Russian).

Harris, R. P., Malej, A., 1986. Diel patterns of ammonium excretion and grazing rhythms in *Calanus helgolandicus* in surface stratified waters. *Mar. Ecol. Progr. Ser*., 31, 75-85.

Harris, R.P., Irigoien, X., Head, R.N., Rey, C., Hygum, B.H., Hansen, B.W., Niehoff, B., Meyer-Harms, B., Carlotti, F., 2000. Feeding, growth, and reproduction in the genus *Calanus*. *ICES J. Mar. Sci*., 57, 1708-1726.

Hirche, H. J., 1983. Overwintering of *Calanus finmarchicus* and *Calanus helgolandicus*. *Mar. Ecol. Progr. Ser*., 11, 281-290.

Hirche, H. J., 1996. Diapause in the marine copepod *Calanus finmarchicus* – a review. *Ophelia*, 44, 129-143.

Hulsemann, K., 1991. *Calanus euxinus*, new name, a replacement name for *Calanus ponticus* Karavaev, 1894 (Copepoda: Calanoida). *Proceedings of the Biological Society of Washington*, 104 (3), 620-621.

Huskin, I., Anadon, R., Alvarez-Marques, F., Harris, R. P., 2000. Ingestion, faecal pellet and egg production rates of *Calanus helgolandicus* feeding coccolithophorid versus non-coccolithophorid diets. *J. Exp. Mar. Biol. Ecol*., 248, 239-254.

Hygum, B.H., Rey, C., Hansen, K., Tande, K., 2000. Importance of food quantity to structural growth rate and neutral lipid reserves accumulated in *Calanus finmarchicus*. *Mar. Biol*. 136, 1057-1073.

Ikeda, T., 1977. The effect of laboratory conditions on the extrapolation of experimental measurements to the ecology of marine zooplankton. IV. Changes in respiration and excretion rates of boreal zooplankton species maintained under fed and starved conditions. *Mar. Biol*., 41, 241-252.

Ikeda, T., Torres, J.J., Hernandes-Leon, S., Geiger, S.P., 2000. Oxygen consumption as an index of metabolism. In: Harris R., Wiebe P., Lenz J., Skjoldal H.R., Huntley M. (Eds.), JCES Zooplankton Methodology Manual. Academic Press, San Diego, San Francisco, New York, Boston, London, Sydney, Tokyo. 684 pp.

Ikeda, T., Kanno, Y., Ozaki, K., Shinada, A., 2001. Metabolic rates of epipelagic marine copepods as a function of body mass and temperature. *Mar. Biol.*, 139, 587 - 596.

Ingvarsdóttir A., Houlihan D.F., Heath M.R., Hay S.J., 1999. Seasonal changes in respiration rates of copepodite Stage V *Calanus finmarchicus* (Gunnerus). *Fish. Oceanogr.*, 8, 73-83.

Irigoien, X., 2004. Some ideas about the role of lipids in the life cycle of *Calanus finmarchicus*. *J. Plankton Res.*, 26, 259-263.

Ivlev, V.S., 1959. The experience in estimation of evolutionary significance of the levels of metabolism. *J. General Biol.*, 20 (2), 94-103 (in Russian).

Ivleva, E.V. 1981. Environmental temperature and the rate of energy exchange in aquatic animals. Naukova Dumka, Kiev, 232 pp (in Russian).

Jaschnov, V.A., 1955. Morphology, distribution and systematics of *Calanus finmarchicus* s.l. *Zool. Zh.*, 34, 1210-1223 (in Russian).

Jiang, H., Meneveau, C., Osborn, T. R., 2002. The flow field around a freely swimming copepod in steady motion. Part II: Numerical simulation. *J. Plankt. Res.*, 24, 191-213.

Johnson, C.L., 2004. Seasonal variation in the molt status of an oceanic copepod. *Prog. Oceanogr.*, 62, 15-32.

Joint, I., Pomroy, A., 1993. Phytoplankton biomass and production in the southern North Sea. *Mar. Ecol. Prog. Ser.*, 99, 169 – 182.

Karavaev, V., 1894. Contributions to the Crustacean pelagic fauna of the Black Sea. Kiev. Universitet. *Obshestvo estestvoispitatelei, Zapiski* 13 (1), 35-61 + pls. 3-5.

Kiørboe, T., Møhlenberg, F. Hamburger, K., 1985. Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. *Mar. Ecol. Prog. Ser.*, 26, 85-97.

Klyashtorin, L.B., 1984. Respiration in the water and oxygen requirements of fishes. Legkaya i pischevaya promyshlenost, Moscow, 168 pp. (in Russian).

Klyashtorin, L.B., Yarzhombek, A.A., 1973. Energy consumption in active movement of planktonic organisms. *Oceanology*, 13, 575-580 (English edition).

Lenz, P.H., Hartline, D.K., 1999. Reaction times and force production during escape behavior of a calanoid copepod, *Undinula vulgaris*. *Mar. Biol.*, 133, 249-258.

Mackas, D.L., Burns, K.E., 1986. Poststarvation feeding and swimming activity in *Calanus pacificus* and *Metridia pacificus*. *Limnol. Oceanogr.*, 31, 383-392.

Marker. T., Andreassen, P., Arashkevich, E., Hansen, B.W., 2003. Lipid deposition and sexual maturation in cohorts of *Calanus finmarchicus* (Gunnerus) originating from Bergen (60^0N), and Tromsø (69^0N) reared in Tromsø, Norway. *Mar. Biol.*, 143, 283-296.

Marshall, S.M., ORR, A.P., 1972. The biology of a marine copepod. Springer-Verlag, New York, 195pp.

Mauchline, J., 1998. The biology of calanoid copepods. Advances in Marine Biology. In: Blaxter, J. H. S., Southward, A. J., Tyler, P. A. (Eds.), Academic Press, San Diego-London-Boston-New York-Sydney-Tokyo-Toronto, 710 pp.

Mayzaud, P., 1976. Respiration and nitrogen excretion of zooplankton. IV. The influence of starvation on the metabolism and the biochemical composition of some species. *Mar. Biol.*, 37, 47-58.

Miller, C.B., Nielsen, R.D., 1988. Development and growth of large calanoid copepods in the Oceanic Subarctic Pacific, May 1984. *Progr. Oceanogr.*, 20, 275-292.

Miller, C.B., Cowles, T.J., Wiebe, P.H., Copley, N.J., Grigg, H., 1991. Phenology in *Calanus finmarchicus*; hypotheses about control mechanisms. *Mar. Ecol. Progr. Ser.*, 72, 79-91.

Miller, C.B., Crain, J.A., Morgan, C.A., 2000. Oil storage variability in *Calanus finmarchicus*. *ICES J. Mar. Sci*., 57, 1786-1799.

Minkina, N.I., 1981. Estimation by hydrodynamic method of energy expenditure of copepods (Copepoda, Crustacea) on swimming. *Doklady Akademiia Nauk SSSR, Biological Sciences Section*, 157, 141-144 (in Russian).

Minkina, N.I., 1983. Space-time characteristics of copepod swimming. *Ekologia Moria*, 14, 38-44.

Morris, M.J., Gust, G., Torres, J.J., 1985. Propulsion efficiency and cost of transport for copepods: a hydromechanical model of crustacean swimming. *Mar. Biol*., 86, 283-295.

Morris, M.J., Kohlhage, K., Gust, G., 1990. Mechanics and energetics of swimming in the small copepod *Acanthocyclops robustus* (Cyclopoida). *Mar. Biol*., 107, 83-91.

Mutlu, E., 2003. Acoustical identification of the concentration layer of a copepod species, *Calanus euxinus*. *Mar. Biol*., 142, 517–523.

Niehoff, B., Hirche, H.-J., 1996. Oogenesis and gonad maturation in the copepod *Calanus finmarchicus* and the prediction of egg production from preserved samples. *Polar Biol*., 16, 601-612.

Niehoff, B., Runge, J.A., 2003. A revised methodology for prediction of egg production *Calanus finmarchicus* from preserved samples. *J. Plankton Res*., 25, 1581-1587.

Omori, M., Ikeda, T., 1984. Methods in marine zooplankton ecology. Wiley-Interscience Publ., John Wiley & Sons, New York, 332 pp.

Ostrovskaya, N.A., 1976. Energy requirements for active motion in adult *Calanus helgolandicus* Claus during migrations. *Biologiya Morya*, 37, 55-61 (in Russian).

Papadopoulos, L.N., Peijnenburg, K.T.C.A., Luttikhuizen, P.C., 2005. Phylogeography of the calanoid copepods *Calanus helgolandicus* and *C. euxinus* suggests Pleistocene divergences between

Atlantic, Mediterranean, and Black Sea populations. *Mar. Biol.*, 147, 1353-1365.

Pavlova, E.V., 1987. The movement and metabolism of marine planktonic organisms. Naukova Dumka, Kiev, 212 pp. (in Russian).

Pavlova, E.V., Minkina, N.I., 1987. Weight-specific respiration of marine planktonic animals with different activity. *J. General Biol.,* 48, 1, 92-103 (in Russian).

Petipa, T.S., 1966. On energy balance of *Calanus helgolandicus* (Claus) in the Black Sea. In: Ivlev, V. S. (Ed.), Physiology of marine animals. Nauka, Moscow, 60-81 (in Russian).

Petipa, T.S., 1981. The trophodynamics of copepods in the marine planktonic communities. Naukova Dumka, Kiev, 245 pp. (in Russian).

Polischuk, V.V., 1984. On significant late-holocene level lift in of the Black Sea and origin of northern elements in its fauna. *Hydrobiological Journal*, 20 (4), 3-13 (in Russian).

Poulet, S. A. Gill, C.W., 1988. Spectral analyses of movements made by the cephalic appendages of copepods. *Mar. Ecol. Prog. Ser.*, 43, 259-267.

Price, H.J., Paffenhofer, G.-A., 1986a. Capture of small cells by the copepod *Eucalanus elongatus*. *Limnol. Oceanogr.*, 31, 189-194.

Price, H.J., Paffenhofer, G.-A., 1986b Effects of concentration on the feeding of a marine copepod in algal monocultures and mixtures. *J. Plankt. Res.*, 8, 119-128.

Raymont, J.E.G., 1983. Plankton and productivity in the oceans. Vol. 2 Zooplankton. Pergamon Press Ltd., 630 pp.

Rey-Rassat, C., Irigoien, X., Harris, R., Head, R., Carlotti, F., 2002. Growth and development of *Calanus helgolandicus* reared in the laboratory. *Mar. Ecol. Prog. Ser.*, 238, 125-138.

Rey-Rassat, C., Bonnet, D., Irigoien, X., Harris, R., Head, R., Carlotti, F., 2004. Secondary production of *Calanus helgolandicus* in the Western English Channel. *J. Exp. Mar. Biol. Ecol.*, 313, 29-46.

Rudyakov, Yu.A., 1986. The dynamics of vertical distribution of pelagic animals. Nauka, Moscow, 135 pp (in Russian).

Sargent, J.R., 1978. Marine wax esters. *Sci. Prog. Oxf.*, 65, 437-458.

Sargent, J.R., Henderson, R.J., 1986. Lipids. In: Corner E.D.S., O`Hara S.C.M. (Eds.) The biological chemistry of marine copepods. Clarendon Press, Oxford, 59-164.

Sazhina, L.I., 1987. Reproduction, growth, productivity of marine Copepoda. Naukova Dumka, Kiev, 156 pp. (in Russian).

Smith, S.L., 1988. Copepods in Fram Strait in summer; distribution, feeding, and metabolism. *J. Mar. Res.*, 46, 145-181.

Stepanov, V.N., Svetlichny, L.S., 1981. Studies on hydromechanic characteristics of planktonic copepods. Naukova Dumka, Kiev, 126 pp (in Russian).

Strickler, J.R., 1977. Observation of swimming performances of planktonic copepods. *Limnol. Oceanogr.*, 22, 165-170.

Suschenya, L.M., 1972. The intensity of respiration in crustaceans. Naukova Dumka, Kiev, 196 pp (in Russian).

Svetlichny, L.S., 1983 a. Calculation of the biomass of planktonic copepods using the coefficients of proportionality between volume and linear dimensions of the body. *Ecologiya Morya*, 15, 46-58 (in Russian).

Svetlichny, L.S., 1983 b. Hydrodynamic resistance of motionless copepods during their passive sinking in the water. *Okeanologia*, 23 (1), 139-144 (in Russian).

Svetlichny, L.S., 1987. The speed, tractive force and energy expenditure of locomotion of copepods. *Okeanologia*, 27 (4), 662-669 (in Russian).

Svetlichny, L.S., 1989. Temperature influence on locomotor activity of *Calanus helgolandicus*. *Okeanologia*, 29 (3), 502-507 (in Russian).

Svetlichny, L.S., 1992 a. Dependence of the specific respiration rate of copepods on mechanical energy of locomotion. *Ekologiya Morya*, 40, 72-77 (in Russian).

Svetlichny, L.S., 1992 b. Concerning biohydrodynamics of the steady and unsteady locomotion of copepods. *Ekologiya Morya*, 40, 84-89 (in Russian).

Svetlichny, L.S., 1993. Locomotor function of mouth appendages in copepods: its kinematics. *Ekologiya Morya*, 44, 84-91 (in Russian).

Svetlichny, L.S., 1996. Locomotion efficiency and maximum values of energy exchange in copepods. *Hydrobiological Journal*, 32, 57-66 (in Russian).

Svetlichny, L.S, Yarkina, I.Ya., 1989. Locomotion rhythms in *Calanus helgolandicus* (Crustacea, Copepoda). *Zool. J*., 68 (6), 50-55 (in Russian).

Svetlichny, L.S., Umanskaya, A.V,. 1991. Oxygen consumption cost and locomotion in *Calanus helgolandicus* (Crustacea, Copepoda). *Okeanologia*, 15, 566-571.

Svetlichny, L.S., Hubareva, E.S., 2005. The energetics of *Calanus euxinus*: locomotion, filtration of food and specific dynamic action. *J. Plankton Res*., 27, 671-682.

Svetlichny, L.S., Kideys, A.E., 2005. Spatial morphological and physiological variability of *Calanus euxinus* (Copepoda). Proceedings of the 4th International Congress of the Hydrobiological Society of Ukraine, 26 - 29 September 2005, Feodosia, Ukraine, 209-211. (in Russian).

Svetlichny, L.S., Hubareva, E.S., Arashkevich, E.G., 1998. Physiological and behavioural response to hypoxia in active and diapausing copepodites Stage V *Calanus euxinus*. *Arch.*

Hydrobiol. Spec. Issues Advanc. Limnol., 52, 507-519.

Svetlichny, L.S., Hubareva, E.S., Erkan, F., Gucu, A.G., 2000. Physiological and behavioural aspects of *Calanus euxinus* females (Copepoda, Calanoida) during vertical migration. *Mar. Biol.*, 137, 963-971.

Svetlichny, L.S., Kideys, A.E., Hubareva, E.S., Besiktepe, S., Isinibilir, M., 2006. Development and lipid storage in *Calanus euxinus* from the Black and Marmara seas: variabilities due to habitat conditions. *J. Mar. Sys.*, 59, 52-62.

Tande, K.S., 1988. Aspects of developmental and mortality rates in *Calanus finmarchicus* related to equiproportional development. *Mar. Ecol. Prog. Ser.*, 44, 51-58.

Tande, K.S., Hopkins, C.C.E., 1981. Ecological investigations of the zooplankton community of Balsfjorden, northern Norway: the genital system *in Calanus finmarchicus* and the role of gonad development in overwintering strategy. *Mar. Biol.*, 63, 159-164.

Thompson, B.M., 1982. Growth and development of *Pseudocalanus elongatus* and *Calanus* sp. in the laboratory. *J. Mar. Biol. Ass. U. K.*, 62, 359–372.

Thor, P., 2000. Relationship between specific dynamic action and protein deposition in calanoid copepods. *J. Exp. Mar. Biol. Ecol.*, 245, 171-182.

Thor, P., 2002. Specific dynamic action and carbon incorporation in *Calanus finmarchicus* copepodites and females. *J. Exp. Mar. Biol. Ecol.*, 272, 159-169.

Thor, P., 2003. Elevated respiration rate of the neritic copepod *Acartia tonsa* during recovery from starvation. *J. Exp. Mar. Biol. Ecol.*, 283, 133-143.

Torres, J.J., Childress, J.J., 1983. Relationship of oxygen consumption to swimming speed in *Euphausia pacifica* 1. Effects of temperature and pressure. *Mar. Biol.*, 74, 79-86.

Unal, E., Frost, B.W., Armbrust, V., Kideys A.E., 2006. Phylogeography of *Calanus helgolandicus* and the Black Sea copepod *Calanus euxinus*, with notes on *Pseudocalanus elongatus* (Copepoda, Calanoida). *Deep-Sea Res. II*, 53, 1911-1922.

Uye, S., 1988. Temperature-dependent development and growth of *Calanus sinicus* (Copepoda: Calanoida) in the laboratory. *Hydrobiologia*, 167/168, 285–293.

Van Duren, L.A., Videler, J.J., 1995. Swimming behavior of developmental stages of the calanoid copepod *Temora longicornis* at different food concentrations. *Mar. Ecol. Progr. Ser.*, 126, 153-161.

Van Duren, L.A., Videler, J.J. 2003. Escape from viscosity: the kinematics and hydrodynamics of copepod foraging and escape swimming. *J. Exp. Biol.*, 206, 269-279.

Vesser, A.V., Jónasdóttir, S.H., 1999. Lipids, buoyancy and the seasonal vertical migration of *Calanus finmarchicus*. *Fish. Oceanogr.*, 8 (Suppl. 1), 100-106.

Vidal, J., 1980 a. Physioecology of zooplankton. II. Effects of phytoplankton concentration, temperature, and body size on the development and molting rates of *Calanus pacificus* and *Pseudocalanus* sp. *Mar. Biol.*, 56, 135–146.

Vidal, J., 1980 b. Physioecology of zooplankton. III. Effects of phytoplankton concentration, temperature and bogy size on the metabolic rate of *Calanus pacificus*. *Mar. Biol.*, 56, 195-202.

Vilenkin ,B.Ya., Vilenkina, M.N., 1979. The respiration of aquatic invertebrates. VINITI, Moscow, 143 pp. (in Russian).

Vinogradov, M.E., Arashkevich E.G., Ilchenko S.V., 1992a. The ecology of the *Calanus ponticus* population in the deeper layer of its concentration in the Black Sea. *J Plankton Res.*, 14, 447-458.

Vinogradov, M.E., Sapozhnikov, V.V., Shushkina, E.A., 1992b. The Black Sea ecosystem. Nauka, Moskow, 112 pp. (in Russian).

Vinogradov, M.E., Shiganova, T.A., Khoroshilov, V.S., 1995. The state of the main organisms in a plankton community in the Black Sea in 1993. *Oceanology*, 35 (3), 387-391.

Vlymen, W. J., 1970. Energy expenditure of swimming copepods. *Limnol. Oceanogr.*, 15, 348-356.

Williams, R., Robins, D.B., 1982. Effects of preservation on wet weight, dry weight, nitrogen and carbon contents of *Calanus helgolandicus* (Crustacea: Copepoda). *Mar. Biol.*, 71, 271-281.

Winberg, G.G., 1968. Growth rate and intensity of metabolism in animals. *Uspehi Sovremennoi Biologii*, 6, 274-293. (in Russian).

Winberg, G.G., 1982. The trophodynamics of copepods in the marine planktonic communities. *Hydrobiological Journal*, 18, 101-105. (in Russian).

Winberg, G.G., 1983. Temperature coefficient of Vant-Hoff and Arrhenius equation in biology. *J General Biol.*, 44, 31-42 (in Russian).

Yen, J., Sanderson, B., Strickler, J.R., Okubo, A., 1991. Feeding currents and energy dissipation by *Euchaeta rimana*, a subtropical pelagic copepod. *Limnol. Oceanogr.*, 36 (2), 362-369.

Yule, A.B., Crisp, D.J., 1983. A study of feeding behavior in *Temora longicornis* Müller (Crustacea: Copepoda*). J. Exp. Mar. Biol.*, 17, 271-282.

Yuneva, T.V., Svetlichny, L.S., Schepkina, A.M., 1997 a. Lipid composition and locomotory activity of diapausing *Calanus euxinus* (*Copepoda*). *Hydrobiological Journal*, 33, 74-84 (in Russian).

Yuneva, T.V., Svetlichny, L.S., Yunev, O.A., Georgieva, L.V., Senichkina, L.G., 1997 b. Spatial variability of *Calanus euxinus* lipid content in connection with chlorophyll concentration and phytoplankton biomass. *Oceanology*, 37 (5), 745-752.

Yuneva, T.V., Svetlichny, L.S., Yunev, O.A., Romanova, Z.A., Kideys, A.E., Bingel, F., Yilmaz, A., Uysal, Z., Shulman, G.E., 1999.

Nutritional condition of female *Calanus euxinus* from cyclonic and anticyclonic regions of the Black Sea. *Mar. Ecol. Prog. Ser.*, 189, 195-204.

Zaitsev, Yu.P., 2006. An introduction to Black Sea ecology. Aven, Odessa, 224 pp. (in Russian).

3. ORGANIC COMPOSITION AND ECOLOGICAL ENERGETICS OF THE JELLYFISH *AURELIA AURITA* L. (CNIDARIA, SCYPHOZOA) UNDER BLACK SEA CONDITIONS

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3.1 Introduction

Aurelia aurita is one of the most well-known species of Scyphozoan jellyfish, with almost cosmopolitan distribution in all neritic areas of the World Ocean between 40°S and 70°N latitude (Kramp, 1961). Dense swarms of this jellyfish can be detected almost anywhere within its natural habitat, causing serious ecological and economical problems - numerous cases have been reported in detail (Möller, 1980; Mills, 2001).

A. aurita's worldwide expansion and prominent abundance are due largely to its high fecundity and ecological tolerance. During its life span each female medusa can generate many thousands of planula-larvae, which develop either into polypoid stage scyphistoms or directly into ephyrae (Schneider, 1988; Arai, 1997). Both ephyrae and medusae are tolerant of a wide range of environmental conditions. For example, medusae is able to survive at temperature of 0.6->31°C and salinities from 4 to 43‰ (Kramp, 1961). However, individual populations of *A. aurita* generally prefer a narrower range of environmental conditions (Hansson, 1997; Båmstedt *et al.*, 1999; Malej *et al.*, 2006). It would appear that wider genetic study of this species could greatly add to the understanding of its biogeography (Mills, 2001).

In the Black Sea, *A. aurita* is usually found at 4-23°C and 16-18‰, although occasionally some individuals can be observed at higher or lower seawater temperatures and salinities (Mutlu *et al.*, 1994; Sorokin, 2002). In the Black Sea, medusae are known to occur at depths of up to

90-100 (Mironov, 1971) and even 160 m (Zaitzev and Polischuk, 1984), suggesting adaptation to reduced oxygen concentrations (<1 mg l^{-1}).

Depending on physiological state, food distribution, hydrology and many other factors, *A. aurita* occupies the oxygenated zone of the sea rather unevenly with a creation of micro- and macroscale aggregations (Mironov, 1971). The biomass of medusae in such aggregations sometimes exceeds 1000 g m^{-2}, although in offshore areas it is usually much less, with seasonal and interannual fluctuations typically from 30 to >300 g m^{-2} (Zaitzev and Polischuk, 1984; Shushkina and Arnautov, 1987; Mutlu *et al*., 1994; Anninsky *et al*., 1998; Weisse and Gomoiu, 2000). In 1978-1987, *A. aurita* attained a biomass 600-1300 g m^{-2} (submersible observations) and completely dominated the gelatinous macrozooplankton of the Black Sea (Gomoiu and Kupriyanov, 1980; Vinogradov and Shushkina, 1982; Shushkina and Arnautov, 1987). However, the introduction of the ctenophore *Mnemiopsis leidyi* to the Black Sea, resulted in this loss of dominance of *A. aurita* in surface waters (above the thermocline). During 1991-1996, jellyfish biomass obtained by net hauls averaged only 86-260 g m^{-2} (Mutlu *et al*., 1994; Anninsky *et al*., 1998). However, comparing the net haul data with submersible observations (Shushkina and Arnautov, 1987), the decreasing biomass of *A. aurita* during 1991-1996 appears less dramatic. During the early-mid 1990s, *A. aurita* and *M. leidyi* had a huge influence on the composition and abundance of planktonic ecosystems of the Black Sea, but since the invasion of the larger predatory ctenonophore, *Beroe ovata* (first discovered in the Black Sea in 1997; Konsulov and Kamburska, 1998), which feeds almost exclusively on *M leidyi* it is tempting to suggest that the influence of *A. aurita* on the Black Sea ecosystem may again begin to increase.

A. aurita usually feeds on micro- and mesozooplankton (Southward, 1955; Mironov, 1967; Möller, 1980; Stoecker *et al*., 1987; Malej *et al*., 2006), but numerous phytoplankton and detrital particles also contribute to its diet. Consequently, the study of its original and adapted food webs, trophic conditions and dietary spectra represent particularly complicated issues (Båmstedt *et al*., 1990; Arai, 1991; Olesen *et al*., 1994; Behrends and Schneider, 1995; Malej *et al*., 2006).

While energy budgets of *A. aurita* in the Black and others seas have been investigated for a long period (Mironov, 1967; Shushkina and Musaeva, 1983; Båmstedt *et al*., 1990; Lebedeva and Shushkina, 1991; Olesen *et al*., 1994; Arai, 1997; Frandsen and Riisgård, 1997; Båmstedt *et al*., 1999), such studies have been based largely on preliminary or generalised data. In essence, no energy budget for this or any other scyphomedusae species has ever been derived based original experimental data. Data concerning the chemical composition, feeding rates, metabolism and growth of the *A aurita* are discussed in the present paper, the main goals of which are to describe the rates and efficiency of food energy transformation for this species at the level of the individual. Data describing the trophic state of *A. aurita* population in the western Black Sea (October, 2005) are also examined in the same context.

3.2. Organic composition and elements of energy balance of *A. aurita*

3.2.1 Materials and methods

Study area, sampling and aquaria maintenance

When the experiments were carried out in 1983-1984, *A. aurita* medusae were caught in the near-shore shelf of Crimea of the Black Sea (2-5 miles off Sevastopol; seawater salinity of 16-18‰). Generally, the animals were sampled using a conical plankton net (mouth diameter 0.2 m^2, mesh size 500 µm), fitted with a 1 litre bucket for the collection of living specimens. Additionally, when aggregations of the jellyfish were observed at the sea surface, some individuals were captured by dipping a small bucket. Within 1-2 hours of capture, animals were taken to the laboratory and placed in aquaria. Depending on season, medusae were maintained at 6-23°C, corresponding to seawater temperature ± 2°C. Medusae were maintained for a maximum of 24 h prior to feeding experiments, during which time they were fed on mesozooplankton (predominantly *Acartia clausi*), collected from the sea using a Jedy net with 100 µm mesh-size. During maintenance *A. aurita*: umbrella pulsation remained rhythmical and full; tentacles looked naturally outstretched as they are usually observed in the sea.

When size-weight structure of *A. aurita* population was studied in 1999-2006, the sampling was undertaken at 3 stations situated along the 50-60 m isobath of the south-western shelf of the Crimea (off Sevastopol Bay). Individuals were counted and measured immediately after capture. The presence of ephyrae in the samples was checked under a microscope in the laboratory.

Size-weight relationships

The umbrella interradial diameter (dimension between statocysts) in *A. aurita* was measured to the nearest 1 mm at the moment of maximal relaxation of the body on a graduated glass plate. For early ephyrae size measurements were made under a dissecting microscope to an accuracy of 0.01 mm. Wet body weight for young and adult *A. aurita* specimens was determined on an analytical balance (accuracy to 1×10^{-2} g) after blotting the animals on nylon gauze to remove excess water. The wet weight of ephyrae was estimated volumetrically after pressing individuals between parallel glass plates (0.2 mm apart; Eiji, 1987). In such calculations, the body density of ephyrae was assumed to be 1 mg/µl.

Dry weight in *A. aurita* was determined after attaining stability for the measurements at 70 °C. To obtain ash-free dry weight subsamples of dry weight of *A. aurita* were heated in a muffle at 480-500°C. Dry and ash-free dry weights were measured to the nearest 1×10^{-5} g.

Chemical composition and organic matter content

Ephyrae, young and adult specimens of *A. aurita* were always analysed individually without any pooling of the material. Homogenised samples of medusa body material were stored at –20 °C in tightly closed vials. All biochemical determinations were completed over the next 1-2 months, usually in no less than three replicates. Subsamples taken from freshly thawed homogenate were in the range 0.1-0.5 g of wet weight.

All the major organic components of jellyfish tissue (protein, lipids, carbohydrates and free amino acids) were quantitatively assayed by routine colourimetric techniques, as described by Anninsky *et al*. (2005).

Protein was measured by the Lowry method, as modified by Hartree, using HSA as a standard (Hartree, 1972). Amino acids (ninhydrin positive substances) were measured by the Pochinok method with D, L-α-alanine as the standard (Kuzmenko, 1975). Carbohydrates were determined by the Dubois method with D-glucose as a standard (Dubois *et al*., 1956). Fractions of mono- and polysaccharides in the total carbohydrates were separated using 80% ethanol (Zaslavsky, 1980). Structural polysaccharides were calculated by difference between total polysaccharide and glycogen content. Glycogen was firstly separated in 30% KOH solution, and then estimated as well by the Dubois method. Total lipids were extracted using 2:1 chloroform/methanol (Folch *et al*., 1957) and later analysed by the method of Amenta (Amenta, 1964; Clarke *et al*., 1992), using triolein/cholesterol (1/1) as standards. Lipid fractions were assayed by TLC on “Silufol” plates (Kopitov, 1983). These methods are reviewed by Clarke *et al*. (1992).

The organic matter content of medusae body was determined as the sum of all aforementioned organic constituents. In addition, the organic content of *A. aurita* ephyrae was individually determined to the nearest 1×10^{-6} g using the dichromate oxidation microtechnique, adapted for gelatinous substances (Anninsky *et al*. 2007).

Energy content

The calorific value (1 cal = 4.186 J) of jellyfish tissue was calculated, using established energy equivalents of each constituent (5.65 cal/mg for proteins, 9.45 cal/mg for lipids, and 4.10 cal/mg for carbohydrates; Omori and Ikeda, 1984).

Feeding

Food selectivity of *A. aurita* was investigated by feeding medusa various combinations of Black Sea zooplankton and large phytoplankton, predominantly Copepoda (*Acartia clausi, Calanus euxinus, Oithona similes, O. nana, Paracalanus parvus, Pseudocalanus elongatus* etc.), Cladocera (*Penilia avirostris, Pseudoevadne tergestina*), Chaetognatha (*Sagitta hispida*), Dinoflagellata (*Noctiluca scintillans, Ceratium fusus*), Appendicularia (*Oikopleura dioica*), together with meroplanktonic organisms (larvae of Mollusca, Annelida, Chordata, Decapoda etc.).

Between 5 and 13 moderately-sized (12.6-136.0 mm in umbrella diameter; WW = 0.1-124.0 g ind.$^{-1}$) *A. aurita* specimens were simultaneously placed into 20-40 l aquaria under dim light conditions, containing 0.4-4.7 (and occasionally 93) mg l^{-1} zooplankton. Generally, medusae began to feed immediately after putting plankton into aquarium. *A. aurita* was allowed for 0.1-1.0 h, depending on seawater temperature (7.8-21.4 °C) and the food concentration, resulting in a maximum 10% decrease in food concentration. Both the number and composition of ingested zooplankton were determined, either by looking through the gastric cavities and oral arms of the medusae under a dissecting microscope, or by measuring the reduction in food abundance in aquaria water during the experiment. The composition of zooplankton in water was analysed by taking subsamples of seawater containing the food organisms before and after the experiment. The subsamples were fixed with Borax buffered 4% formalin and later treated like typical net plankton.

The selectivity of *A. aurita* feeding on heterotrophic bacteria was assumed to be the same as that for their clearance. For this purpose, 5 *A. aurita* individuals (of 0.5-29.4 g of wet weight) were separately fed on the same food under similar conditions (jar volume = 2 l; temperature = 17°C; food concentration = 0.01-0.20 mg l^{-1}; duration of the feeding = 1 h). The clearance of bacteria was measured in relation to that obtained for 3 copepod species (*A. clausi, O. similis, P. parvus*) by the decrease in their concentration during the experiment. The concentration of heterotrophic bacteria in seawater was estimated by the number of colonies which grew upon beef-extract agar before and after the experiment. Colony counting was undertaken using a dissecting microscope.

For the determination of chemical composition of the gut content of medusa in the sea, recently captured medusae were provoked to reject undigested food. Food particles embedded into mucous filaments were collected, homogenised and analysed for protein and carbohydrate contents, using the same methods described above. “Pure” mucus, obtained from starving individuals, was used to provide a control baseline against which “food” protein and carbohydrate values could be calculated.

Feeding and clearance rates by *A. aurita* were investigated at the same time as food electivity in the above experiments. Standard morphometric equations for different species of the Black Sea zooplankton (Petipa, 1957) were used to convert number of organisms ingested into their wet weight.

When the daily ration of *A. aurita* was studied over a long period of time (up to 7 days), a single jellyfish of wet weight 4-74 g was placed into 20-40 l aquarium filled with fresh seawater containing excess zooplankton (up to 10 mg l^{-1} of predominantly *A. clausi* and *C. euxinus* added every 1-2 days during the course of the experiment). Every day, one quarter of the original seawater was replaced, and the aquarium water carefully stirred. The daily ration was calculated from the reduction in zooplankton concentration in the seawater. Triplicate samples of zooplankton (0.1-0.3 l) were taken from aquarium with 5-12 hours and 1-2 days periodicity in the first and following days, respectively. When final calculations of rations were made, copepods removed by sampling were also taken into account.

For jellyfish feeding on zooplankton, assimilation efficiency was estimated by the ratio in calorie equivalents of ingested and undigested food, using the dichromate oxidation technique of Giginyak (1979).

Medusae (WW = 0.4-35.8 g) were initially starved for a day and then fed on two types of food:

1. A mixture of small copepods with the predominance of *A. clausi* (at seawater temperature of 21.4-23.6 ºC); and
2. *C. euxinus* (at seawater temperature of 7.8 ºC).

After one-hour of feeding, each medusa was placed into a 1-4 l jar containing filtered seawater. Simultaneously, uneaten zooplankton was collected and later analysed alongside the initial samples in order to determine the amount of food ingested. In addition, zooplankton samples were taken to determine their calorie content. The assimilation efficiency of *A. aurita* was measured over a one-day period. Seawater from each "experimental" and "control" (without *A. aurita*) jar was filtered through a "Nucleopore", 0.46 μm membrane filter, and the trapped solids were washed off with distilled water, dried and oxidized with dichromate. The calorie content of zooplankton between 0.5 and 0.6 cal $mgWW^{-1}$ - rather less then that generally accepted for the Black Sea Copepoda (0.7-0.8 cal $mgWW^{-1}$) (Shushkina and Musaeva, 1983; Sorokin, 2002). Perhaps, this could be due to incomplete oxidation (~ 40%) for some organics constituents, such as some proteins, lipids and amino acids (Giginyak, 1979).

Respiration

Oxygen uptake by *A. aurita* was determined using the Winkler technique (Omori and Ikeda, 1984). Measurements were undertaken at 6.1-23.0 ºC over 4-8 h periods. Respirometers volumes of 0.1-1.0 l were chosen according to the number (1-15) and body weights of medusae (2×10^{-2}-100 g) used in the experiments. Oxygen concentration decrease during the experiments was a maximum of 10% of initial content. The caloric equivalent of oxygen respired was accepted as 4.86 cal mlO_2^{-1} (Winberg, 1971).

Growth

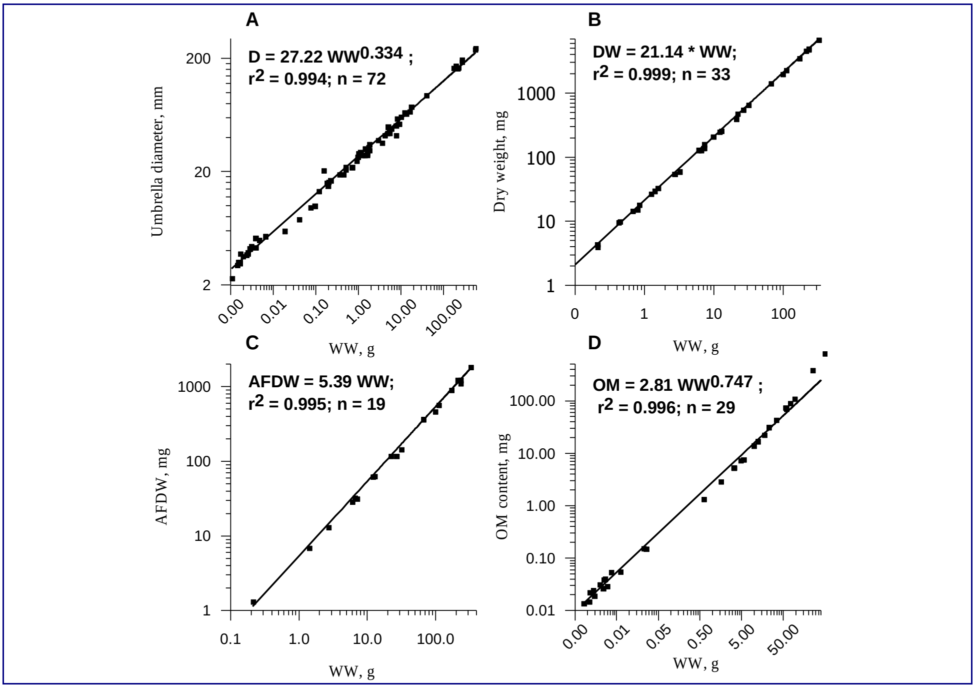
In 1983-1984, the growth rate of A. *aurita* was measured using modal classes in the frequency distributions of umbrella diameter during discrete time intervals. Additionally, in 1983-1984 and 1999-2006, data on medusae growth were obtained from seasonal measurements of population mean umbrella diameter. Using randomised sampling, 150 to 250 and from 30 to 100 individuals were collected on each occasion during 1983-1984 and 1999-2006, respectively.

3.2.2 Results and discussion

Size-weight relationships

Because, the dry weight of gelatinous zooplankton depends on both salinity and drying temperature (Larson, 1986), body wet weight is used as the basis for calculating functional parameters. Linear characteristics of individuals, such as umbrella diameter, often provide an unreliable basis for the measurement/reporting of chemical and metabolic parameters, due to body elasticity and pulsation while measuring. However, other morphometric and physiological indicators can benefit if expressed in relation to linear characteristics. Nevertheless, since so many calculations in the literature have been reported in relation to umbrella diameter, dry, ash-free dry, and organic body weight, the direct comparison of results gained in this investigation with values reported in the literature requires relationships between these different basal parameters to be established.

According to our data for the Black Sea *A. aurita*, the allometric relationship between body wet weight and umbrella diameter, reveals a regression slope factor of 0.334 (Fig. 3.1A). So, umbrella diameter for the specimens with wet weight within the range 0.001-558 g will always be proportional. Probably, morphometric proportions in this species do not vary essentially with the development of sexual dimorphism. Maturity is usually observed in medusae with a minimum wet weight close to 50 g (~100 mm umbrella diameter). The same is true also for wet weight-specific amount of dry and ash-free dry weight, which seems to be constant in *A. aurita* regardless of body wet weight and maturation stage (Fig. 3.1B,C). Therefore, intercept coefficients in linear equations calculated for these data were close to the arithmetic means for wet weight-specific content of dry and ash-free dry weight which have made up (± SD) 20.94 ±□1.04 and 5.14 ± 0.47 mg g^{-1} WW, respectively. In contrast to above parameters, the dependence for organic matter content in *A. aurita* better corresponds to non-linear function, which can be expressed as the power equation with a slope factor of 0.747 (Fig. 3.1D). This and some others peculiarities of organic matter content became more clear, when organic composition of the jellyfish are examined in details.



. Figure 3.1. Relationships between umbrella diameter (A), dry weight (B), ash-free dry weight (C), organic matter content (D) and individual wet weight of the jellyfish, *Aurelia aurita* from the Black Sea

Chemical composition

Generalised data on the chemical composition and organic matter content of freshly-captured specimens of *A. aurita* from the Black Sea are shown in Table 3.1.

Table 3.1. Organic matter content and proximate chemical composition of the jellyfish, *Aurelia aurita* from the Black Sea

Size, mm	Organic compounds	Content		N
		mg g^{-1} wet weight (±SD)	%	
2.3 – 7.9	Organic matter	13.595 ± 4.302	100	14
23 – 211	Organic matter	1.504 ± 0.254	100	17
23 – 211	Protein	1.074 ± 0.148	71.4*	17
23 – 211	Lipids	0.188 ± 0.055	12.5*	17
45 -126	Phospholipids	0.051 ± 0.007	29.7**	7
45 -126	Sterols	0.043 ± 0.004	25.1**	7
45 -126	Unidentified neutral lipids	0.045 ± 0.003	26.5**	7
45 -126	Triacylglycerols	0.014 ± 0.003	8.2**	7
45 -126	Sterol esters and wax	0.008 ± 0.003	4.5**	7
45 -126	Minor fractions	0.010 ± 0.003	6.0**	7
23 – 211	Carbohydrates	0.112 ± 0.036	7.4*	17
45 -126	Polysaccharides	0.063 ± 0.016	60.0***	7
45 -126	Glycogen	0.015 ± 0.003	24.4****	7
45 -126	Monosaccharides	0.042 ± 0.009	40.0***	7
23 – 211	Amino acids	0.129 ± 0.056	8.6*	17

* Expressed as % organic matter
** Expressed as % of total lipids
*** Expressed as % of total carbohydrates
**** Expressed as % of polysaccharides

A. aurita's organic composition is similar to those reported for other Cnidarians, in that protein makes up the greatest proportion of total organic content, lipids occupy a lesser amount, and carbohydrates are present only in small amounts (Clarke *et al*., 1992; Lucas, 1994; Bailey *et al*.; 1995; Arai, 1997). According to Table 3.1, the organic matter of Black Sea *A. auralia* consists of 65-78% protein, 9-17 % lipids, 6-10% carbohydrates and 3-12% free amino acids. The large difference between protein and lipids contents testifies that *A. aurita* has limited resources for accumulation of storage compounds. Indeed, the lipid fractions of medusae of this species from the Mediterranean Sea contained large amounts of phospholipids and sterols (37.5%), i.e. the main components of cellular membranes, while triacylglycerols and waxes were less than 12.7% (Mastronicolis *et al*., 1991).

A similar story is true of carbohydrates in *A. aurita*, the content of which is approximately half that of lipids, and glycogen constitutes only about 14% of the total carbohydrates. Glycogen and reserve lipids in *A. aurita* did not exceed 3% of the total organic matter.

The high free amino acids content, of *A aurita*, co-ordinates well with the hypothetical calculations of Clarke *et al*., (1992), revealing that up to 12-30% of the total nitrogen can be included in non-protein components. Perhaps, a major part of these compounds consist of amino sugars, which are under-represented by colorimetric (ninhydrin-based) techniques. The presence of amino sugars in mesogloea of this jellyfish has been confirmed by histological and biochemical investigations (Arai, 1997).

Values obtained for the composition and total organic content of the Black Sea *A. aurita* are similar to those obtained other researchers (Table 3.2).

The total organic matter content of this species is only one third of the ash-free dry weight. This large difference is partly caused by so-called "residual" or "bound" water which is still retained in "dried" jellyfish at 60 ºC, but released by further heating (Madin *et al*., 1981). The increase of drying temperature for *A. aurita* from 60 ºC to ~ 200 ºC results in a decrease in weight of 12% (Larson, 1986). Concerning such ash-free dry weight (~2.60 mg g^{-1} WW) the organic matter of *A. aurita* is only by 43% less. A lesser discrepancy may also caused by "bound" water, some amounts of which become free only after burning of the organic matter. However, numerous minor composites missed by way of cumulative evaluation of organic matter content, also could be the reason for such a discrepancy.

Table 3.2. Organic matter content and proximate chemical composition of *Aurelia aurita* from different regions. All values are expressed as mg g^{-1} of wet weight

Area	Protein	Lipids	Carbohydrates	Organic matter	Source
The Black Sea	-	-	-	13.60*	P.S.
The Black Sea	1.07	0.19	0.11	1.50	P.S.
Kiel Bight	-	-	-	2.55	1
Kiel Bight	1.18	0.38	0.56	1.98	2
Saanich Inlet, British Columbia	1.90**	-	-	3.10***	3
Southampton Water	11.08*	-	0.33*	(11.41*)	4
Southampton Water	0.84 – 3.70	0.53 – 1.62	0.04 – 0.22	1.5 – 5.4	4
The Mediterranean Sea	-	0.20	-	-	5

Sources: P.S. present study; (1) Möller, 1978; (2) Schneider, 1988; (3) Larson, 1986; (4) Lucas, 1989; (5) Mastronicolis *et al.*, 1991.

* Estimated for ephyrae; (11.41*) calculated without carbohydrates

** Calculated assuming protein = nitrogen × 5 (Schneider, 1989).

*** Calculated assuming organic matter = carbon × 1.9 (Omori and Ikeda, 1984).

Both ephyrae and young medusae contain much more organic matter than is characteristic of mature specimens (P<0.001; t-test). The concentrations of proteins, lipids, carbohydrates, free amino acids and organic matter as a whole gradually decrease with an increase in size up to ~100 mm in umbrella diameter, above which the decreasing trend may stabilise or even reverse, particularly in the case of lipids (Fig. 3.2).

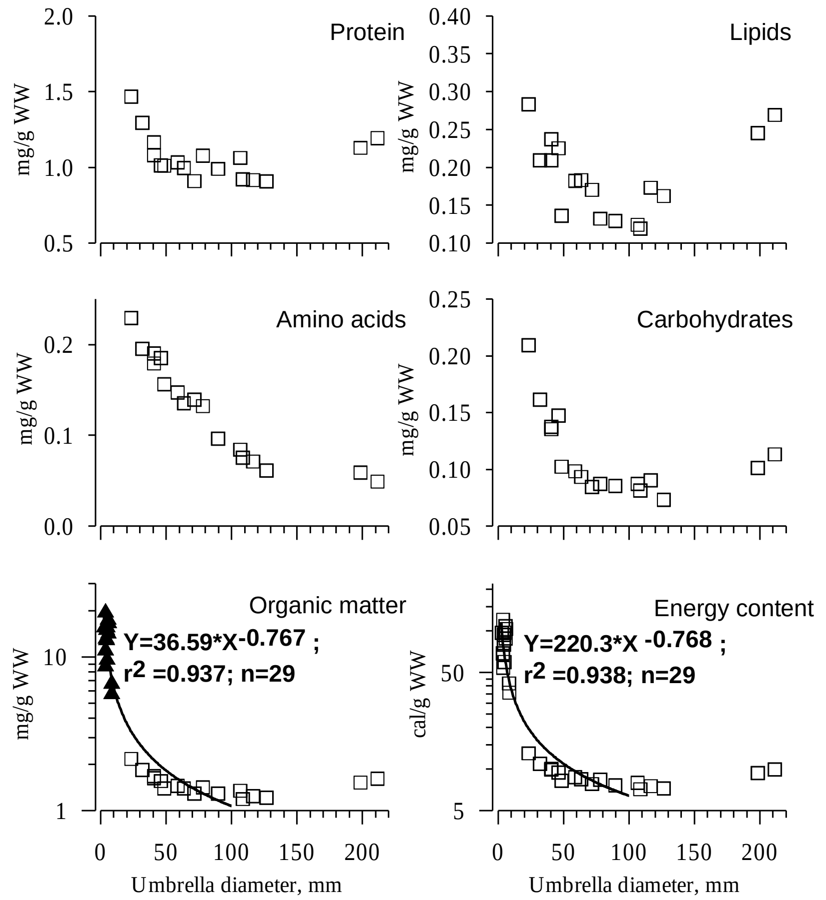


Figure 3.2. Proximate biochemical composition, organic matter and calorific contents of the jellyfish *Aurelia aurita* from the Black Sea

A maximum organic content (20.3 mg/ g WW) was observed in ~2 mm ephyrae, while the minimum amount (1.20 mg/ g WW) was detected in medusae with 108 mm umbrella diameter. Upon release from the parent, ephyrae are only 0.8 mm wide (Hernroth and Gröndahl, 1983), and have an organic content close to 40 mg/ g WW. While growing up to an adult individual the weight-specific organic content of *A. aurita* decreases 30-fold. However, the organic content of medusae between 23 and 211 mm in size is rather stable, rarely showing more than a two-fold difference.

Sexual maturity is another significant factor influencing organic content of medusae. Usually, this process in *A. aurita* starts at an umbrella diameter of ~100 mm (Fig. 3.3A). The wet mass of gonads in both males and females increases allometrically up to 2-3 g/ind. in medusae of ~200 mm size (Fig. 3.3B). After fertilisation, some 100-200 thousands planula - larvae begin to develop in pockets along the oral arms of females (Fig. 3.3C).

At some point, after attaining sexual maturity, *A aurita* completely stops its feeding. These starving, have to additionally expend their internal organic resources onto development of gonads.

In the ctenophores *B. ovata* and *M. leidyi*, starvation may be followed not only by a decrease in total organic matter content, but, due to catabolism of the gelatinous matrix, also by an increase in weight-specific organic content (Anninsky *et al*., 2005). So, individuals appear to become more compact during food shortage. Perhaps, a similar phenomenon occurs in starving *A. aurita* medusae, which utilise components of the most hydrated mesogloea as an energy source, thereby accounting for the higher weight-specific organic content of larger mature specimens.

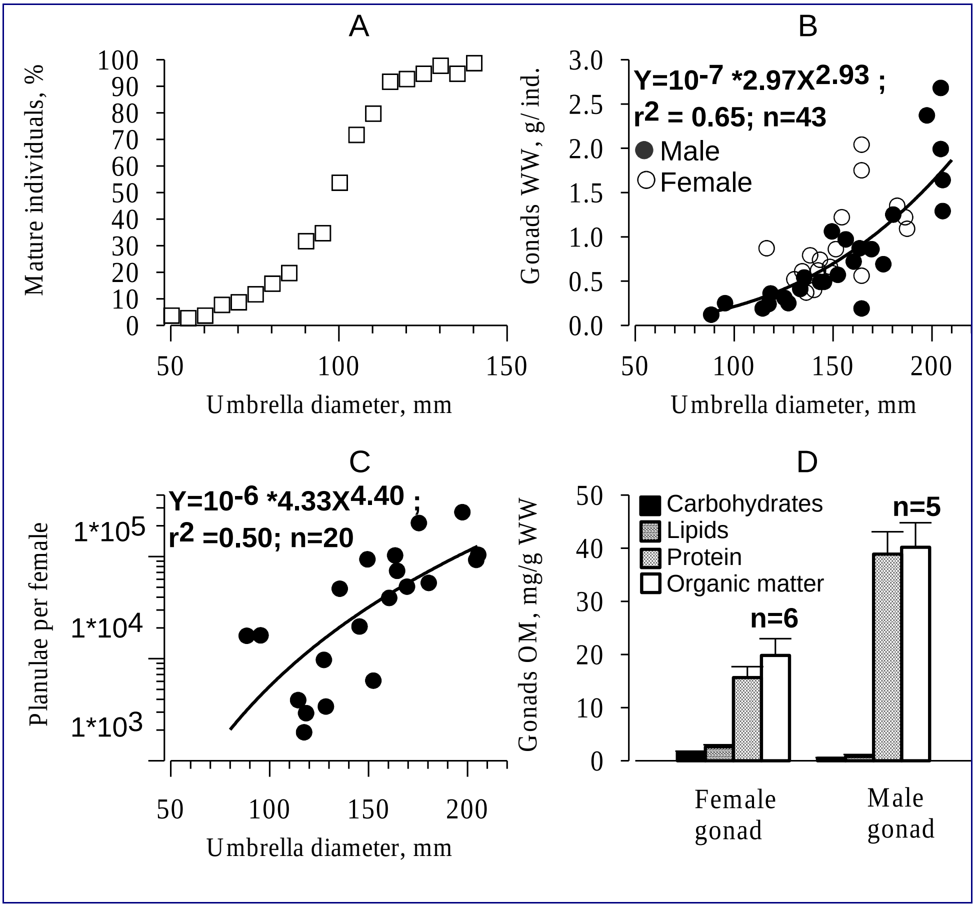


Figure 3.3. Adulthood (A), fecundity (C), gonads wet weight (B) and gonads proximate biochemical composition (D) of the jellyfish, *Aurelia aurita* from the Black Sea

Energy content

The calorific content of organic matter in *A. aurita* of 23-211 mm umbrella diameter is (± SD) 6.01 ± 0.08 cal/mg OM (1cal = 4.186 J). If the composition of organic matter in *A. aurita* does not vary greatly in individuals up to the size of ephyra, the energy content of wet weight (Y, cal g^{-1}) for any individual with a diameter of less than 100 mm may be calculated according to the equation $Y = 220.3\ X^{-0.768}$, where X is the umbrella diameter (mm) (See Fig. 3.2). In specimens between 23 and 211 mm umbrella diameter, the calorific content averages (± SD) 9.04 ± 1.54 cal g^{-1} WW. Calorific values of 28-30 cal g^{-1} WW (Mironov, 1967; Shushkina and Musaeva, 1983; Shushkina and Arnautov, 1987) for *A. aurita* are, apparently, mistakenly overstated, as they are unattainable even if organic content is considered on the level of an ash-free dry weight (16.63 cal g^{-1} WW).

Types of prey

Numerous representatives of the marine zoo- and phytoplankton, particles of detritus and various organic fragments can be detected in the gastric cavity of *A. aurita* (Southward, 1955; Mironov, 1967; Möller, 1980; Stoecker *et al*., 1987; Båmstedt, 1990; Arai, 1991; Mills, 1995; Behrends and Schneider, 1995; Malej *et al*., 2006). If the probability of a potential food item being found in the immediate environment of *A aurita* is n_i, and the probability of this ingredient being found in the gastric cavity is r_i, then the probability of capture of such food objects by the jellyfish (E^*) is determined by the expression:

$$E^* = r_i/(r_i + n_i),$$

Where $0 \le E^* \le 1$. When the jellyfish captures any food without selection, $E^* = 0.5$, if $E^* > 0.5$ the food object is being selected by the jellyfish, and if $E^* < 0.5$ the food item is actively avoided/rejected. Probabilities n_i and r_i for each ingredient of the food can be calculated from a proportion Ni/N_Σ between the number Ni of food type i and number of all food items N_Σ, separately for the environment and gastric cavity of the jellyfish. In essence, E^* value represents bias along the electivity index (E) scale, proposed by Ivlev (1961):

$$E = (r_i - n_i)/(r_i + n_i);$$

$$E^* = 0.5E + 0.5.$$

However unlike *E*, *E** characterises the probability of preference, instead of a conditional value of the index ($-1 \leq E \leq 1$). As the probability of preference of whatever food component is being evaluated in relation to other offered components, *E** as well as the value of the Ivlev's index, depends on present food composition. Therefore, the true food spectrum of *A. aurita* can only be objectively determined when a very wide range of potential food components are available to select from.

An experimental study of food selectivity (*E *)* of ~150 individuals of *A. aurita* offered 18 natural combinations of Black Sea plankton has shown, that the jellyfish captures different food components with various probabilities (Figs. 3.4 and 3.5). Decapoda larval stages, eggs and early larvae of fishes, Bivalvia veligers, and *Paracalanus parvus* from Copepoda are amongst the most favoured organisms. *Oithona similis, O. nana* and the cladoceran *Penilia avirostris* are usually eaten by the jellyfish with a lower preference than that for zooplankton on average. Some species of Pyrrophyta, such as *Noctiluca scintillans* and *Ceratium fusus*, being detected in the diet only sporadically, are predated by *A. aurita* with an order of magnitude lesser probability than for zooplankton as the whole. Nevertheless these protozoans, and perhaps some bacterial aggregates, may also be eaten. Preliminary data for heterotrophic bacteria (*E** ± SD) (0.16 ± 0.05) suggest that they are preferred to representatives of Pyrrophyta (0.05 ± 0.08).

The food spectra of *A. aurita* vary depending on the predator’s size. Usually, with an increase in umbrella diameter, larger food items (larvae Decapoda, copepods *Pseudocalanus elegans* and *Acartia clausi*) are more prevalent in the diet of *A. aurita*. Among the most preferred food (*E**) for *A. aurita* with an umbrella diameter of ~100 mm are *P. parvus* (0.72), larvae of Decapoda & ichthyoplankton (0.69), *P. elegans* (0.60) and *A. clausi* (0.59); while for jellyfish with an umbrella diameter ~10 mm, favoured prey include Bivalvia veligers (0.89), *P. parvus* (0.60) and *P. avirostris* (0.53).

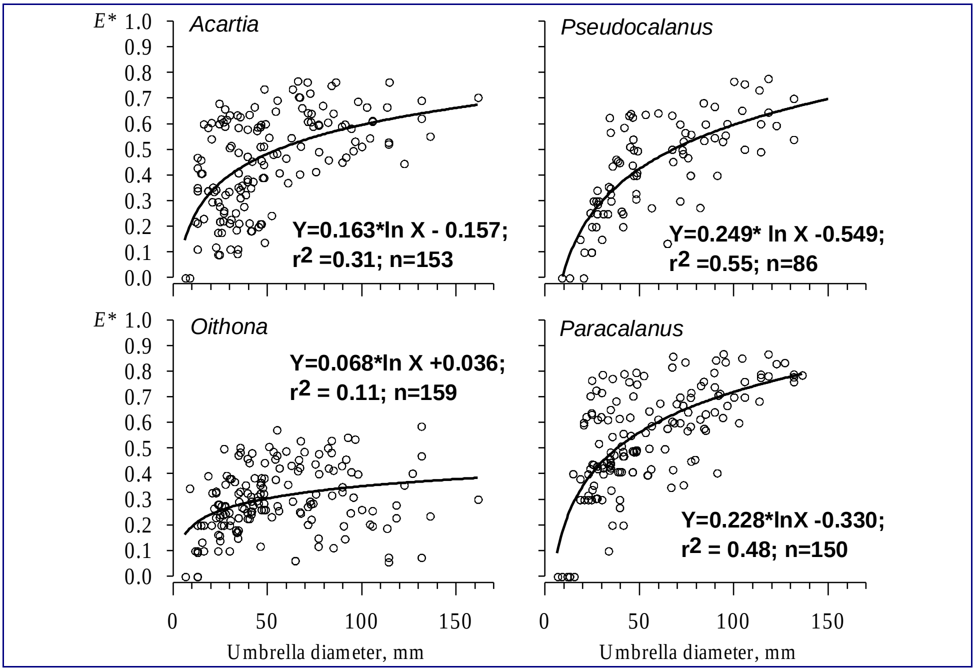


Figure 3.4. Probabilistic selection (*E) among four species of copepods, captured by the jellyfish *Aurelia aurita* from the Black Sea**

Differences in the preference for various kinds of food could be due to locomotor, morphological and/or chemical characteristics of the food components, as well as to the jellyfish's ability to select or avoid such objects. As a rule, those kinds of zooplankton which are preferred by the jellyfish lack avoidance behaviour (Bivalvia veligers, fish eggs), or such behaviour is poorly expressed (early fish & Decapoda larval). Since food particles and organisms are captured not only by jellyfish tentacles but also over all body surfaces, adhesive features of particles or integuments of food organisms also contribute to their relative preference. *P. parvus* among copepods appears to be especially predisposed to adhesion; correspondingly the preference of this species by *A. aurita* has been found the highest.

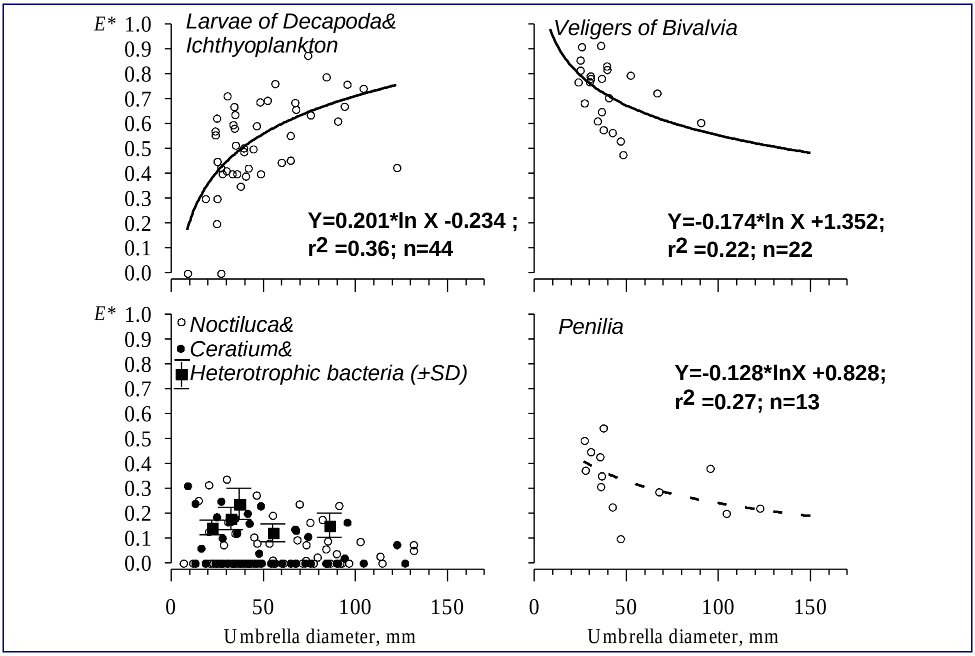


Figure 3.5. Probabilistic selection (*E) among some representatives of micro- and mesoplankton captured by the jellyfish *Aurelia aurita* from the Black Sea**

After retention of potential food items, food recognition is continued at the terminal part of the oral arms (Southward, 1955), which display a typical feeding reaction to protein, amino acids and extracts of a muscle tissue, but not to carbohydrates or inorganic particles (Arai, 1991; 1997). A substance known to activate feeding behavior in this species is the amino acid alanine (Lenhoff, 1971). Probably due to this, algae and vegetative particles, composed mainly of carbohydrates, are usually consumed by *A aurita* with a lower preference than for zooplankton. In particular, *N. scintillans* (Pyrrophyta) having a similar spherical shape, density and size to fish eggs, but with a cellullar wall formed of carbohydrates, has a ten-times lower probability of capture by the jellyfish.

Nevertheless, even at such low selectivity of algae or detrital particles, their prevalence among suspended matter of the sea can result in them being (by mass or energy content) comparable with zooplankton in the diet of *A. aurita*. According to early examination of 40 medusae from different regions of the Black Sea, the preponderance of *N. scintillans* amounted to them contributing 32% by mass of all organisms in the jellyfish diet (Mironov, 1967). In Crimean coastal watrers in spring 2007, the gastric cavities of medusae contained so much of the algae *Proboscia alata* (Bacillariophyta), that gastric filaments of many individuals acquired a deep-green colouring. *Coscinodiscus janischii* (Bacillariophyta) also is quite often discovered in *A. aurita* gastric cavities in large quantities. Similar observations have also been made in relation to detrital particles and some microorganisms (Southward, 1955). Moreover, algae are also registered not only in medusal but scyphistom diet too (Hernroth and Gröndahl, 1983).

"Vegetative" (algae + detritus) particles, however, while less preferred to zooplankton, still contribute significantly to the diet of *A. aurita*. In the food of specimens examined, the mean ratio of protein: carbohydrates was 2 (Table 3.3).

Table 3.3. Protein and carbohydrates in the mucus and the food of the jellyfish, *Aurelia aurita* from the Black Sea near the Crimea coast in March of 1986 (±SD)

Substance	Protein		Carbohydrates		N	Prot./ Carb.
	mg/g	%	mg/g	%		
Food & Mucus*	0.54 ± 0.08	77.1	0.16 ± 0.04	22.9	10	3.4
Mucus	0.22 ± 0.03	100	$(0.16 \pm 0.03)\times10^{-4}$	0.0	10	1.4×10^{4}
Food	0.32 ± 0.08	66.5	0.16 ± 0.04	33.5	10	2.0

*Overall sample for ~50 specimens of 80-130 mm in umbrella diameter

The food spectra of *A. aurita* vary depending on the predator's size. Usually, with an increase in umbrella diameter, larger food items (larvae

Decapoda, copepods *Pseudocalanus elegans* and *Acartia clausi*) are more prevalent in the diet of *A. aurita*. Among the most preferred food (*E**) for *A. aurita* with an umbrella diameter of ~100 mm are *P. parvus* (0.72), larvae of Decapoda & ichthyoplankton (0.69), *P. elegans* (0.60) and *A. clausi* (0.59); while for jellyfish with an umbrella diameter ~10 mm, favoured prey include Bivalvia veligers (0.89), *P. parvus* (0.60) and *P. avirostris* (0.53).

In calanoid copepods, as well as many other representatives of zooplankton, the value of this ratio is usually about 10 (Raymont, 1963), and for spring detritus of the Black Sea the average is 0.25 (Sorokin, 2002). Knowing the ratios of protein (Prot.) to carbohydrates (Carb.) in different food components allows the proportion of “vegetative” (veg.) and “animal” (an.) fragments in the gastric cavity of *A. aurita* to be calculated exactly. The initial equation set is as follows:

$$\text{Prot. an.} + \text{Prot. veg.} = 66.5\%$$

$$\text{Carb. an.} + \text{Carb.veg.} = 33.5\%.$$

Or, after substitutions the equations can be rewritten as:

$$\text{Carb. an.} \times 10 + \text{Carb. veg.} \times 0.25 = 66.5\%$$

$$\text{Carb. an.} + \text{Carb. veg.} = 33.5\%.$$

Then we obtain:

$$\text{Prot. \& Carb an.} = 65.6\%$$

$$\text{Prot. \& Carb veg.} = 34.4\%.$$

Therefore, in the coastal zone of Crimea in the spring of 1986, detrital particles formed 34.4 % of jellyfish food. Moreover, if it is assumed that phytoplankton constitute 10% (Sorokin, 2002) of the “vegetative” component, the share of “vegetative” fragments of the total food consumed increases up to ~ 40%.

These values differ from many visual estimations of the food spectrum of this species. In some investigations, the presence of “vegetative” fragments in the food of *A. aurita* has been entirely ignored (Behrends and Schneider, 1995; Mills, 1995) or considered to be at a level of less than 5% (Mőller, 1980). Such large differences in reported values could be explained, partly at least, by differences in feeding behaviour brought about by changes in food availability. In a study of feeding behavior of cubomedusae, some organic compounds that originally failed to provoke any feeding reaction, later stimulated such reflexes after a short period of a starvation (Larson, 1976). Similar occurrences were sometimes observed in aquaria with *A. aurita*: small fragments of macrophytes that were rejected as a food when other types of food were available, were captured and eaten by fasting specimens.

Clearance rate

Already the definition of clearance rate (the volume of water cleared by each animal per unit of time) implies that the body mass, physiological activity and capturing of the foods are undoubtedly to be among the most significant factors of this characteristic of feeding for *A. aurita*. The dependence of clearance rate (*C*) on the wet weight of medusa (*WW*) can be expressed by the power function:

$$C = a\, WW^{b},$$

where *a* and *b* are coefficients.

In the equations ($r^2 = 0.851$-0.910; $p < 0.001$) the clearance rates of *A. aurita* (*C*, l $\text{hour}^{-1}\text{ind.}^{-1}$) at three different temperatures (7.8, 12.2, 21.4 ºC) were determined, and the coefficient values obtained within the ranges 0.10-0.29 and 0.837-0.901 for intercept (*a*) and slope (*b*), respectively (Fig. 3.6). The mean value of *b* is (±SD) 0.861±0.035.

Under such value of the coefficient the intercept factors' *a* at temperatures of 7.8, 12.2 and 21.4 ºC, and a comparable range of food concentrations (<10 mg l^{-1}) are about 0.09 (n = 58), 0.16 (n = 18) and 0.33 (n = 48), correspondingly. Therefore, the clearance rates for a jellyfish of wet body weight 1 g (~27 mm in umbrella diameter) under these conditions are 0.09 (n = 58), 0.16 (n = 18) and 0.33 (n = 48) l $hour^{-1}$, accordingly.

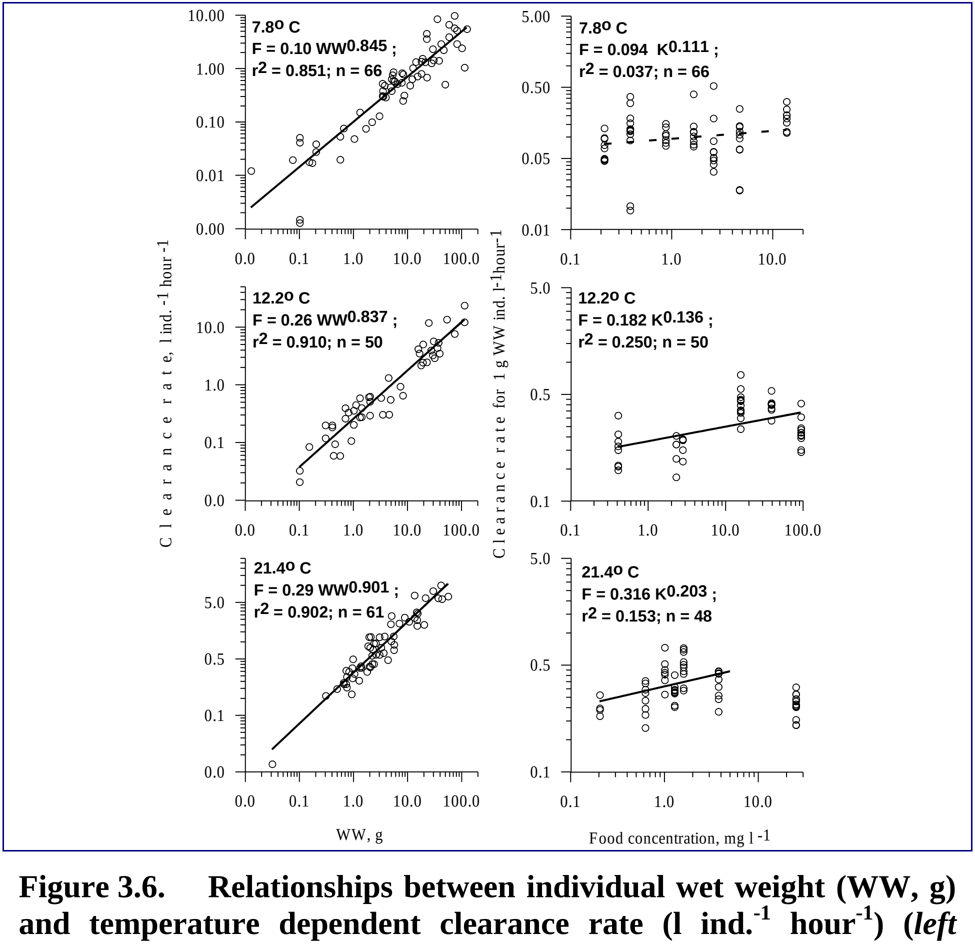


Figure 3.6. Relationships between individual wet weight (WW, g) and temperature dependent clearance rate (l $ind.^{-1}$ $hour^{-1}$) (*left blocks*) and between temperature dependent clearance rate of 1g WW individual (l $ind.^{-1}$ $hour^{-1}$) and food concentration (mg l^{-1}) (*right blocks*) of the jellyfish *Aurelia aurita* from the Black Sea

The clearance rate of this species is positively dependent on seawater temperature, but not linearly over the temperature range investigated. At lower temperatures (7.8-12.2 ºC) the value of temperature acceleration (Q_{10}) of clearance rate is on average higher ($Q_{10} = 3.4$) than at the higher temperature range of 12.2-21.4 ºC ($Q_{10} = 2.3$). However, in similar experiments with ephyrae of *A. aurita* fed on *Artemia* nauplii, Q_{10} values varied from 2.5 to ~2.0 within the temperature ranges 6-12.2 and 12.2-18 ºC, respectively (Båmstedt *et al.*, 1999).

The concentration of zooplankton can also influence the clearance rate of *A. aurita*. At lower food concentrations the relationship is positive, but at food concentration above 10 mg l^{-1}, an inverse correlation exists between food concentration and clearance rate. This might be caused by losses of food due to the excessive aggregation of captured particles in food pockets around the umbrella margin, due to an increase in mucus secretion and/or umbrella pulsation. The existence of such a relationship between food concentration and clearance rate appears to be expedient considering usually patchy distribution of a zooplankton in the sea (Sorokin, 2002).

It is not known how the composition of zooplankton offered as food for the jellyfish can affect *A. aurita* clearance rates, but in individuals collected from the Western Baltic clearance rates for 8.5 mm sized medusae feeding on the rotifer *Brachionus plicatilus* at 15 ºC were within the range 0.010-0.015 l hour^{-1} (Olesen *et al.*, 1994). Black Sea clearance rate data for *A aurita* of the same size feeding on mesozooplankton were similar (0.010-0.018 l hour^{-1}).

Feeding rate

As well as in case of clearance rate, the relationship between feeding rate (*F*, mg ind.$^{-1}$ hour^{-1}) and wet body weight (*WW*) for *A. aurita* is usually expressed by the power function:

$F = d\, WW^{f}$,
where *d* and *f* are constants.

As the rates of both processes (feeding and clearance) are determined by the same factors, there is no reason to believe that the slope factor' f may significantly deviate from the value of 0.86, obtained for the clearance rate. Then, the last equation can be rewritten as

$$F = d\ WW^{0.86}.$$

Thus, feeding rates numerically equal to factor d (F, mg ind.$^{-1}$ hour^{-1}) (or feeding rate for 1 g of wet weight individual) can vary widely, depending on food concentration and seawater temperature (Fig. 3.7).

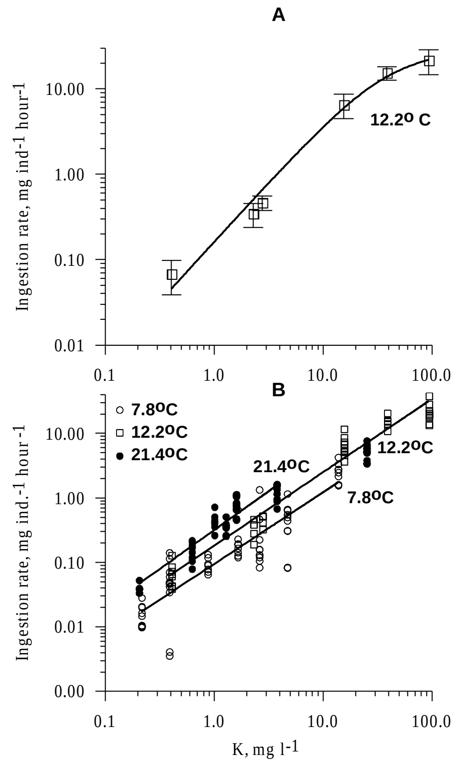


Figure 3.7. Relationships described by the mono equation (A) and the power functions (B) between temperature dependent ingestion rate (mg ind.$^{-1}$ hour^{-1}) and food concentration (mg l $^{-1}$) of 1 g WW jellyfish, *Aurelia aurita* from the Black Sea

Probably *A. aurita* starts it's feeding at a very low food concentration, and at higher food concentrations the feeding rate reaches a maximum. From this it follows that the relationship between feeding rate and food concentration for *A. aurita* is probably sigmoidal. This relationship is described by the Mono equation (Monod *et al.*, 1965), which is more often applied to biochemical kinetic relationships:

$$F = F_{max}\, K^c / (K^c + M),$$

Where: F_{max} is maximum value of feeding rate; K is food concentration; c is the interaction constant; and $M = (K_{max}/2)^c$.

At 12.2 ºC the equation parameters will be as follows

$$F = 28.8\, K^{1.4} / (K^{1.4} + 178) \qquad (r^2 = 0.99).$$

Therefore, the maximum value of feeding rate for 1 g of wet weight jellyfish at 12.2 ºC is 28.8 mg ind^{-1} hour^{-1}. A rather high interaction factor value (c=1.4) is required to achieve unity, meaning that the capture of any food particle favours the consumption of another. So, the assumption of a direct relationship between feeding rate and food concentration would be wrong; *A. aurita* is able to intensify its feeding. In the case of a dependent relationship between feeding rate (F) and food concentration (K) the following power equation would apply:

$$F = g\, K^h,$$

Where g and h are constants (Table 3.4).

At each of temperatures the value of h in the equations is greater than unity. Furthermore, this factor increases rising seawater temperature. Thus, *A. aurita* accelerates its feeding rate not only with respect to food concentration, but with the temperature too; a jellyfish penetrating into a zooplankton swarm at ~20ºC intensifies its feeding rate more quickly than at lower temperature.

Judging from values of constant' *g*, the feeding rate of a of 1 g wet weight jellyfish at a food concentration of 1 mg l^{-1}, temperature of 7.8-21.4 ºC for a short duration is 0.094-0.316 mg $ind.^{-1}$ $hour^{-1}$. Or, at a zooplankton concentration of about 0.25 mg l^{-1} (average value for the North-West shelf of the Black Sea, October 2005), the feeding rate at 21.4 ºC amounts to 0.06 mg $ind.^{-1}$ $hour^{-1}$, corresponding to ~4% of bodies' energy equivalent per day.

Table 3.4. ***Aurelia aurita*** **feeding rate parameters** $(F = g\ K^h)^*$ **in relation to food concentration (*K*, mg** l^{-1}**) and temperature (ºC)**

ºC	*K*, mg l^{-1}	*g*	*h*	N	r^2
7.8	0.2 - 13.6	0.094	1.11	66	0.80
12.2	0.4 - 93.1	0.182	1.14	50	0.96
21.4	0.2 - 3.7	0.316	1.20	48	0.86

**F* in the equation is ingestion rate (mg ind^{-1} $hour^{-1}$) for 1g WW jellyfish.

Daily ration

A. aurita is able to maintain a high feeding rate almost indefinitely at food concentrations that can not provide nutritional satiety. However, when the food is provided in excess of requirements, feeding rate gradually decreases to a level required to meet 'current' physiological/metabolic needs. In experiments, when 9 specimens *A. aurita* of wet weight of 4-74 g were maintained over a long period at excessive zooplankton concentrations (average±SD = 3.5 ± 2.0 mg l^{-1}), adjustments in feeding rate occurred predominantly during the first day (Fig. 3.8**).** Values of daily ration for individuals studied at 1-2 days duration of feeding were practically the same (p> 0.05; t- test) as those at 3-8 days duration of feeding.

Being a result of initial overeating of individuals under conditions of zooplankton excess the relationship between daily ration and food concentration for *A. aurita* remains positive regardless of the term of feeding. Similar observations have been made for this species by other workers, when medusae were kept at high rotifer concentrations (Olesen *et al.*, 1994). In fact long-term acceleration of feeding rate in conditions of the food excess for *A. aurita* makes little physiological sense. [It would do if the excess nutrients could be converted to storage compounds, but as discussed earlier in this chapter, there is no evidence of this being the case.] This is statement is supported the decrease trend of slope coefficient m in the following equation:

$$I = n\,K^{m},$$

Where: I is daily ration (mg ind.$^{-1}$) for 1g of wet weight individual; and n is constant (Table 3.5).

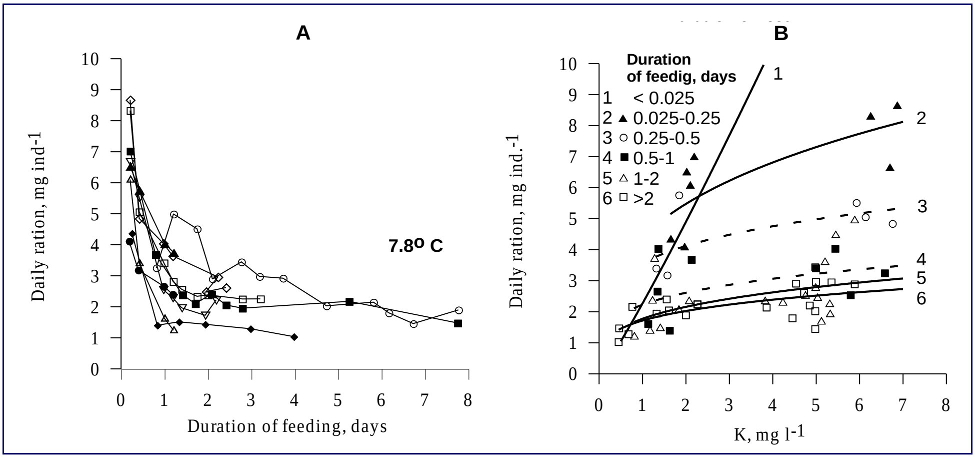


Figure 3.8. Variations in daily ration (mg ind $^{-1}$) of 1g WW *Aurelia aurita* from the Black Sea depending on duration of feeding (days) (A) and food concentration (mg l $^{-1}$) (B). (7.8°C)

Values for coefficient m decreased from 1.11 to 0.32, 0.28 and 0.24 with an increase in the term of feeding from <0.025 to 0.025-0.25, 1-2 and >2 days, respectively. In ctenophores, true food requirements at excess food levels can also be accounted for by a decrease in assimilation efficiency (Reeve *et al.*, 1989).

Table 3.5. ***Aurelia aurita*** **daily ration parameters ($I = n K^m$)* in relation to food concentration (K, mg l^{-1}) and duration of feeding (day) (7.8ºC)**

Duration of feeding, day	K, mg l^{-1}	n	m	N	r^2	p
<0.025	0.2 - 13.6	2.268	1.11	66	0.80	<0.001
0.025-0.25	1.6 - 6.9	4.404	0.32	8	0.54	<0.05
0.25-0.5	1.3 - 6.8	3.570	0.21	6	0.40	>0.05
0.5-1	1.1 - 6.6	2.211	0.25	8	0.18	>0.05
1-2	0.8 - 5.9	1.775	0.28	18	0.27	<0.05
>2	0.4 - 5.9	1.716	0.24	21	0.47	<0.001

*I in the equation is daily ration (mg ind^{-1}) for 1g WW jellyfish.

When maintained at the same temperature, a comparison of the daily ration at a maximum feeding rate with that occurring after feeding >2 days shows that both rations begin to change at food concentrations of >0.73 mg l^{-1}. At food concentrations >0.73 mg l^{-1} *A. aurita* maximal feeding rate is suppressed, the daily ration at K = 0.73 mg l^{-1} can be defined as the minimal sufficient for nutritional satiety (*SI*), corresponding to the physiological/metabolic requirements of the jellyfish. For an individual wet of weight 1g, *SI* =1.60 mg $ind.^{-1}day^{-1}$ or, at 7.8ºC:

$$SI = 1.60\ WW^{0.86}.$$

Higher values for *SI* of medusa (at K >0.73 mg l^{-1}) indicates that mechanisms to prevent excessive feeding by *A. aurita*, are under-developed.

Assimilation efficiency

Al food items are catabolised by *A. aurita* in 2 stages: initially in the gastric cavity and then intracellulary in amoebocytes of the mesogloea. Judging by discoloration of blood cells offered to *A. aurita* as food, it takes about a day to complete their utilisation (Vetokhin, 1930). Probably, no less time is needed for this species to assimilate natural food, although it is frequently accepted that digestion has essentially been completed by the time of evacuation of undigested food particles or their visual disappearance. By these criteria, herring larvae at 10-12ºC are digested by *A. aurita* within 3-10 hours (Möller, 1980). Observations in the current study, suggest a similar time-scale for the digestion of small-medium sized copepods (*A. clausi* and *P. parvus*) by medusae, while *C. euxinus* with its coarser body's covering is usually digested over 10-24 and 7-21 hour periods at 9.8 and 21.6ºC, respectively. Therefore, the digestion time for *A. aurita*, when feeding on zooplankton, should be taken as ~1 day.

On the basis of this assumption, the assimilation efficiency (*AE*) of this species ranged from 0.39 to 0.86, with a mean value of (±SD) 0.59 ± 0.15 (Fig. 3.9).

No significant differences (p>0.05; t-test) were found between the assimilation efficiencies of net plankton dominated by *A. clausi* (*AE* = 0.57 ± 0.13) and a copepod *C. euxinus* (*AE* = 0.61 ± 0.18). On the whole, irrespective of food type, the efficiency of zooplankton utilisation by *A. aurita* was inversely correlated with both weight–specific feeding rate and food concentration. Maximum values of assimilation efficiency (*AE* = ~0.80) are more likely to occur at low weight-specific feeding rates and low food concentration, while lower assimilation (*AE* = ~0.40) is more often observed at the higher values of these parameters. Furthermore, with regard to the weight-specific feeding rate, this dependence has manifested itself a bit more definitely (r^2 = 0.484) than in relation to a food concentration (r^2 = 0.449). It seems not unlikely, as against of a food concentration, the weight-specific feeding rate characterizes a direct input of a food into the jellyfish' organism. The analogous data on assimilation efficiency variability are known also for ctenophore *M. leidyi* (Reeve *et al*., 1989).

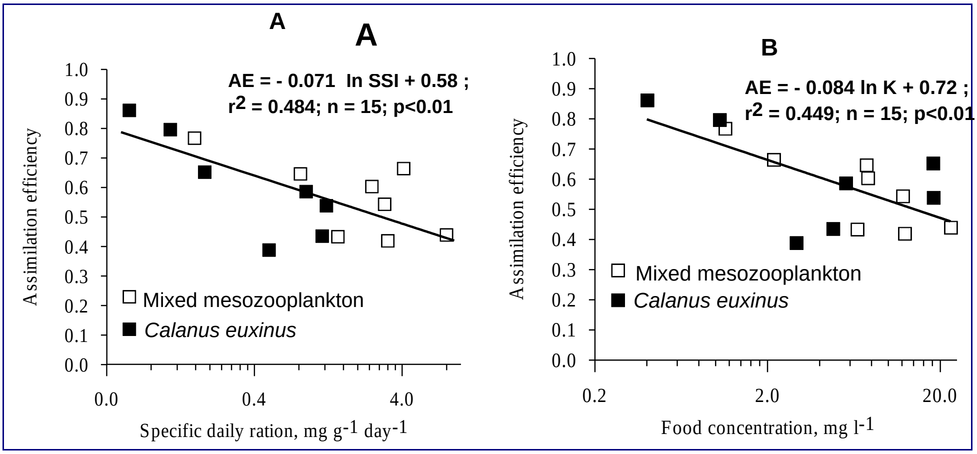


Figure 3.9. Variations in assimilation efficiencies of mixed zooplankton and copepod *Calanus euxinus* depending on weight-specific daily ration (*SSI,* mg g^{-1} day^{-1}) (A) and food concentration (mg l^{-1}) of the jellyfish, *Aurelia aurita* from the Black Sea (B)

Insufficient data are available to determine the influence of seawater temperature on assimilation efficiency in *A. aurita*. However, as the temperature can appreciably affect metabolic rates, some dependence of medusae assimilation efficiency on seawater temperature seems to be logical. Assuming that the relationship between assimilation efficiency and temperature is the same as that for the clearance rate (Q_{10} = 2.6 in the range 7.8-21.4°C), then the dependence of assimilation efficiency on feeding rate can be calculated with greater precision (taking into account temperature correction of the data). In particular at 21.4°C, assimilation efficiency can be estimated as:

$$AE = -0.095 \ln SSI + 0.63 \qquad (r^2 = 0.584; n=15; p<0.001),$$

where *SSI* is weight-specific daily ration.

These calculations are evidence of possible increase of *A. aurita* assimilation efficiency with rising temperature.

Size-age development of the digestive system can also affect the assimilation efficiency of some marine organisms (Omori and Ikeda, 1984). However, no relationship between assimilation efficiency and wet weight (range 0.4-35.8 g) has been detected in Black Sea *A. aurita* ($p>0.05$; t-test).

Respiration rate

As a function of body wet weight (*WW*, g), the respiration rate (R, μl O_2 ind.$^{-1}$ hour^{-1}) for *A. aurita* can be represented by the power equation: $R = q\ WW^{k}$ ($r^2 = 0.882 - 0.966$; $p < 0.001$), where values of the coefficients q and k at 7.6 - 23.0ºC are between 0.810-0.886 and 3.21-9.92, respectively (Fig. 3.10). These data agree well with other calculations in which respiration rates for the jellyfish are based on power equations with the slope (k) and intercept (q) constants varying at 10-20ºC between 0.83-0.92 and 5.48-10.53, respectively (Table 3.6).

The mean value of $k \pm$ SD calculated from our data (0.840 ± 0.029) is not statistically different ($p > 0.05$; t-test) from the most probable value of this coefficient from other studies (0.868 ± 0.039), nor from the value of this factor for *A. aurita* clearance rate (0.861 ± 0.035). Using all collated data for *A. aurita*, k is inversely related to temperature (t; $p < 0.05$):

According to the linear regression obtained, k values can range from 0.89 to 0.81 within the temperature range 7-23ºC. A negative relationship between k and temperature exists for many poikilothermic organisms (Ivleva, 1981), and with regard to *A. aurita,* testifies to better temperature tolerance in young small-size medusae which enhance respiration greater than adults. It is possible that early developmental stages of this species gain some advantages in temperature tolerance due to maintenance of high level of active metabolism. The data concerning the same coefficients for clearance rate, which depend on feeding electivity and conflict with this assumption, cannot be sufficient argument against it.

Table 3.6. *Aurelia aurita* respiration rate parameters ($R = q\ WW^k$)* from different sources. Values *R* and *WW* are expressed as μl O_2 ind.$^{-1}$ hour^{-1} and g, respectively

°C	*WW*, g	*q*	*k*	Source
7.6-23.0	0.02 - 96.87	3.21-9.92	0.81-0.89	Present study
13-15	18.92 - 189.21	7.70	0.83	Thill, 1937
20	5.35 - 48.63	6.90	0.83	Yakovleva, 1964
20	0.81 - 259.40	7.20	0.86	Kuzmicheva, 1980
10-15	~0.27 - 173.80*	5.60 – 10.53*	0.91-0.92	Larson, 1986
15	~0.02 - 93.72**	5.48**	0.86	Frandsen and Riisgard, 1997

* Calculated assuming DW = 3.74% of WW at seawater salinity 34‰

** Calculated assuming DW = 18.14% of WW at seawater salinity 15‰.

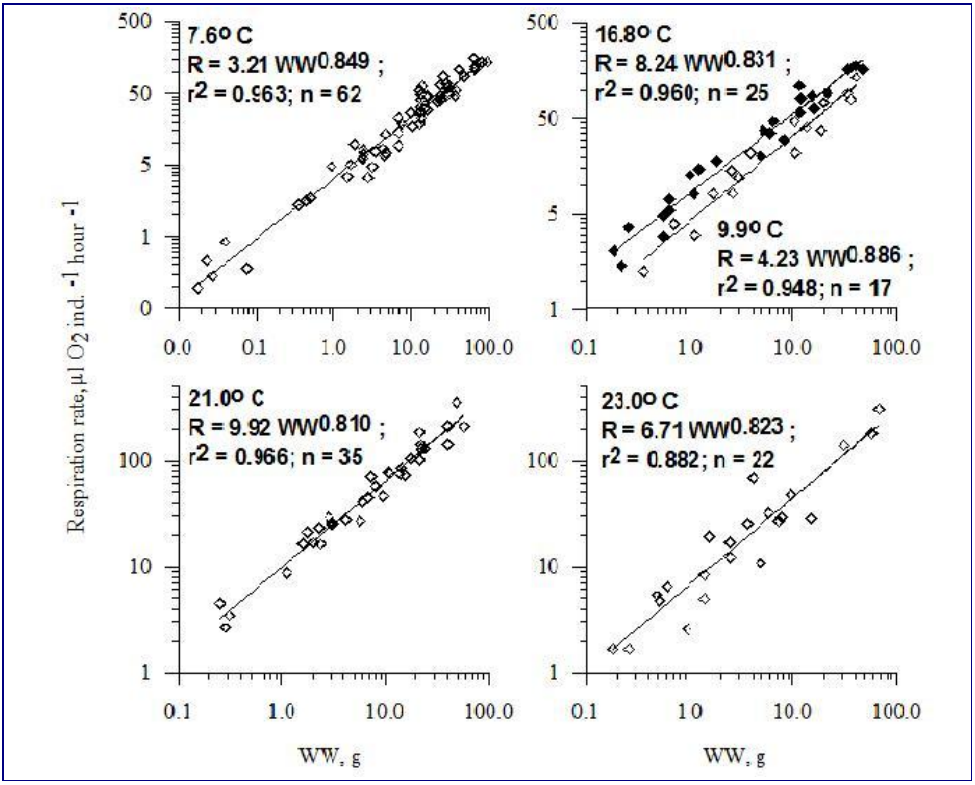


Figure 3.10. Relationship between temperature dependent respiration rate (µl O_2 ind.$^{-1}$ hour^{-1}) and body wet weight (WW, g) of the jellyfish *Aurelia aurita* from the Black Sea

$$k = -0.00425\, t + 0.922 \qquad r^2 = 0.351;\ n = 11.$$

The variation of *k* with temperature suggests that the influence of temperature on respiration rates of individuals of different wet weight may differ. If we restrict the analysis for this dependence by the general trend (when *k* = 0.84), then a temperature increase from 7.6 to 23.0ºC will result in a 2-3-fold increase in respiration rate. However, this increase in respiration rate is not linear over this temperature range (Fig. 3.11). An increase in *A. aurita*'s oxygen consumption between 7.6 and

9.9ºC continues up to 20°C, and levels off at 20-21ºC. Between 21 and 23ºC the respiration rate falls sharply.

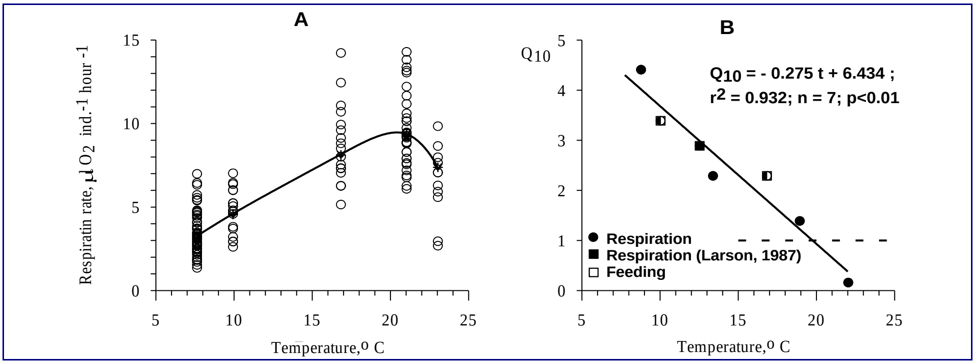


Figure 3.11. Variations in respiration rate (μl O_2 ind.$^{-1}$ hour^{-1}) (A) and temperature acceleration of metabolic rate (Q_{10}) (B) for 1g WW *Aurelia aurita* depending on seawater temperature (°C). Dotted line corresponds to the maximum rate of the metabolism (Q_{10} = 1)

The temperature at which the maximum respiration rate (t_{max}) occurs can be approximately calculated from the regression equation:

$$Q_{10} = -0.275\,t + 6.344 \qquad r^2 = 0.932;\ p < 0.01,$$

Where Q_{10} is the Vant Hoff coefficient and *t* is the temperature.

When Q_{10} = 1, the respiration rate in *A. aurita* reaches the plateau. Hence the temperature corresponding to the maximal value of metabolic rates (t_{max}) is 19.75ºC. The predicted respiration rate for a jellyfish of 1 g wet weight at this temperature and *k* = 0.84 amounts to (±SD) 9.36 ± 0.47 μl O_2 ind.$^{-1}$hour^{-1}. At t >19.75ºC, a temperature influence on *A. aurita* metabolic rates seems to be the depressive one. In the Black Sea environment, medusae usually prefer to avoid such warm-water conditions. Indeed, this may be one of the reasons contributing to aggregations of medusae near the thermocline. In August, 1991, 70% of *A. aurita* biomass was concentrated within the thermocline zone (data for the North-West Black Sea, 34 cruise of sci. vessel "Professor Vodyanitsky"; Anninsky and Abolmasova, 2000).

Feeding of medusae at a zooplankton concentration of about 1 mg l^{-1} for 1 hour had no significant effect ($p > 0.05$; t-test) on *A. aurita* respiration rates. On average, the respiration rates for fed specimens as compared with unfed ones were higher by only 1.5% (Fig. 3.12). Similar data are known from other experiments (Frandsen and Riisgard, 1997). Therefore, as for the ctenophore *M. leidyi* (Kremer *et al.*, 1986), feeding at moderate concentrations of zooplankton has little effect on *A. aurita* respiration rates.

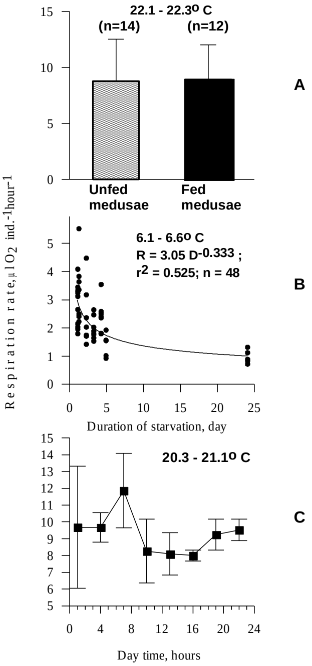


Figure 3.12. Variations in respiration rate (µl O_2 ind.$^{-1}$ hour^{-1}) of 1g WW *Aurelia aurita* from the Black Sea depending on state of the food supply (fed, unfed) at 22.1-22.3°C (A) duration of starvation at 6.1-6.6°C (day) (B) and day time (hours) at 20.3-21.1°C (C)

However, prolonged periods of food shortage results in a decrease in *A. aurita* metabolic decrease. Under experimental starvation at 6.1-6.6ºC the rate of oxygen consumption in medusae decreased, starting 2-3 days into the period of starvation. On the 5th day of starvation, the metabolic level in fasting individuals diminished by ~40% in relation to their initial oxygen demands. With more prolonged starvation, the metabolic rate continued to decrease slowly, accompanied by visual changes in medusae morphometry and coloration. Over the whole starvation time (24 days) the metabolic rate of *A. aurita* decreased by 65%. Thus, the basal metabolic rate appears to be only 35% of that occurring in fed jellyfish.

The active metabolic rate can be calculated for *A. aurita* from daily respiration dynamics. Respiration rates during the morning exceed those measured during other times of the day. These variations are presumably caused by migrating activity, which is especially prominent during sunrise and sunset periods (Malej *et al.*, 2006). This activity enhancement increases the mean metabolic rate of *A. aurita* by ~33%.

Growth

In contrast to feeding and respiration rates which are easily monitored in experiments, the growth rates for post-ephyra stages of *A. aurita* almost never attain the values adjusted to natural habitat. Hence, only field measurements of medusa growth rates can provide evidence of how much of the physiological potential of this species is realised in reality.

A. aurita populations display great size-age variability (Fig. 3.13). This is caused by many reasons, including the continuous and irregular renewal of populations by the production of ephyrae, spatial and temporal fluctuations in growth rates of ephyrae and adults, gradual shrinkage (or “degrowth”) and elimination of senescent individuals. Although ephyrae of *A. aurita* are observed in the Black Sea plankton over the whole year (albeit seldom in July – August), their mass separation from polyps usually occurs with a seasonal increase in seawater temperature. Thus, ephyrae numbers reach a maximum between January and March (in 1984 and 2000-2005). Each year, just these generations of ephyrae make up a modal size-age class in *A. aurita* population. In the process of the growth, this group of individuals tends to increase in both mean umbrella diameter and size spectrum, finally becoming more and more dissipated. In the Black Sea, *A. aurita* can grow up to ~360 mm in umbrella diameter; however, <1% of individuals are >200 mm in size. This means that medusae extremely rarely grow up to this size and their growth rate cannot be correctly determined by the date of appearance of ephyrae and the biggest adults (Mironov, 1967).

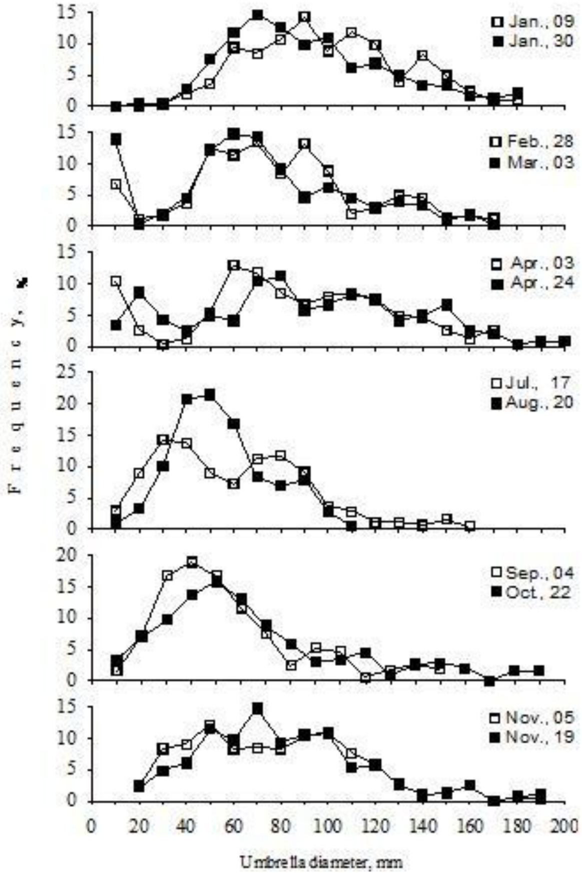


Figure 3.13. Size frequency distribution of jellyfish *Aurelia aurita* within Crimean shelf of the Black Sea in 1984

For various scyphomedusae, growth rates in the sea are initially exponential (or close to it), but gradually deviates from this pattern with increasing age (Zaika, 1983). Nevertheless during active phase of the growth a weight-specific decrease in growth rate can be expressed by the power equation:

$$P = dW/dt = p\ W^{q},$$

where p and q are constants.

As the slope factor of this equation (q) corresponds to the same coefficient in equations for respiration rate (k) (Winberg, 1986), then this equation can be rewritten as

$$dW/dt = p\ W^{0.84}.$$

Or, if the weight increments for the period covered are not large, the equation may be given as:

$$(W_2 - W_1)/\ (t_2 - t_1) = p\ W^{0.84},$$

Where W_1 and W_2 are values of wet weight at times $t_1$and t_2, respectively, and $W = (W_1W_2)^{0.5}$.

Hence, the growth rate for a jellyfish of wet body weight of 1 g, being numerically equal to p coefficient, can be calculated as follows:

$$p = (W_2 - W_1)/\ (t_2 - t_1)/W^{0.84}.$$

According to 1983-1984data , the growth rate for *A. aurita* of 1 g wet weight on the Crimean shelf amounted to 3.1-31.6 mg WW^{-1} day^{-1} (Fig. 3.14), corresponding to a weight-specific growth rate of 0.003-0.032 day^{-1}. At the same time, for *A. aurita* ephyrae (with a wet weight of 1.4×10^{-3} g and umbrella diameter ~3 mm) the growth rate should hypothetically be between 0.009 and 0.09 day^{-1}. In fact, under favourite conditions, the growth of ephyrae can be even faster than this, due largely to body hydration. Because of this, ephyrae of *A. aurita* are able to grow at a weight-specific growth rate of up to 0.7 day^{-1} (Båmstedt *et al.*, 1999), which is virtually unattainable following developmental stages (Table 3.7). Higher weight-specific growth rates are also found for *A. aurita* medusae of earlier (rather than later) developmental stages from different regions of the North Atlantic.

During 1983-1984, in the Black Sea, *A. aurita* grew fastest during May to July and slowest during the winter months. However, it is not clear how this much this seasonality in growth rates was influenced by temperature or seasonal dynamics in the food supply.

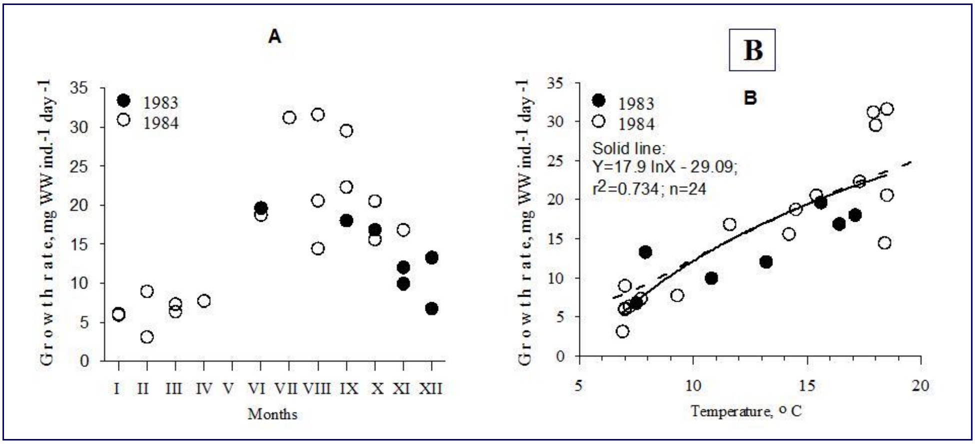


Figure 3.14. Variations in growth rate (mg WW ind.$^{-1}$ hour^{-1}) of 1g WW *Aurelia aurita* from the Black Sea depending on season (A) and seawater temperature (B) during 1983-1984. Solid line corresponds to statistical trend for growth; dotted line corresponds to Q_{10} trends for feeding and respiration

Table 3.7. Weight-specific growth rate (day^{-1}) estimated for jellyfish *Aurelia aurita* from different regions

Area	Specific growth rate, day^{-1}	Source
The Black Sea	0.003 – 0.032*	Present study
The Black Sea	0.009 - 0.090**	Present study
The Black Sea	0.03 – 0.04	Shushkina *et al*., 2000
Kiel Bight	0.087 – 0.12	Möller, 1980
Kiel Bight	≤0.20	Schneider, 1989
Kertinge Nor	<0 - 0.092	Olesen *et al*., 1994
Kertinge Nor (lab.exp.)	<0 - 0.25**	Olesen *et al*., 1994
Kertinge Nor (lab.exp.)	0.14 – 0.24**	Frandsen and Riisgard, 1997
Southampton Water	≤0.28	Lucas and Williams, 1994
Gullmar Fjord	0.028 – 0.119	Hernroth and Gröndahl, 1983
Gullmar Fjord	0.11 – 0.16	Hansson, 1997
Gullmar Fjord (lab.exp.)	0.05 – 0.15	Hansson, 1997
The North Atlantic	0.09 – 0.12	Verwey, 1942
The North Atlantic (lab.exp.)	0.175 – 0.345**	Båmstedt *et al*., 1999

* Calculated for 1g WW jellyfish
**Calculated for ephyrae

Assuming that 73–94% of the *A. aurita* population is found in the uppermost 30 m layer of water (Mironov, 1971; Vinogradov and

Shushkina, 1982; Anninsky and Abolmasova, 2000), then temperature appears to be of vital importance for the growth of this species. As shown previously, the relationship between jellyfish growth rate and temperature is strong and positive (up to ~20°C). On average, with the temperature enhancement from 7.5 to 17.4ºC the daily growth rate of a 1g WW individual increases from 7.1 to 22.54 mg WW. Although empirically this dependence can be described as semi-logarithmic one ($r^2 = 0.74$), it agrees well ($p>0.05$; Mann-Whitney – test) with similar dependences for both feeding and respiration rates. So, there are no reasons to believe that temperature dependence for *A. aurita* growth rate has Q_{10} coefficients which are different from coefficients calculated for other physiological parameters. Consequently, using 1983-1984 data, the most probable value of growth rate for an individual of 1g WW at t_{max} = 19.75ºC is 24.31 mg WW^{-1} day^{-1}.

The dependence of *A. aurita*'s growth rate on food supply is well illustrated by seasonal dynamics of mean umbrella diameter during 1983-1984 and 1999–2006 (Fig. 3.15). However, these two periods the Black Sea plankton community differed substantially. Prior to 1988, *A. aurita* was the overwhelmingly dominant gelatinous predator in near-surface horizons, with a plentiful supply of zooplankton. But in 1988-1990 a mass outbreak of *M. leidyi* occurred in the Black Sea, resulting in a rapid decrease in abundance of mainly warm-water zooplankton species, and even to the disappearance some of them (*Oithona nana*). The jellyfish population is also believed to have diminished, although available data are too scarce to confirm this statistically. The penetration into the sea of the ctenophore' *B. Ovata,* which feeds exclusively on other ctenophore species, appears to have resulted (in 1999-2006) in the gradual recovery of the whole planktonic community almost up to the level of 1960-1980. Nevertheless a peak abundance of *M. leidyi* occurs in July-August each year, and is generally accompanied by a lowering of numbers and biomass of prey zooplankton (Finenko *et al*., 2006). Such seasonal reductions in zooplankton can result in depression, stagnation or even negative growth rates of *A. aurita*.

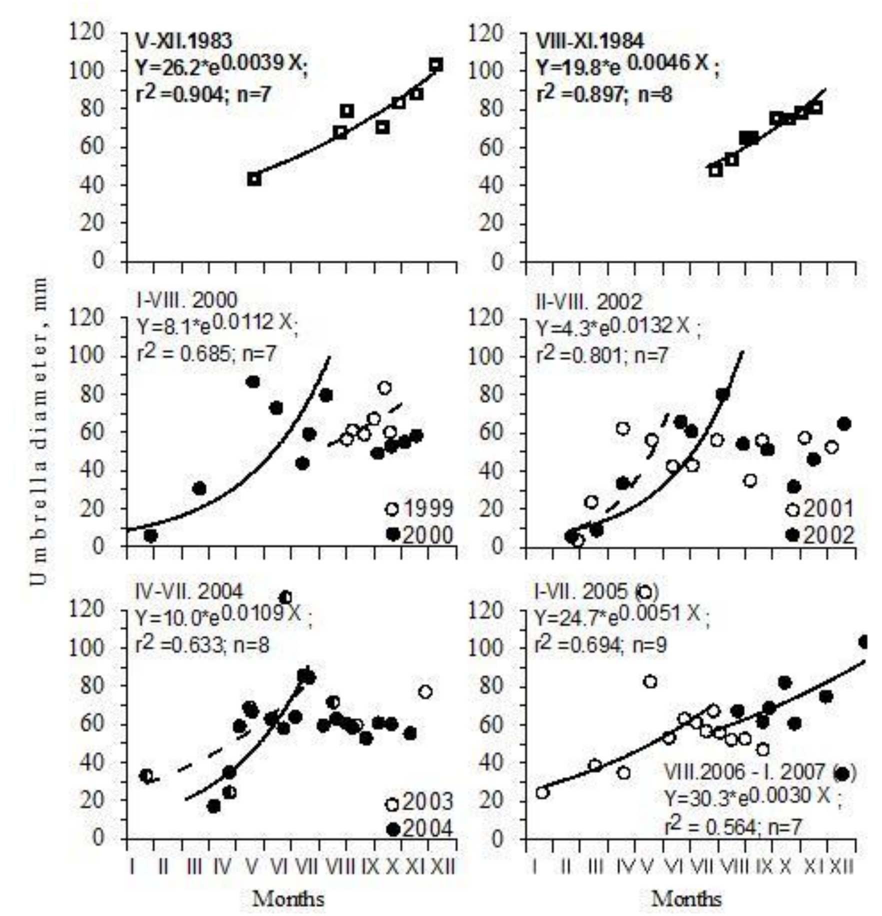


Figure 3.15. Dynamics in average umbrella diameter (mm) of jellyfish *Aurelia aurita* within Crimean shelf of the Black Sea during 1983-1984 and 1999-2006

According to long-term observations, during spring months in 1983-1984 and 1999-2006, *A. aurita* had similar growth rates to those occurring at the beginning of June, resulting in a mean umbrella diameter within the range 40-60 mm. However, due to a decrease in growth rates during summer 1999-2006, the mean umbrella size at the beginning of September did not exceed, 50-60 mm, compared to 60-70 mm in 1983-1984. This change in medusa growth rate is even more evident when December values are considered: in 1983-1984, the mean diameter size was 90-100 mm, compared to 40-80 mm in 1999-2006.

Under favourable conditions *A. aurita* growing for 9-10 months achieves an umbrella diameter of 90-100 mm. There is then a lessening in growth rate, but by 12 months, umbrella diameter is typically 110-120 mm. At such a size, most individuals become sexually mature. Taking into account that medusae of 110-120 mm represent size class close to the modal, then larger medusae (at least up to 150 mm umbrella diameter) may represent faster-growing individuals that achieve this size within the same 12 month timescale. The ages of the biggest individuals (up to 360 mm) are not clear: they could also be very fast-growing individuals or could be 12 months old.

Energy budget

The data obtained for *A. aurita* can be used to derive a variety of energy budgets, represented by the following generic equation:

$$I = P + R + U,$$

Where: I = ingestion (being equal to feeding rate, F or daily ration, SI); P = production (~growth); R = respiration; and U = excretion.

Unlike some previous calculations of energy or carbon budgets of medusae (Lebedeva and Shushkina, 1991; Shushkina *et al.*, 2000; Olesen *et al.*, 1994; Frandsen and Riisgard, 1997) the following analysis was undertaken predominantly using experimental (feeding and respiration) and field (growth) data, where possible negating the use of general assumptions. Nevertheless, some generally accepted conversion factors have. The calorie content for zooplankton ingested by the jellyfish has been taken as 0.7 cal mg WW^{-1} (Shushkina and Musaeva, 1983), and the value of the calorific equivalent of oxygen consumed, which varies depending on the type of substrate), is accepted as 4.86 cal. ml O_2^{-1} (Winberg, 1971; Omori and Ikeda, 1984).

When the energy budget is studied at the level of individual, two situations are of particular interest:

1. The partition of energy at sufficient food supply (optimal budget)
2. The partition of energy at a limited food supply that allows only compensating metabolic expenditures (maintenance budget).

Positive budgets developed for medusae in the sea depend on food conditions. These could be close to either of these variants, or fall some way between the two. Negative budgets are also realistic for *A. aurita* during some months, having been identified periodically during the summer period of 2000-2005.

Energy budgets for jellyfish of 1 g wet weight (27 mm in umbrella diameter) at conditionally “winter” (8ºC) and “summer” (20ºC) temperature are given in Table 3.8.

Table 3.8. Energy budget parameters for *Aurelia aurita* of 1g WW (27 mm in umbrella diameter) under conditions of the food excess (*) and at the food sufficient for maintenance of the respiration (**). *I* – daily ration; *AE* –assimilation efficiency; *R* –respiration rate; *P* - production; A_1, A_2 – assimilation; K_1 –gross growth efficiency; K_2 – net growth efficiency

ºC	*I*, cal. day^{-1}	*AE*	*R*, cal. day^{-1}	*P*, cal. day^{-1}	$A_1 = I \times AE$, cal. day^{-1}	$A_2 = R+P$, cal. day^{-1}	K_1	K_2
8*	1.15	0.54	0.36	0.22	0.62	0.58	0.19	0.37
8**	0.61	0.59	0.36	0	-	0.36	-	-
20*	3.45	0.47	1.09	0.51	1.62	1.60	0.15	0.32
20**	2.18	0.50	1.09	0	-	1.09	-	-

Since the assimilation values (A_1 and A_2) are the same for both of the optimal budgets and both of the maintenance budgets, all above data and dependences being used for the budget make up are internally consistent. So, in case of the optimal budget, the daily ingestion for a jellyfish of 1 g of wet weight in relation to body' energy equivalent (16.89 cal. ind^{-1}) ranges daily from 6.8 to 20.4 %. Such values are approximately half of those for the maintenance budget (3.6-12.9%). An almost halving of the ingestion rate is not fully reflected in assimilation efficiency, which variy from 0.47 to 0.59. These values are much lower than used previously in *A. aurita* budget calculations (0.7-0.9) (Shushkina *et al*., 2000; Olesen *et al*., 1994; Frandsen and Riisgard, 1997).

Using the growth rate calculated for 1983-1984, the daily rations for a jellyfish of 1 g wet weight at 8 and 20ºC would be ~0.90 and ~3.17 cal day^{-1}, respectively. Therefore, in respect to sufficient food supply, the actual food requirements of this species in these years were food limited by at least 8% in summer and by 22% in winter months. The daily ration of *A. aurita* was found to be only ~53-63% of that required to achieve the maximum growth rate, but even such daily maintenance rations could not be periodically supplied following the *M. leidyi* invasion into the Black Sea. Obviously, the period of negative growth rates of *A. aurita* during June-September 2005, the daily ration of medusae was less than that of the maintenance level.

Under conditions of non-limited feeding both gross (K_1) and net (K_2) growth efficiencies were relatively constant, with values ranging from 0.15 to 0.19 for K_1, and from 0.32 to 0.37 for K_2. Over the whole size range of growing medusae, both of these efficiencies are close to the maximum. Ephyrae may have higher growth efficiencies than adult medusae. If, in the initial growth phase, the maximum $K_2 \leq 0.8$ (Winberg, 1986), then from the point of view of the energy budget the weight increment in ephyrae, expressed as an organic or energy content, can hardly ever exceed 0.15 day^{-1} ($K_2 = 0.75$).

K_2 values close to the theoretical maximum (0.68 – 0.83) were obtained in experiments with *A. aurita* ephyrae from Kerting Nor (a shallow Danish fjord; Frandsen and Riisgard, 1997). However, other experimental data have shown that the net growth efficiency of ephyrae from Kerting Nor does not exceed 0.38, and under natural conditions it never exceeds 0.24 (Olesen *et al*., 1994). Similar K_2 values (0.37 and 0.18, under experimental and natural conditions, respectively) have been btained for *A. aurita* from Kiel Bight (western Baltic Sea) (Schneider, 1989).

The gross growth efficiency for *A. aurita* from the Black Sea (K_1 = 0.15-0.19) also well agrees with that (K_1 = 0.11-0.25) obtained for ephyrae of the same species fed on excess *Artemia* nauplii at a salinity of 35‰ and temperature of 6-18°C from coastal waters of the North Atlantic (Båmstedt *et al*., 1999). These experiments did not detect of any co-variability of K_1 with seawater temperature. For *A. aurita* from the Black Sea, a weak downtrend of K_1 and K_2 with decreasing temperature is also questionable; indeed this could be due to data variability.

On the whole, dependent on the food supply, indices of *A. aurita’* growth efficiency are similar to those of other gelatinous and non-gelatinous species of zooplankton and micronekton (Omori and Ikeda, 1984; Winberg, 1986; Bailey *et al*., 1995; Arai, 1997).

3.3 Populational bioenergetics of *A. aurita*

A. aurita’s survival under the Black Sea conditions are of vital importance when food-webs and and/or energy budget are analysed at the ecosystem level. As an example, it is of interest to observe the current state of the jellyfish population in the western Black Sea (studied during the cruise of scientific vessel “Vladimir Parshin” on October 6-14, 2005).

3.3.1 Materials and methods

The distribution of zooplankton was studied at 31 stations betweeb 41°14-46°06 N and 28°38-32°30 E during 6-14 of October, 2005. At each station, sampling was performed with a standard JOM and/or Jedy net by vertical hauls from upper boundary of the hydrogen sulphide zone (where σ_t = 16.2) to the surface. The density gradient was measured using a CTD probe (Seabird). A JOM net (mouth diameter 80 cm, mesh size 300 μm) was usually used for collecting gelatinous macrozooplankton a the Jedy net (mouth diameter 36 cm, mesh size 112 μm) for mesozooplankton. Community composition data were corrected for the catchability of individual species using different nets. Immediately, after collection, all medusae were measured to the nearest millimetre (umbrella diameter between statocysts) and zooplankton samples were fixed with Borax buffered 4% formalin. Jellyfish wet weight and biomass was calculated from a pre-determined length-weight relationship. Zooplankton samples were analysed for abundance and species composition, taking into account routine morphometric ratios (Petipa, 1957). No correction factors were applied to these data (Shushkina and Arnautov, 1987; Sorokin, 2002) to adjust net hauls to submersible observations.

3.3.2 Results and discussion

In the autumn of 2005, as in previous years (Mironov, 1971; Zaitzev and Polischuk, 1984; Mutlu *et al.*, 1994; Sorokin, 2002), the spatial distribution of *A. aurita* was highly aggregated. As a result of this, the biomass of medusae varied from <1 to 5600 g m^{-2}, (Fig. 3.16). Measured as the geometric mean, the biomass increased from coastal waters (8.4 g m^{-2}) through the continental slope zone (30.0 g m^{-2}) to the deep-see area (34.3 g m^{-2}). Since ~90% of *A. aurita* medusae can be found in the top 30 m layer of water during autumn (Mironov, 1971; Vinogradov and Shushkina, 1982; Anninsky and Abolmasova, 2000), the mean biomass values calculated per cubic metre in the upper warm horizon amount to ~0.26, 0.90, and 1.03 g m^{-3}, for these regions, respectively. In spite of rather strong spatial variability in medusa size, the mean umbrella diameter in the western Black Sea was quite stable (±SE) (47.4 ± 4.2 mm), differing little ($p > 0.05$; t-test) from that measured near the Crimean coast on September 20, 2005 (47.1 ± 3.9 mm).

Starting from these structural parameters of the population, the food requirements of the jellyfish at a seasonal mean temperature of ~15ºC can be calculated as 17, 62, and 70 cal. m^{-2} day^{-1}, for the shelf, intermediate, and deep-sea areas, respectively. At the same time, the production of non-gelatinous prey zooplankton with a weight-specific growth rate of ~0.1 day^{-1} (Zaika, 1983) and biomass (±SE) in these areas being 3.99 ± 1.00, 4.11 ± 1.28, and 8.72 ± 1.25 g m^{-2}, is approximately 279, 288 and 610 cal. m^{-2} day^{-1}, respectively. Hence, the production of prey zooplankton was theoretically more than enough to supply the food requirements of medusae. But, as the wet weight/biomass of medusae did not increase during autumn 2005, *A. aurita* did not obtain sufficient food for growth.

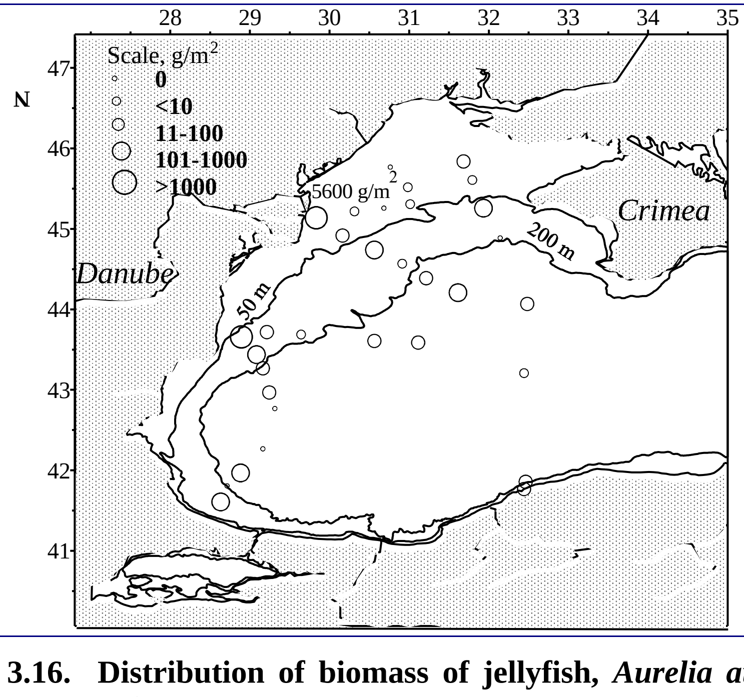


Figure 3.16. Distribution of biomass of jellyfish, *Aurelia aurita* in western sector of the Black Sea in October, 2005

For many reasons, but notably because of the patchy distribution of medusae and their food components, the simulation of real food conditions for *A. aurita* in the Black Sea is an extremely complicated issue. Assuming that most of the zooplankton permanently (in shelf area) or periodically (in deep-sea area) inhabits the uppermost 30 m layer, mean concentration of zooplankton in this water body could be about 0.13, 0.14, and 0.29 g m^{-3}, for the shelf, intermediate, and deep-sea areas, respectively. Hence, daily rations for a jellyfish of 5.3 g mean wet weight (~47 mm in umbrella diameter) at such food concentrations and temperature ~15ºC are approximately 1.7, 1.8, and 3.7 cal. $ind.^{-1}$. The daily maintenance ration for this individual under such conditions is 7.2 cal. $ind.^{-1}$. Clearly, the concentrations of zooplankton present were not sufficient for *A. aurita* metabolic requirements even assuming the plankton nets trapped only half of the zooplankton present (Sorokin, 2002).

According to our calculations, the concentration of zooplankton needed to maintain *A. aurita* is 0.45-0.50 g m^{-3}. A weak trend of decreasing umbrella diameter in July-September suggests that food concentrations during this time medusae were slightly lower than those required for maintenance of *A. aurita*. Higher food concentrations than those integrated by the plankton nets, could exist at the thermocline (*A. clausi, O. similis, P. parvus, P. elongates, O. dioica*), above (*A. clausi, P. avirostris, P. tergestina*), or below (*C. euxinus, P. elongates*) it (Vinogradov & Shushkina 1982; Zagorodnaya, 1988; Sorokin, 2002). Besides, the food requirements of *A. aurita* are, to some extent, met by protozoans (in size range, 20-200 μm) (Stoecker *et al*., 1987; Olesen *et al*., 1994), algae (Mironov, 1967; Möller, 1980) detritus and bacterial aggregates (Southward, 1955). By weight, about ten times as much detritus is present in the sea as zooplankton (Sorokin, 2002), and the complex of microorganisms associated with detrital particles could provide a major additional food source for *A. aurita*. Nevertheless, judging by the summer decrease in medusal growth rates during 1999-2006, when zooplankton in the sea was almost totally eaten up by *M. leidyi*, detritus and other "vegetative" food components were not present at a high enough level to fully support *A. aurita*'s metabolic requirements.

The jellyfish predatory impact on various species and kinds of zooplankton seems more specific then it might be supposed. In October 2005, in the western Black Sea, the biomass of medusa was generally positively correlated with numbers and biomass of almost all species and kinds of zooplankton (Fig. 3.17). Theoretically this pattern of dependence could be explained by different circumstances:

1. The same character of predator-prey variability in the distribution;
2. Common food source;
3. Dependence of the jellyfish growth rate on productivity of zooplankton;
4. Other factors.

In any case, it is evident that predatory impact of medusae on most of copepods was absolutely compensated by these species productivity. At the same time, the negative, although statistically insignificant ($p > 0.05$) probabilistic trends relative to *O. similis* and Bivalvia veligers, may be evidence of a strong predatory selection of these species. Some other observations suggest the same: in October 1984, nearby the Crimea coast, many *A. aurita* individuals within the size range 40-100 mm contained in their gastric cavitities about 30-150 Bivalvia veligers, and one jellyfish of 143 mm size contained 470 veligers. In Kiel Bight, mollusc larvae also constituted perhaps the predominant food item of this species (Schneider, 1988). According to other data, the populational growth of the jellyfish in Kiel Bight was followed by decrease in biomass of *Pseudo-*, *Paracalanus*, and *O. similis*, whereas copepods, *Centrophages* and *Acartia* had no visible indications of suppression (Behrends and Schneider, 1995). To understand the reasons underlying the prey-specific predatory impact of *A. aurita*, more detailed information about the ecology and life history of this and others zooplankton species is needed.

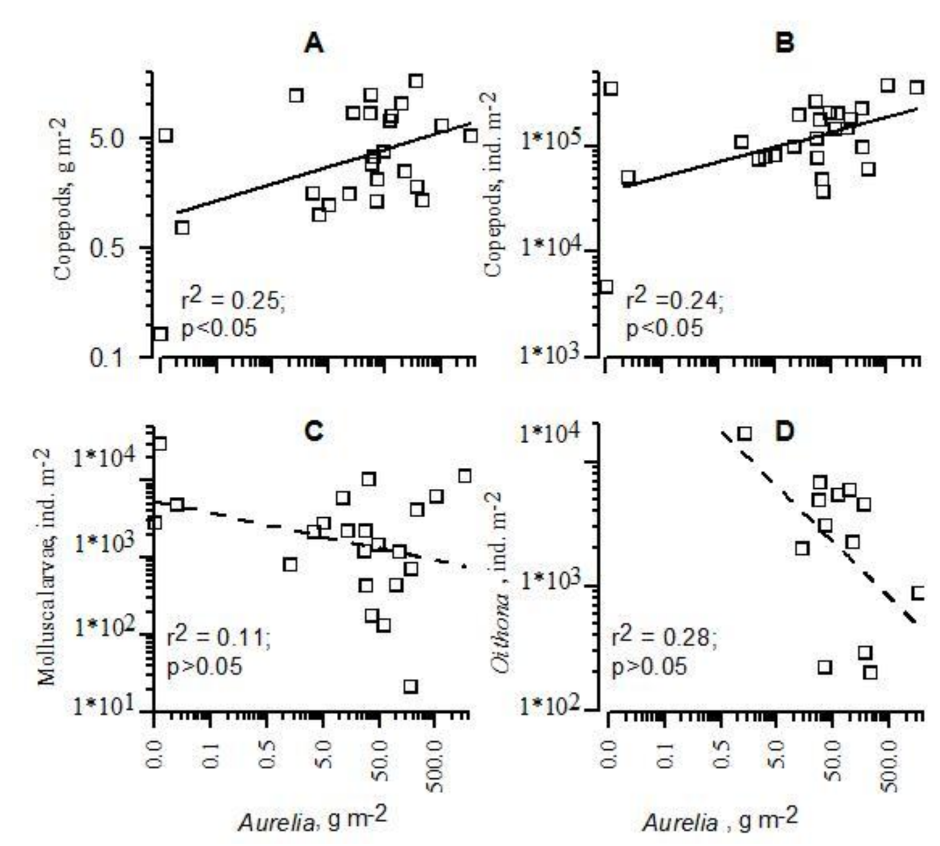


Figure 3.17. Variations in average Copepoda biomass (g m^{-2}) (A), Copepoda abundance (ind. m^{-2}) (B), Mollusca larvae numbers (ind. m^{-2}) (C) and *Oithona similis* numbers (ind. m^{-2}) (D) depending on biomass (g m^{-2}) of jellyfish *Aurelia aurita* in the western sector of the Black Sea in October, 2005

References

Amenta J.S. 1964. A rapid chemical method for quantification of lipids separated by thin layer chromatography. *J. Lipid Res.* 5, 270-273.

Anninsky B.E., G.I. Abolmasova, 2000. Temperature as a factor influencing the ctenophore *Mnemiopsis leidyi* metabolic intensity and expansion over the Black Sea. *Okeanologiya*. 40, 729-735 (in Russian).

Anninsky B.E., Finenko G.A., Abolmasova G.I., Hubareva E.S., Svetlichny L.S., Bat L., Kideys A.E.. 2005. Effect of starvation on the biochemical compositions and respiration rates of ctenophores *Mnemiopsis leidyi* and *Beroe ovata* in the Black Sea. *J. Mar. Biol. Ass. U.K.* 85, 549-561.

Anninsky B.E., Finenko G.A., Abolmasova G.I., Romanova Z.A. 2007. Body organic content of ctenophores *Mnemiopsis leidyi* (Ctenophora, Lobata) and *Beroe ovata* (Ctenophora, Beroida) on the early life stages of the ontogenesis. *Russ. J. Mar. Biol.* 33(6), 417-424.

Anninsky B.E., Romanova Z.A., Abolmasova G.I., Gucu A.C., Kideys A.E. 1998. The ecological and physiological state of the ctenophore *Mnemiopsis leidyi*. In: L.I. Ivanov and T. Oguz (eds.), Ecosystem modelling as a Management Tool for the Black Sea, Vol. I, Kluwer Acad. Publ., Netherlands, pp. 249-261.

Arai M.N. 1991. Attraction of *Aurelia* and *Aequorea* to prey. *Hydrobiologia*. 216/217: 363-366.

Arai M.N. 1997. A functional biology of Scyphozoa. Chapman and Hall, London.

Bailey T.G., Youngbluth M.J., Owen G.P. 1995. Chemical composition and metabolic rates of gelatinous zooplankton from midwater and benthic boundary layer environments off Cape Hatteras, North Carolina USA. *Mar. Ecol. Progr. Ser*.122,121-134.

Båmstedt U. 1990. Trophodynamics of scyphomedusae *Aurelia aurita*. Predation rate in relation to abundance, size and type of prey organism. *J. Plankton Res*. 12, 215-229.

Båmstedt U., Lane J. Martinussen, M.B. 1999. Bioenergetics of ephyra larvae of the scyphozoan jellyfish *Aurelia aurita* in relation to temperature and salinity. *Mar. Biol.* 135, 89-98.

Behrends G., Schneider G. 1995. Impact of *Aurelia aurita* medusae (Cnidaria, Scyphozoa) on the standing stock and community composition of mesozooplankton in the Kiel Bight (western Baltic Sea). *Mar. Ecol. Prog. Ser*. 127, 39-45.

Clarke A, Holmes L.J., Gore D.J. 1992. Proximate and elemental composition of gelatinous zooplankton from the Southern Ocean. *J. Exp. Mar. Biol. Ecol*. 155, 55-68.

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A., Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28, 350-356.

Eiji Y. 1987. A method of measuring the volume of soft tissue. *Zool. Sci*. 4, 747-749.

Finenko G.A., Romanova Z.A., Abolmasova G.I., Anninsky B.E., Pavlovskaya T.V., Bat L., Kideys A.E. 2006. Ctenophora invaders and their role in trophodynamics of the planktonic community in the coastal waters of the Crimea Area of the Black Sea (Sevastopol Bay). *Okeanologiya*. 46, 507-517 (in Russian).

Frandsen K.T., Riisgård H.U. 1997. Size dependent respiration and growth of jellyfish, *Aurelia aurita*. *Sarsia*. 82, 307-312.

Folch J., Lees M., Sloane-Stanley G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol. Chem*. 226, 251-262.

Giginyak Yu.G. 1979. Caloricity of aquatic invertebrate animals. In: G.G. Vinberg (ed.) General basis of aquatic ecosystems study, Nauka, Leningrad, 43-57.

Gomoiu M-T., Kupriyanov S.S. 1980. Evaluation of numbers and distribution of medusae *Aurelia aurita* in the eastern Black Sea. In: M.E. Vinogradov (ed.), The Black Sea pelagic ecosystem, Nauka, Moscow, 191-198 (in Russian).

Hansson L.J. 1997. Effect of temperature on growth rate of *Aurelia aurita* (Cnidaria, Scyphozoa) from Gullmarfjorden, Sweden. *Mar. Ecol. Prog. Ser*. 161, 145-153.

Hartree E.F, 1972. Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.* 48, 422-427.

Hernroth L. Gröndahl F. 1983. On the biology of *Aurelia aurita* (L.). 1. Release and growth of *Aurelia aurita* (L.) ephyrae in the Gullmar Fjord, western Sweden, 1982-1983. *Ophelia*. 22, 189-199.

Ivlev V.S. Experimental ecology of the feeding of fishes. 1961. Yale University Press, New Haven.

Ivleva I.V. Environment temperature and rate of energy metabolism in aquatic animals. 1981. Nauk. Dumka, Kiev (in Russian).

Konsulov A., Kamburska L. 1998. Ecological determination of the new Ctenophora - *Beroe ovata* invasion in the Black Sea. *Trudi Instituta Oceanology BAN*. 2, 195─197.

Kopitov Yu.P. 1983. A new variant of thin layer chromatography of lipids and carbohydrates. *Ecologiya Morya*. 13, 76-80 (in Russian).

Kramp P.L. 1961. Synopsis of the medusae of the world. *J. Mar. Biol. Ass. U.K.* 40, 1-469.

Kremer P., Canino M.F., Gilmer R.W. 1986. Metabolism of epipelagic tropical ctenophores. *Mar. Biol*. 90, 403-412.

Kuzmenko M.I. 1975. The determination of amine nitrogen by ninhydrin reagent of new composition. In: L.A. Sirenko (ed.), Methods of physiological-biochemical investigation of algae in hydrobiological practice, Naukova Dumka, Kiev, 116-118 (in Russian).

Kuzmicheva V.E. 1980. Respiration rates of jellyfishes from the Black Sea. In: M.E. Vinogradov & R. Heerkloss (eds.), Bioproductivitat des Pelagials in Schwarzen Meer, Wilhelm-Peck-Universitat, Rostock, 91-94.

Larson R.J. 1976. Cubomedusae: feeding-functional morphology, behaviour and phylogenetic position. In: G.O. Mackie (ed.), Coelenterate ecology and behaviour, Plenum Press, New York and London, 237-245.

Larson R.J. 1986. Water content, organic content and carbon and nitrogen composition of medusae from the northeast Pacific. *J. Exp. Mar. Biol. Ecol.* 99, 107-120.

Larson R.J. 1987. Respiration and carbon turnover rates of medusae from the NE Pacific. *Comp. Biochem. Physiol.* 87A, 93-100.

Lebedeva L.P., Shushkina E.A. 1991. The estimation of population characteristics of the medusa *Aurelia aurita* in the Black Sea. *Oceanology, Wash.* 31, 314-319.

Lenhoff H.M. 1971. Research on feeding, digestion, and metabolism in coelenterates: some reflections. In: H.M. Lenhoff, L Muscatine and L.V. Davis (eds.), Experimental coelenterate biology, University of Hawaii Press, Honolulu, pp. 75-83.

Lucas C.H. 1994. Biochemical composition of *Aurelia aurita* in relation to age and sexual maturity. *J. Exp. Mar. Biol. Ecol.* 183, 179-192

Lucas C.H., Williams J.A. 1994. Population dynamics of scyphomedusa *Aurelia aurita* in Southampton water. *J. Plankton Res.* 16, 879-895.

Madin L.P., Cetta C.M., McAlister V.L. 1981. Elemental and biochemical composition of salps (Tunicata: Thaliacea). *Mar. Biol.* 63, 217-226.

Malej A., Turk V., Lučić D., Benović A. 2006. Direct and indirect trophic interactions of *Aurelia* sp. (Scyphozoa) in stratified marine environment (Mljet Lakes, Adriatic Sea) *Mar. Biol.* 151, 827-841.

Mastronicolis S.K., Minladis S., Nakhel L., Smirniotopoulou A. 1991. Biochemical and ecological research on jellyfish and other organisms in the Mediterranean Sea. *Map Tech. Rep. Ser.:* 47, 268-282.

Mills C.E. 1995. Medusae, siphonophores and ctenophores as planktivorous predators in changing global ecosystems. *ICES J. Mar. Sci.* 52, 575-581.

Mills C.E. 2001. Jellyfish blooms: are populations increasing globally in response to changing ocean conditions? *Hydrobiologia*. 451,55-68.

Mironov G.N. 1967. Nutrition in planktonic predators. III. Food requirement and daily rations of *Aurelia aurita* L. In: V.A. Vodyanitsky (ed.), Biology and distribution of plankton in southern seas, Nauk. Dumka, Kiev, 124-137 (in Russian).

Mironov G.N. 1971. Biomass and distribution of jellyfish *Aurelia aurita* L. on data drag-net hauls in 1949-1962. *Biologiya Morya. (Kiev).* 24, 49-69 (in Russian).

Möller H. 1980. Scyphomedusae as predators and food competitors of larval fish. *Meeresforsch.* 28, 90-100.

Monod J., Wyman J., Changeux J.-P. 1965. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol*. 12, 88-118.

Mutlu E., Bingel F., Gücü A.C. Melnikov V.V., Niermann U., Ostrovskaya N.A. and V.E. Zaika. 1994. Distribution of the new invader *Mnemiopsis* sp. and the resident *Aurelia aurita* and *Pleurobrachia pileus* populations in the Black Sea in the years 1991-1993. *ICES J. Mar. Sci.* 51, 407-421.

Olesen N.J., Frandsen K.T., Riisgård H.U. 1994. Population dynamics, growth and energetics of jellyfish *Aurelia aurita* in a shallow fjord. *Mar. Ecol. Prog. Ser.* 105, 9-18

Omori M.S. and Ikeda T. Methods in marine zooplankton ecology. 1984. Wiley-intersci. Publ. John Wiley and Sons, New York.

Petipa T.S. 1957. On the average weight of principal kinds of zooplankton from the Black Sea. *Proc. Sevas. Biol. Stn*. 9, 46-57 (in Russian).

Raymont J.E.G. 1963. Plankton and productivity in the oceans. Pergamon Press, New York.

Reeve M.R., Syms M.A., Kremer P. 1989. Growth dynamics of a ctenophore *(Mnemiopsis*) in relation to variable food supply. I. Carbon biomass, feeding, egg production, growth and assimilation efficiency. *J. Plankton Res*. 11, 535-552.

Schneider G. 1988. Chemische Zusammensetzung und Biomasseparameter der Ohrenqualle *Aurelia aurita*. *Helgol. Wiss. Meeresunters*. 42, 319-327.

Schneider G. 1989. Zur chemischen Zusammensetzung der Ctenophore *Pleurobrachia pileus* in der Kieler Bucht. *Helgol. Wiss. Meeresunters*. 43, 67-76.

Schneider G., Weisse T. 1985. Metabolism measurements of *Aurelia aurita* planulae larvae, and calculation of maximal survival period of the free-swimming stage. *Helgol. Wiss. Meeresunters*. 39, 43-47.

Shushkina E.A., Musaeva E.I. 1983. The role of jellyfishes in energetics of plankton communities of the Black Sea. *Okeanologiya*. 23, 125-130 (in Russian).

Shushkina E.A. and G.N. Arnautov. 1987. *Aurelia* medusae in the Black Sea plankton in May 1984. In: M.E. Vinogradov & M.V. Flint (eds.), Current state of the Black Sea ecosystem, Nauka, Moscow, 186-196 (in Russian).

Shushkina E.A., Musaeva E.I., Anokhina L.L., Lukasheva T.A. 2000. The role of the gelatinous macroplankton: jellyfish *Aurelia* and invading ctenophores *Mnemiopsis* and *Beroe* in the plankton communities of the Black Sea. *Okeanologiya*. 40, 859-866 (in Russian).

Sorokin Yu.I. The Black Sea. Ecology and oceanography. 2002. Backhuys Publ., Leiden.

Southward A.J. 1955. Observations on the ciliary currents of the jellyfish *Aurelia aurita* L. *J. Mar. Biol. Ass. U.K.* 34, 201-216.

Stoecker D., Michaels A.E., Davies L.H. 1987. Grazing by the jellyfish, *Aurelia aurita,* on microzooplankton. *J. Plankton Res*. 9, 901-915.

Thill H. 1937. Beiträge zur Kenntnis der *Aurelia aurita*. *Z. Zool.* 150, 51.

Verwey J. 1942. Die Periodizität im Auftreten und die aktiven und passiven Bevegungen der Quallen. *Arch. Neerland. Zool*. 6, 363-468.

Vinogradov M.E., Shushkina E.A. 1982. Assessment of abundance of the Black Sea zooplankton with the use of submersible ”Argus”. *Okeanologiya*. 22, 473-479 (in Russian).

Weisse T., Gomoiu M.-T. 2000. Biomass and size structure of the scyphomedusa *Aurelia aurita* in the northwestern Black Sea during spring and summer. *J. Plankton Res*. 22, 223-239.

Wetokhin I.A. 1930. Capture of the food and nutrition of *Aurelia aurita* medusae in relation to work of ciliary epidermis. *Russ. Physiol. J*. 8, 585-599 (in Russian).

Winberg G.G. 1971. Methods for the estimation of production of aquatic animals. Academic Press, New York.

Winberg G.G. 1986. Growth efficiency and production of marine animals. In: N.N. Khmeleva (ed.), Growth efficiency of hydrobionts, Gomel University Publ., Gomel, 20-61 (in Russian).

Yakovleva K.K. 1964. Respiration rate of some Black Sea medusae. *Proc. Sevas. Biol. Stn.* 17, 364-367. (*Fish. Res. Bd. Can. Trans. Ser*. No. 2218).

Zagorodnaya Yu.A. 1988. Mesozooplankton and macrozooplankton distribution in the Black Sea in summer 1980. In: V.I. Zatz and Z.Z. Finenko (eds.), Hydrodynamics and planktonic productivity in the Black Sea, IOAN Publ., Moscow, 340-355 (in Russian).

Zaika V.E. 1983. Comparative productivity of hydrobionts. Nauk. Dumka, Kiev (in Russian).

Zaitzev Yu.P., Polischuk L.N. 1984. Outburst of jellyfish *Aurelia aurita* in the Black Sea. *Ecologiya Morya. (Kiev).* 17, 35-46 (in Russian).

Zaslavsky E.M. 1980. The determination of the forms of carbon location in the sea sediments. In: E.A Romankevich (ed.), Methods of investigation of organic matter in the ocean, Nauka, Moscow, 121-128 (in Russian).

4. FUNCTIONAL ROLE OF THE CTENOPHORE INVADERS *Mnemiopsis leidyi* AGASSIZ AND *Beroe ovata* MAYER IN INSHORE PLANKTONIC COMMUNITIES

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4.1 Introduction

In recent decades the numbers of alien species and their introduction rate to aquatic systems have been increasing over the world. Most extend biodiversity of the system and most of them don’t have negative influence on its functioning. Only some of them can attain massive occurrence, suppressing or totally replacing the native species due to food competition and consumption. As a result the community structure is simplified, tolerance to external impact decreases and the system becomes open to injury. Ability to successful invasion of alien species, along with other factors, is determined with their ecological–physiological features. The characteristics of these species are short life cycle, high fecundity and growth rate, earlier maturation, wide food spectrum, eurybiotics and high tolerance to pollution.

The North Atlantic ctenophore *Mnemiopsis leidyi* Agassiz A., 1865 (Ctenophora, Lobata) introduction of which caused real catastrophe in the Sea of Azov and the Black Sea in the late 80-s, have the most of these traits. The consequence of introduction of this species was changing of energy pathway in the plankton pelagic system (Vinogradov *et al*., 1989; Shushkina *et al*. 1990; Shushkina and Vinogradov, 1991; Kideys, 1994). Instead of the trophic chain“ zooplankton – planktivorous fish” the energy flow has been directed to “zooplankton – *M. leidyi*” chain that resulted in fishery collapse and great economic damage of the Black Sea countries. *M. leidyi* invasion has brought to reconstruction of the structure and functioning of different food levels of the Black Sea ecosystem. Feeding on herbivorous zooplankton, egesting the nutrients and mucus it defined to significant extent the phytoplankton and microzooplankton dynamics in summer. High *Mnemiopsis* pressure on herbivorous zooplankton resulted in phytoplankton bloom occurred to be more often in inshore waters. Uneaten superfluous phytoplankton sinking has lead to oxygen deficiency and demise of fish, mussels and other valuable bottom species.

The first appearance of *M. leidyi* in the Black Sea was recorded in November 1982 near Sudak, the south-eastern Black Sea (Pereladov, 1988**);** massive occurrence in different regions of the sea was observed in 1988–1989. Such long period between its appearance and bloom possibly is a result of extremely low water temperature in the Black Sea that years (Oguz, 2005): the coldest and severe winters of the last century were noted in 1985–1987 with mean winter (December – March) surface water temperature about 7.2°C.

At the end of 80-s there was a bloom of this ctenophore: in July – September 1989 its biomass in the open Black Sea was on average about 1.5 kg m^{-2}, in the inshore waters and on the northwestern shelf it was more than 3 kg m^{-2} with maximum of 5.2 kg m^{-2} (Shushkina *et al.*, 1990; Bogdanova and Konsulov, 1993) The total *M. leidyi* stock in the sea was as high as 780 billion tons. In parallel with the other factors that destabilised the Black Sea ecosystem (eutrophication, various types of pollution) massive occurrence of this ctenophore has changed sharply species structure and biodiversity of mesozooplankton, the main food of *M. leidyi* (Vinogradov *et al.*, 1992; Kovalev *et al.*, 1998). By the beginning of 90-s biomass of previously numerous warm water crustaceans – Copepoda *Paracalanus parvus* and Cladocera *Penilia avirostris, Pleopis polyphemoides* **–** has decreased some times, *Sagitta* – more than tenfold, *Aurelia aurita* – three times as compared to the previous period while *Acartia clausi* and *Acartia tonsa* became dominant in inshore regions.

Being the food competitor and consumer of fish eggs and larvae the ctenophores have caused tremendous damage to pelagic fish stock and as a result the catches of mass planktivorous fish and, first of all, *Engraulis encrasicolus ponticus* and *Trachurus mediterraneu ponticus* sharply diminished (Niermann *et al*., 1994; Gücü, 1997). Economical losses for all the countries around the Black Sea amounted to 240 billion dollars per year (Caddy and Griffith, 1992).

The role of the ctenophores in the Black Sea ecosystem degradation in the whole and in catch decreases in particular, is a point at issue. Quite a number of factors like over fishing (Gücü and Oguz, 1998; Gücü, 2002), natural climate fluctuations due to change of North Atlantic water circulation at the beginning of 1990-s (Niermann *et al*., 1999; Bilio and Niemann, 2004; Oguz**,** 2005), eutrophication and anthropogenic pollution (Zaitsev and Alexandrov, 1997) may have been the reason but *M. leidyi* invasion is considered as a main factor that induced such sharp shifts in the Black Sea ecosystem (Zaika, 1994; Kideys, 2002; Kideys *et al*., 2005).

As a result of lack of natural predators food and temperature conditions only have controlled *M. leidyi* abundance in the Black Sea. Here it showed the typical features of a new colonizer: after blooming during the initial period of settling (1989–1990) with biomass of 3-4 kg m^{-2}, its biomass and abundance have decreased to moderate level. The reason of this decrease in 1991–1993 may have been colder climatic period: that years were very specific for the Black Sea ecosystem with the most cold winters with temperature 1.8^0 C below the average value (Oguz, 2005). In parallel with *M. leidyi* biomass the biomass of zooplankton also dropped sharply: from 17 g m^{-2} in 1990 to 2-4 g m^{-2} in 1991-1993. It coincided with the lowest level of fish stock and the highest phytoplankton biomass when compared to 1960-s. After the period of decrease a new peak of *M. leidyi* abundance followed in 1995, that was comparable with the peak of the late 1980-s. Like the first bloom the significant decrease of the biomass has followed for the successive years (1996–1997) that may have been the result of food lack. As Anninsky *et al*., 1998a showed content of glycogen, the main storage compound in autumn 1996 in the *M. leidyi* population in the southern Black Sea corresponded to its content in 2 days starveling ctenophores. *M. leidyi* decreased pressure resulted in zooplankton and ichthyoplankton biomass restoring. That years when the general ecological situation became better because of economic collapse in the former social countries some positive changes in the Black Sea pelagic ecosystem in the whole were observed: zooplankton biomass in the Central Black Sea started to increase since 1994, some zooplankton species that had disappeared in the recent years appeared again. During 1996–1998 *M. leidyi* average biomass stabilized on the level of 200–300 g m^2.

In October 1997 a new invader ctenophore *Beroe ovata* Mayer 1912 a known feeder on *M. leidyi* in native North Atlantic waters, appeared in shallow waters of Bulgaria (Konsulov and Kamburska, 1998) and in August – September 1999 it was observed in other regions of the Black Sea (in Sevastopol Bay and in the northeastern Black Sea). They believe that *B. ovata* massive occurrence in August – September 1999 was a result of abnormal warm winters of 1997–1998 and1998–1999 as well as very hot summer of 1999 (Shushkina *et al*., 2000).

Beroe introduction had initiated some new changes in the plankton community (Finenko *et al*., 2000, 2001, 2003; Shiganova *et al*., 2000, 2001, 2004; Shushkina *et al*., 2004; Vinogradov *et al*., 2000; Vinogradov and Shushkina, 2002). Being a monophage, which under the conditions of the Black Sea consumes almost exclusively *M. leidyi* it regulates its abundance and, in this way, controls other components of the ecosystem (Finenko *et al*., 2000, 2001; Shiganova *et al*., 2000, 2004; Vinogradov *et al*., 2000).

This chapter aims to analyzse ecological–physiological features of both alien ctenophores, that enabled their massive occurrence under the conditions of the Black Sea, the seasonal and inter annual dynamics as well as trophic interactions in the planktonic community at the new stage of its development.

4.2 Chemical composition and elements of *M. leidyi* energy budget

4.2.1 Chemical composition

M. leidyi dry weight in the Black Sea amounts 2.0 -2.2% of wet weight (Finenko and Romanova, 2000; Vinogradov *et al.*, 2000; Vostokov *et al.*, 2001), that is significantly lower than in the other regions due to low salinity of the Black Sea.

Organic matter content in *M. leidyi* ranges from body ranges from 0.060 to 0.15% wet weight and depends on the ctenophore size, being maximal in small ctenophores with length less than 10 mm (Vinogradov *et al.*, 2000; Shushkina *et al.*, 2004). The energy content in adult *M. leidyi* with size range of 15–70 mm on average is 6.7±1.0 cal g^{-1}wet weight (Anninsky *et al.*, 2005). Predominant fraction is protein that forms about 80% of the total organic matter, lipids amount to about 8.7–10.0%. Carbohydrates and amino acids separately make up less than 6.5% of the total organic matter. Polysaccharides dominate in carbohydrates (4.4%) and the glycogen content reaches 3.0%. Phospholipids and sterins prevail in lipids amounting to 3.1% and 2.7% respectively. The main storage compound in *M. leidyi* is glycogen, which content is 4 times as great as all storage lipids (triacylglycerols and waxes). Glycogen content, as it was noted earlier, is an index of ctenophore food supply (Anninsky and Gubanova, 1998). Using this carbohydrate as a main source of accumulated energy is a characteristics of the species with low activity.

4.2.2 Feeding

The food of *M. leidyi* is the most of zooplankton species with size from tens and hundreds microns to 10–15 mm; their share in the ration depends on their mobility, predator and prey size as well as on time and place. According to Zaika and Revkov (1998), in summer in the coastal regions off the Crimea and the Caucasus Coasts Cladocera *Pleopis polyphemoides*, *Oikopleura dioica*, Bivalvia larvae, Copepoda and their eggs may amount to 85–90% of the total number of prey in gut content of the ctenophores while tintinnids, Cirripedia nauplii, *Noctiluca scintillans*, Polychaeta, *Sagitta setosa* and Gastropoda and fish larvae are less than 1%. In offshore waters off the Caucasus coasts Copepoda and Cladocera prevailed in the ration in summer and winter (75 and 56%; 23 and 20% respectively), in inshore regions in winter eggs of invertebrates composed up to 85% of the total ration (Tsikhon–Lukanina *et al*., 1993). Phytoplankton that was found sometimes in gut contents may had been originated from the Crustacean guts. In the experiments it was revealed that *M. leidyi* was starving when algae and detritus were the only food offered (Baker and Reeve, 1974). Survival rate of the ctenophores feeding on phytoplankton is similar to this on the filtered water.

It seems that small number of ciliates in the gut contents is not result of their low ingestion but of poor conservation and difficulties of identification. The ingestion rate of Cirripedia nauplii that can be observed into *M. leidyi* gut content probably is low. In our experiments ctenophores lost their weight when Cirripedia nauplii were ordered as a food in the same numbers as adult *Acartia clausi* (Finenko *et al*.,1995). Low moving activity of these nauplii may not stimulate the lobes to such an extent as Copepoda do due to their higher vibration rate.

It is worth noting that fish larvae have been found rarely into *M. leidyi* gut content in the sea (only in 2.6% of total ctenophore number) (Tsikhon–Lukanina *et al*., 1993) but predatory effect of *M. leidyi* population on them is possible to be high in the period of ctenophore maximum (more than 70% of total larvae abundance per day).

The daily rhythm in *M. leidyi* feeding was observed in nature: feeding intensity is significantly higher at night. Zaika and Revkov (1998) have revealed that at night the share of Ctenophores with some food in gut content was 4 times as high as during the day that may be a result of their migration to the surface sea layers with high food concentration at night (Vereshchaka, 2002).

4. FUNCTIONAL ROLE OF THE CTENOPHORE INVADERS Mnemiopsis leidyi AGASSIZ AND Beroe ovata MAYER IN INSHORE PLANKTONIC COMMUNITIES

An important characteristic of lobate ctenophore nutrition is continuous feeding, and therefore digestion rate is one of the factors that define their feeding rates. According to Reeve, 1978, Larson, 1987, Tsikhon–Lukanina *et al.*, 1995 digestion time values in *M. mccradyi* and *M. leidyi*, feeding on Copepoda, vary in wide range from 0.33 to 6.6 h and depend on temperature and prey number in gut content. Besides digestion time is a function both prey and predator sizes. Small prey (< 1 mm – Copepoda and Cirripedia nauplii, *Oitnona* sp.) are digested by 50-mm *M. leidyi* for 0.3 - 0.6 h at 10-50 prey in their gut content (Larson, 1987). Large Copepoda (*Pontella mediterranea*, *Calanus ponticus*) were digested in the experiments much slowly than small ones (*A. clausi*), and digestion time decreased as *M. leidyi* size increased (Tsikhon–Lukanina *et al.*, 1995). For instance, 10- mm ctenophores digested *P. mediterranea* for 6.6 h, while in 70-mm *M. leidyi* the digestion time reduced by half under the same temperature conditions. All sized Ctenophores digested *A. clausi* of 1–2 mm for 1.0–1.7 h, that is 2 -3 times faster than digestion of *P. mediterranea* and *C. ponticus* (3–3.5 mm length). *Artemia salina* nauplii of 1 mm length were digested with the same rate as *A. clausi* There was no a significant difference in digestion time at different prey number in the gut content (1.0–1.7 h), but the difference in the average digestion times of *A. salina* nauplii in two size groups of predators was significant: 0.86±0.32 h in 20-30-mm ctenophores and 1.29±0.38 h in 35–50 mm ($p<0.01$) (Fig. 4.1.).

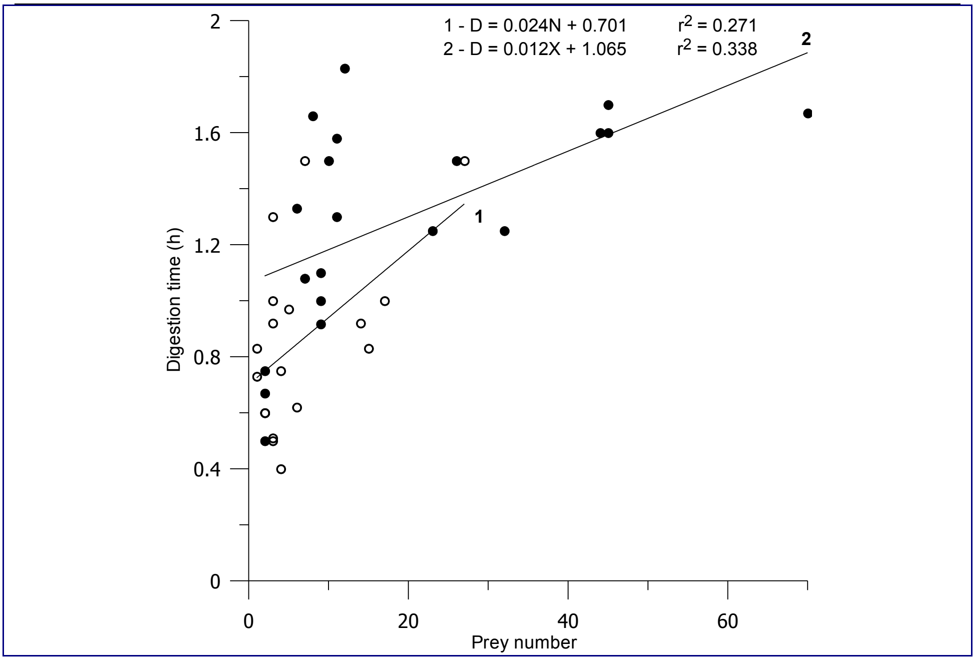

Figure 4.1. Relationship between digestion time (D) and prey number in gut content (N) in *M.* l*eidyi:* 1 = 20-30mm, 2 = 35-50mm

Digestion time of fish larvae (length of 3–32 mm) by adult *M. leidyi* are close to digestion time of large planktonic Crustacea and range from 1.0–4.7 h (mean value is 2.8 h at 18–20°C) (Tsikhon–Lukanina *et al*., 1993).

Ability for food selectivity of *M. leidyi* is not clear. Although some authors revealed that *Acartia* sp., Bivalvia veligers, Cirripedia nauplii and Polychaeta larvae were more numerous in the diet than in environment (Burrell and Van Engel; 1976; Larson, 1987), our calculations from Zaika and Revkov (1998) data showed that ingestion rate of different prey species in the sea was proportional to their share in total zooplankton abundance.

Daily ration value - number or weight of prey ingested for a day - and clearance rate - volume that ctenophore have to process to get certain amount of food - are two important indices of feeding intensity of *M. leidyi.* The feature of food behavior of lobate ctenophores is a linear relationship between ingestion and prey number over a wide range of food concentrations, i.e. there is no critical (threshold) concentration under which food saturation is reached (Bishop, 1968; Reeve, 1980; Gibbson and Painting; 1992; Finenko *et al*., 1995)**.**

In the experiments the maximum concentration of Copepoda up to which the ration of *M. leidyi* increased was more than 3000 ind l^{-1} (Reeve *et al*, 1978). Zaika and Revkov (1998) revealed also that in the Black Sea the food ingestion of *M. leidyi* of 20–40 mm increased in parallel with the food concentration increasing (Cladocera *P. polyphemoides* and Bivalvia larvae) in the range of 125–1100 ind m^{-3}. The same character of ration dependence we found when *M. leidyi* was fed on *A. salina* nauplii of much higher densities from 5 to 50 ind l^{-1} or 0.1-1.0 mg l^{-1} (Fig. 4.2). Under such effect of prey concentration on the ration value the clearance rate is constant and does not depend on food density.

In our experiments clearance rate of 20–30 mm *M. leidyi* has not changed over a wide range of food concentrations (5–50 *A. salina* nauplii l^{-1}), being equal to 0.234±0.076 l g WW^{-1} h^{-1} and slightly increased in 40–50 mm ctenophores (Fig.4.3). Meantime *M. leidyi* weight (or energy content) affects on clearance rate value and correlation between these two values at 23°C is as follows:

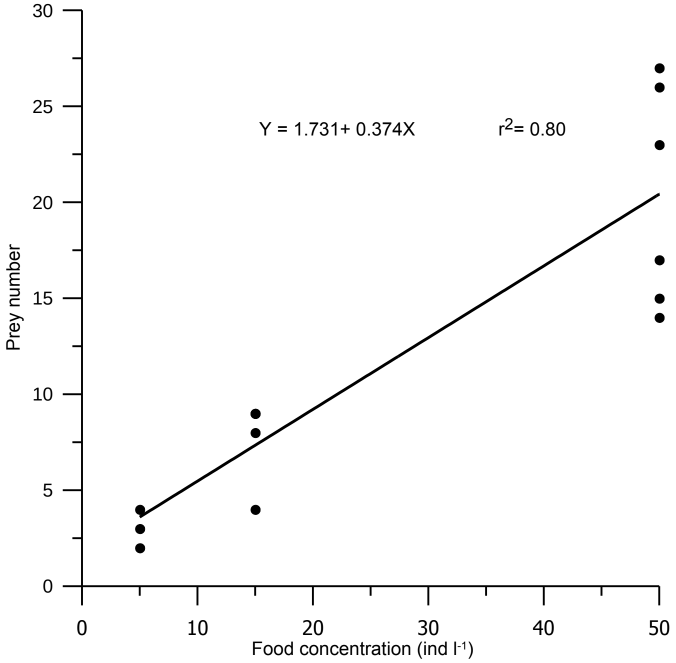

Figure 4.2. Effect of food concentration (ind l $^{-1}$) on ingestion rate in *M. Leidyi*

$CR/WW = 18.39WW_1^{-0.595}$ $0.32<WW_1<29.2$, $n = 18$, $r^2 = 0.616$,

Where CR/WW is clearance rate, $l \cdot g^{-1} \cdot h^{-1}$; WW_1 is body energy content, $cal \cdot ind^{-1}$ (Fig. 4.4).

Clearance rate depends also on prey size: it is twice as high when Ctenophore feed on Copepoda nauplii than on adult *A. clausi*: (Fig. 4.5)

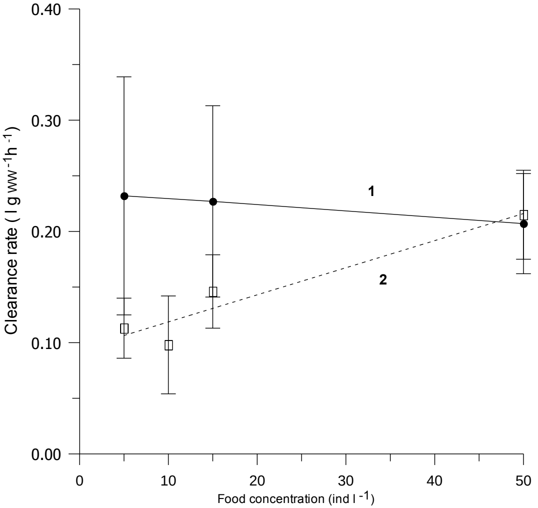

Figure 4.3. Effect of food concentration on clearance rate in 20 – 30 mm (1) and 40-50 mm (2) *M. leidyi*

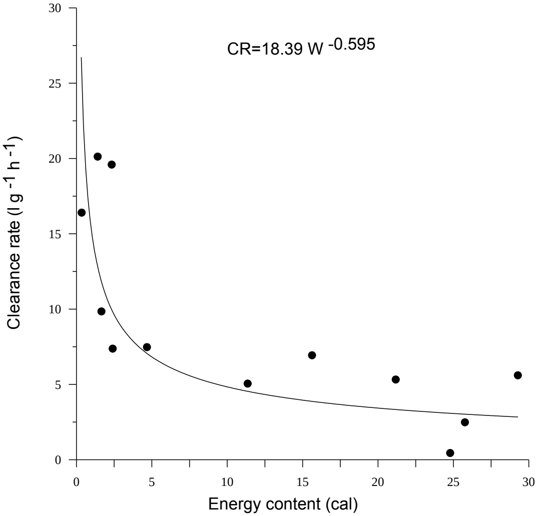

Figure 4.4. Relationship between specific clearance rate (l g $^{-1}$ DW h^{-1}) and body energy content (cal ind.$^{-1}$) in *M. leidyi*

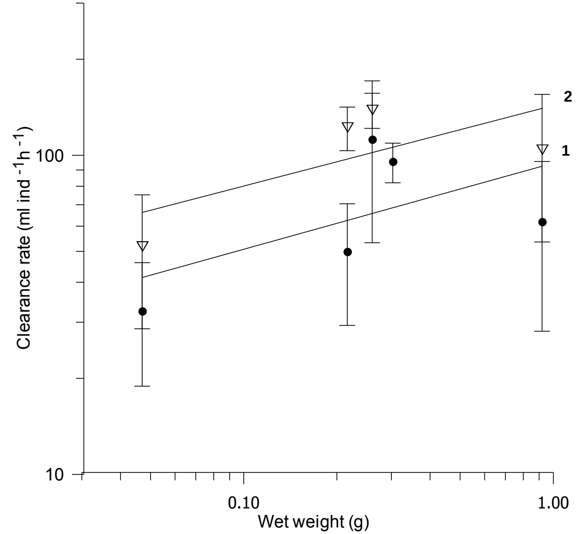

Figure 4.5. Relationship between clearance rate (ml ind. $^{-1}$ h $^{-1}$) and wet weight of *M. leidyi* fed on *Acartia* sp .copepods and adult (1) or Copepoda nauplii (2)

$CR_A = 94.55WW^{0.270}$ $r^2 = 0.670$
$CR_n = 168.08\ WW^{0.284}$ $r^2 = 0.397$

Where CR_A is clearance rate of *M. leidyi* when they fed on adult and copepodites of *A. clausi*, CR_n is the same at feeding on Copepoda nauplii (ml ind^{-1} h^{-1}) (Finenko *et al*., 2006)

The feature of *M. leidyi* is superfluous feeding when under high food concentration some share of prey is not digested, but it is covered with mucus and is released to water. So not only consuming but killing their prey *M. leidyi* can decrease the prey abundance.

Growth

Our laboratory experiments on *M. leidyi* growth at two food concentrations
(60 and 100 *A. clausi* l^{-1} or 0.35 and 0.60 mg DW l^{-1}) revealed the different food provision of small and large ctenophores under the same food conditions: while both prey densities were sufficient for growth of

small *M. leidyi* with dry weight of less than 20 mg, they could not meet growth requirements of large ones (Finenko and Romanova, 2000). Under the same food concentration (0.35 mg DW·$l^{-1)}$ the specific growth rate decreased six time: from 0.382 day^{-1} in the smallest (0.15mg DW) to 0.06 day^{-1} in the ctenophores with dry weight of 13-18 mg. The same pattern was observed for gross growth efficiency values that were 0.50-0.88 in the former and 0.30 in the latter.

The large ctenophores of dry weight > 20 mg at both food concentrations consumed 21.1-4.6% of body energy content per day, but this energy was not sufficient to meet their growth requirements: they lost from 3 to 19% their weight in a day and these losses were proportional to ctenophore weights (Table 4.1**).**

Thus in the experiment the small *M. leidyi* of dry weight < 20 mg at food density of 60 ind l^{-1} could meet their food requirements as well as realize their high growth potential while for the ctenophore of > 20 mg dry weight even the experimental concentration of 100 prey l^{-1} was insufficient for their growth.

Table 4.1. Food consumption and growth rates of *M. leidyi* at two food concentrations

Food concentration,(mg DW l $^{-1}$)	**Duration of experiment (day)**	**W_o**	**W_t**	**Body energy content (cal ind $^{-1}$)**	**Weight specific growth rate (day $^{-1}$)**	**Ration**	**Growth**	**K_1**	**CR ($l \cdot g^{-1} \cdot h^{-1}$)**
		(mg DW)				(cal·cal^{-1}·day $^{-1}$)			
0.35	8.6	0.15	4.0	1.38	0.38	0.43	0.21	0.50	20.15
- « -	5.8	0.76	5.2	2.31	0.31	0.74	0.27	0.36	19.62
- « -	8.6	1.02	5.6	2.38	0.20	0.29	0.16	0.55	7.40
- « -	5.7	13.74	19.7	11.33	0.06	0.24	0.07	0.29	5.08
- « -	5.7	17.92	27.2	15.60	0.07	0.28	0.08	0.30	6.96
- « -	4.7	47.27	27.9	24.76	-0.11	0.05	-0.11	-	0.47
- « -	5.7	48.39	26.1	25.74	-0.11	0.15	-0.08	-0.56	2.51
0.60	8.0	20.40	15.5	11.39	-0.03	0.24	-0.03	-0.14	4.35
- « -	7.0	40.60	22.9	21.15	-0.08	0.28	-0.08	-0.03	5.35

W_o = initial weight
W_t = final weight
K_1 = gross growth efficiency
CR = clearance rate

As our experiments showed the *M. leidyi* specific growth rate especially in early stages was very high under conditions of plenty of food and exceeded zooplankton growth rate. According to our observations, *M. leidyi* larvae in the laboratory during 9 days at temperature 23-24°C have grown from 0.7 to 9.5 mm, i.e. growth coefficient value was 1.2 per day that is close to phytoplankton growth rate.

Energy budget of small *M. leidyi* in the dry weight range of 2.1–22.6 mg, calculated from our experimental data at 23°C showed that under conditions of sufficient food abundance the daily ration was 40±20% of body energy content, assimilation efficiency was 58±14% and high gross and net efficiencies for growth were observed (K_1 = 0.40±0.12 and K_2 = 0.69±0.10) (Table 4.2).

Table 4.2. Daily energy budget of *M. leidyi* at 23°C (from Finenko and Romanova, 2000, Abolmasova, 2001)

DW	***W***	***R***	***C***	***G***	***A***	***a***	***K_1***	***K_2***	***C/W***
2.07	1.38	0.103	0.591	0.295	0.398	0.67	0.50	0.74	42.8
2.98	2.31	0.14	1.705	0.617	0.757	0.44	0.36	0.81	73.8
3.31	2.38	0.153	0.702	0.386	0.539	0.77	0.55	0.72	29.5
16.72	11.33	0.606	2.731	0.793	1.399	0.51	0.29	0.57	24.1
22.56	15.6	0.781	4.415	1.326	2.107	0.48	0.30	0.63	28.3
Mean ±SD						0.58 ±0.14	0.4 ±0.12	0.69 ±0.10	39.7 ±20.3

DW = dry weight (mg)
W = ctenophore body energy content (cal ind^{-1})
R = respiration rate (cal $ind^{-1}day^{-1}$)
C = ration (cal $ind^{-1}day^{-1}$)
G = growth (cal $ind^{-1}day^{-1}$)
A = assimilated food (cal $ind^{-1}day^{-1}$)
a = assimilation efficiency (%)
K_1 = gross growth efficiency
K_2 = net growth efficiency
C/W = energy specific daily ration (% of body energy content)

In Sevastopol Bay the maximum population specific growth rate (0.092 day^{-1}) occurred in summer period of intensive reproduction of adult ctenophores and growth of larvae (Table 4.3). In spring and autumn when the population consisted from wintered large ctenophores it was minimal. Calculated average daily specific growth rate of the *M. leidyi* population in a year in Sevastopol Bay was 0.039.

M. leidyi can survive during the periods of food lack using their own body tissues for metabolic requirements (Anninsky *et al.*, 2005). Under experimental starving (8 days at 12.4°C) they lost 9.3% wet weight day^{-1}. The rate of organic matter loss was half as much (5.9%). While the percentage of four main biochemical components (proteins, lipids, carbohydrates and free amino acids) was practically constant during starvation, glycogen content in polysaccharides and monosaccharide content in total carbohydrates have reduced sufficiently (from 86.6% to 3–28.8% and from 45.8% to 14.3–23.2% from the start to the end of fasting correspondingly). This fact demonstrates once again that glycogen content is an indicator of ctenophore trophic status and can be used as an index of food provision in the sea (Anninsky *et al.*, 1989a).

Table 4.3. Daily specific production (μ) of *M. leidyi* population in Sevastopol Bay in 1995

Period	Days	W_0	W_t	μ
10.08–11.09	31	0.015	0.126	0.092
26.09–30.10	35	0.020	0.126	0.073
26.09–21.12	85	0.020	0.280	0.031
30.11–28.03	120	0.007	0.460	0.035
28.03–29.06	60	0.460	1.020	0.013

W_0 = initial weight
W_t = final weight

Reproduction

In the Black Sea *M. leidyi* reproduces the year round in temperature range from 8 to 28°C (Vinogradov and Shushkina, 2002; Romanova *et al.*, 2005). In winter ctenophore fecundity is low (300–500 eggs in a clutch); due to low abundance of adults and long embryonic period ctenophore eggs and larvae in plankton are very rare. Intensive reproduction starts from the middle of April – early May and lasts until middle October – November. The most mass egg release is observed at surface water temperature of 23°C (Zaika and Revkov, 1994; Finenko *et al*., 2006b). This time reproductive activity (share of reproduced ctenophores and egg number in a clutch) achieve their maximum (to 2000–3000 eggs in the largest *M. leidyi*). Reproduction takes place synchronously at night, the ctenophores are fully prepared for spawning after 3–4 h of darkness (Zaika and Revkov, 1994). Reproduction in the population is wavy with 1–14 day periodicity during the intensive reproduction and more prolonged intervals other time (Vinogradov and Shushkina, 2002). Egg number in a clutch is a function of trophic conditions. Effect of daily ration on a clutch size is described as $E = 0.052F^{1.69}$, where E is egg number and F is daily ration, joule ind^{-1}(Vinogradov *et al*., 2000).

M. leidyi can reproduce being in larvae state. Already after 7–11 days after hatching at the length of 5–7 mm the first eggs appear. As larvae and juvenile ctenophores grow egg number increases and relation between *M. leidyi* length (L, mm) and egg number in a clutch (E) is described with power function (Romanova *et al*., 2005).

$$E= 0.513\ L^{1.86} \qquad \begin{array}{l} 5 \le L \le 20 \\ 5 \le E \le 178 \end{array} \qquad (4.4)$$

Duration of embryonic development in the temperature range of $17–24^0$ C reduces from 24 to 12 h with Q_{10} of 2.34 (Zaika and Revkov, 1994; Romanova *et al*., 2005).

Respiration.

M. leidyi respiration rate (R, ml O_2 ind^{-1} h^{-1}) can be described as $R = 0.112\ DW^{0.772}$ in a wide range of dry weights ($0.0005 < DW < 0.41$ g) at 23°C (Abolmasova, 2001). Similar relationship ($R= 0.11\ DW^{1.02}$) was found also by other authors in the whole length range of adult ctenophores – 10–70 mm (Vinogradov *et al*. 2000; Vostokov *et al*. 2001). The most important factor affected on the respiration rate along with their weight is a temperature. *M. leidyi* is the most sensible to low temperature: in the range of temperature of 7–12°C Q_{10} coefficient was as high as 6.7 and decreased to 1.8 between 12–23°C (Abolmasova, 2001). The respiration intensity value depends on food supply not so much: the maximum value twice as high as minimum one (Finenko *et al*., 1995). Under 8–day starving at 12.4°C respiration rate also was half as high as in freshly collected Ctenophora (Anninsky *et al*., 2005).

4.3 Chemical composition and the elements of ctenophore *Beroe ovata* energy budget

4.3.1 Chemical composition

B. ovata dry weight amounts 2.4–2.5% of wet weight (Vinogradov *et al*., 2000; Finenko *et al*., 2001) that is close to the value in *M. leidyi.* In the chemical composition *B. ovata* is also similar to *M. leidyi* but organic matter content per unit of wet weight two times higher (Anninsky *et al*., 2005). This determines its higher energy content -14.8±2.8 cal $g–^{1}$ wet weight against 6.7±1.0 cal g^{-1} in *M. leidy*i. Protein, the main organic component, amounts to 80% of total organic matter; lipids, carbohydrates and free amino acids are 10 and 5.3–5.6%, correspondingly. Chemical composition is almost the same in the ctenophore size range of 17–43 mm, although in the *B. ovata* of the same size protein, carbohydrate and amino acid content can vary 2–3 fold, proteins and lipids – 3–4 times (Anninsky *et al*., 2005).

4.3.2 Feeding

B. ovata is a monophage, that feeds almost exclusively on *M. leidyi* in the Black Sea. Obviously, the another, indigenous Black Sea ctenophora *Pleurobrachia pileus* that is disconnected with *B. ovata* in space can be used as a supplementary food item during some periods of the population migration to the *B. ovata* inhabitation layers at night or in winter under extremely low *M. leidyi* concentration.

The most important biotic factor that determines the *B. ovata* ration value is their weight (Finenko *et al*., 2003). The effect of *B. ovata* wet weight (WW, g) on specific daily ration (F/WW) in the experiments under food concentration of 2 g l^{-1} was described with power function:

$F/WW = 25.9\ WW^{-0.703}$ $(3 < WW < 30\ g)$

and the ration value changed from 15 to 460% of wet weight (WW, g), being the maximum in the smallest ctenophores (Fig. 4.6).

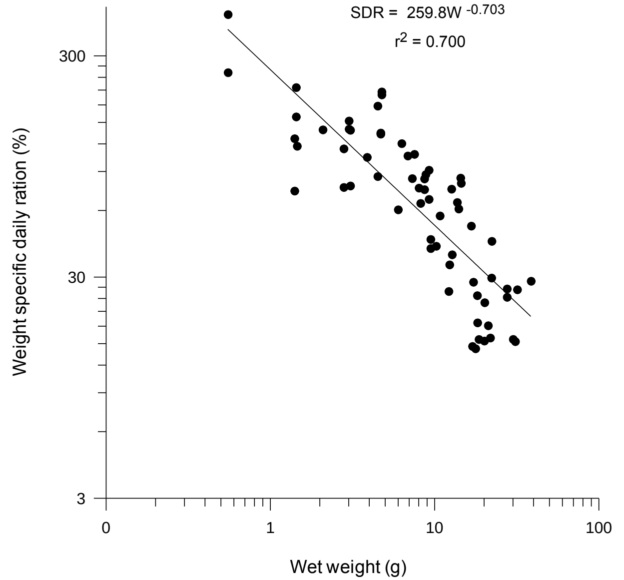

Figure 4.6. Relationship between weight-specific daily ration (SDR, %WW) and wet weight (g) in *B. ovata* at food concentration of 1 g l^{-1}

Probably such high ration values should be considered as a momentary greatest possible ration at high food concentration. But such situation is quite possible in the sea because as underwater observations showed *B. ovata* occur into *M. leidyi* aggregations. In such a way *B. ovata* can consume sufficient prey number to meet their food requirements for a long time and to minimize energy losses for food searching (Kideys *et al*., 2004).

A periodicity in feeding is adaptation to sporadically food availability, it is not related to light conditions or to the time of day, but more to the intensity of the previous feeding: ingestion of a large portion of food is followed by a low feeding rate (Fig.4.7). All sizes of *B. ovata* consume intensively all sizes of *M. leidyi* but under conditions of possible selection and high prey density (number per volume), *B. ovata* preferred large and medium size *M. leidyi.* When there are no selection (the different size prey were offered in the experiment in the same biomass but various number separately) they could ingest the small *M. leidyi* too and ranges of specific daily rations were very close to each other upon feeding on different sizes of *M. leidyi.*

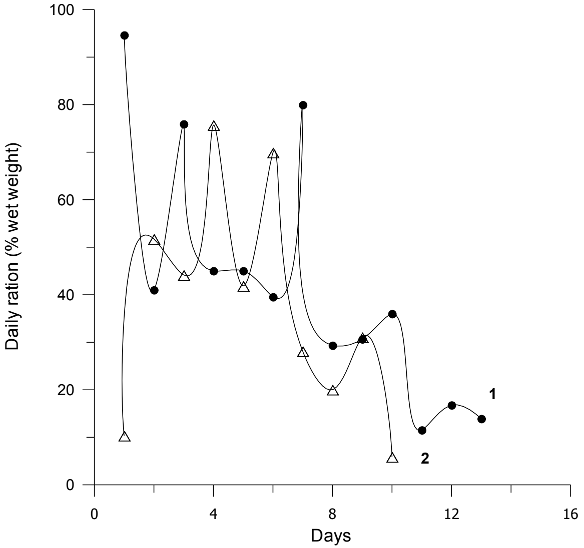

Figure 4.7. Dynamics of *B. ovata* feeding rate in long–term experiments at 26°C. 1 and 2 – *Beroe* specimens with initial length 30 mm. Average daily ration in *Beroe* 1 is 40.2 ± 27.0%, in *Beroe* 2 is 37.9 ± 23.4%

B. ovata digestion time varies from 0.75 to 5 hours at 20–22°C (Finenko *et al.*, 2000, 2001; Shiganova *et al.*, 2000) and the prey - predator weight ratio appeared to be effective on digestion time. It can be described by the following equation with a high coefficient of determination:

$DT = 2.956\ P^{0.59}$ $r^2 = 0.87$ $0.03 < P < 3.23$ (4.6)

Where DT is digestion time, min, P is prey/predator weight ratio (Fig. 4.8).

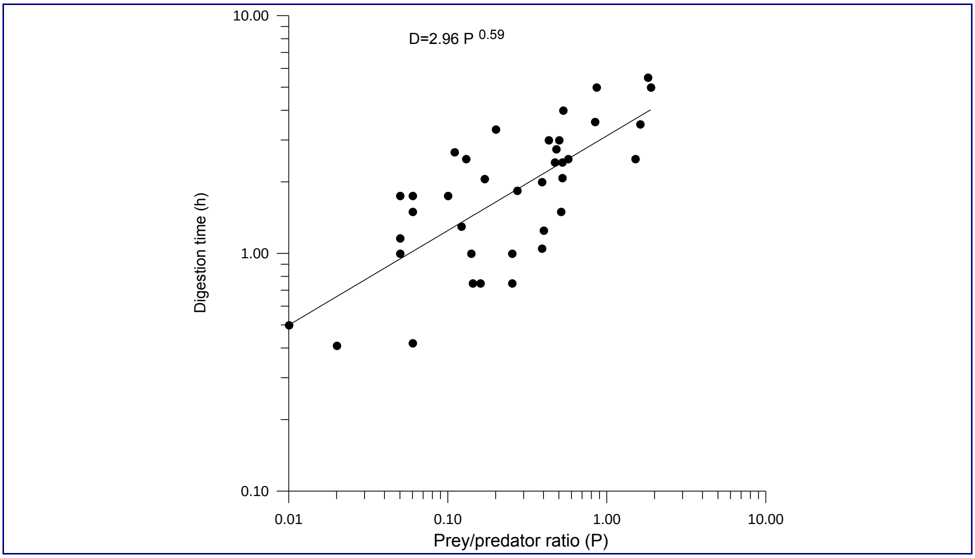

Figure 4.8. Effect of prey/predator weight ratio (P) on digestion time (D, h) in *B. ovata* at 21°C

It is important because of different *M. leidyi* population structure throughout an year. In the Black sea *B. ovata* appear in plankton after *M. leidyi* reproduction and small ctenophores make up the most population. *B. ovata* ability to ingest small prey results in sharp decrease of *M. leidyi* abundance during the shortest period.

4.3.3 Growth

B. ovata high potential feeding rate can result in very high growth intensity: in Sevastopol Bay under maximum prey concentration and high temperature maximum daily specific population growth rate ranged from 0.33 to 0.67, that is higher than the growth rate of planktonic Crustacea. It is a reason of explosive development of *B. ovata* population as well as *M. leidyi* growth rate of which can be even higher than in *B. ovata*.

B. ovata demands both high food rations (not less than 20% of body weight per day) and a high prey biomass for growth. Probably the low prey abundance in late autumn is a major reason for disappearance of *B. ovata* from the plankton.

In our laboratory experiments the specific growth rate increased from -0.05 to 0.20 while the ration increased from 27 to 150% of body weight (Fig. 4.9). The same relation was found for gross growth efficiency (K_1) that was negative (about – 0. 26) at the ration value less than 27% of wet weight (Fig. 4.10). Increasing of the ration resulted in rise of the growth efficiency.

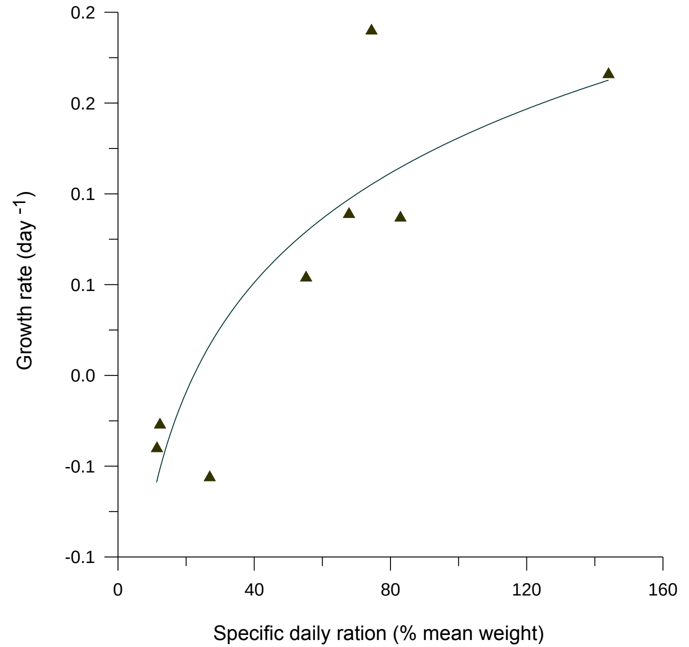

Figure 4.9. Effect of specific daily ration (%mean wet weight) on specific growth rate (day^{-1}) in adult *B. ovata*

Like *M. leidyi B. ovata* can endure long- term starving. Under 18- day experimental starving at temperature 16–18°C ctenophore wet weight have been decreasing by 9.4% in a day, while rate of organic matter decrease was half as much of that (5.9%) (Anninsky *et al.*, 2005). As our calculations showed at that rate of organic matter loss (with temperature correction) some *B. ovata* specimens are able to survive for rather long period (about 9 months when we don't find them in plankton) without any food.

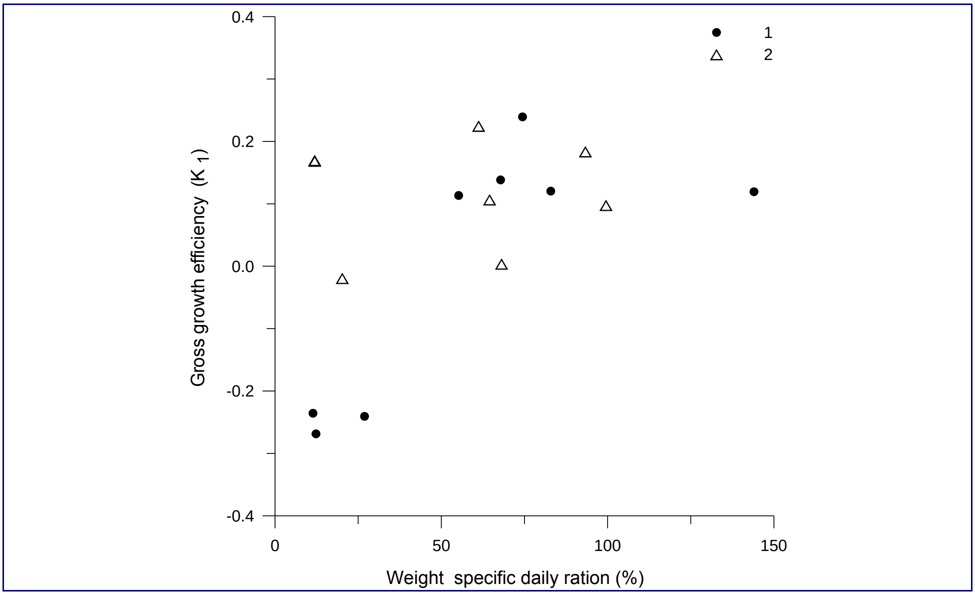

Figure 4.10. Effect of specific daily ration (% mean wet weight) on gross growth efficiency (K_1) at two food concentrations: 1.68 ± 0.47 g l^{-1} (1), 2.54 ± 0.80 g l^{-1} (2)

4.3.4 Respiration

The comprehensive researches of *B. ovata* energy metabolism in the Black Sea have been conducted by Svetlichny *et al.*, 2004, which cover their whole life cycle. As their experiments showed *B. ovata* had different relationship between respiration rate (R, $\mu g\ O_2\ ind^{-1}\ h^{-1}$), and wet weight (WW, mg) during ontogeny in contrast to most of marine invertebrates when these relations were unified for the whole life cycle. In small *B. ovata* with length of 0.4-3.0 mm and wet weight of 0.021–100 mg at 20°C it is expressed by the equation $R = 0.093WW^{0.62}$, according which the weight - specific respiration rate reduces 20 times as wet weight raises; in ctenophores with length of 4–60 mm and wet weight of 100–30000 mg the expression $R = 0.016\ WW^{0.99}$ shows that weight specific respiration rate is steady – state in this range of ctenophore size. The same character of relation between respiration rate and wet weight in adult *B. ovata* have been recorded earlier (Finenko *et al.*, 2001; Shiganova *et al.*, 2001; Vostokov *et al.*, 2001), but they have not conducted their researches on early stages of ctenophores.

B. ovata respiration rate is 2–3 times as much as in *M. leidyi* at the same temperature. Thus, specific oxygen consumption by adult *B. ovata* at 20^0C ranges from 0.010 to 0.020, while by *M. leidyi* from 0.003 to 0.007 $\mu g\ O_2\ mg\ WW^{-1}\ h^{-1}$ (Shushkina *et al.*, 2000; Abolmasova, 2001, Shiganova *et al.*, 2001; Vostokov *et al.*, 2001; Svetlichny *et al.*, 2004; Anninsky *et al.*, 2005). Probably this is a result of larger locomotion activity as well as high organic matter content in *B. ovata* (Vostokov *et al,*. 2001; Anninsky *et al.*, 2005).

Respiration rate in adult ctenophores with length of 10–50 mm (200–12000 mg) in the temperature range of 10–28^0 C increases continuously and Q_{10} coefficient is equal to 2.17±0.5. The basal metabolism of ctenophores narcotized with chloral hydrate was 4.5±0.9 times lower than total metabolism that is characteristics of aquatic animals with high locomotion activity (Svetlichny *et al.*, 2004).

The study of effect of food conditions on respiration rate revealed that *B. ovata* weight specific respiration rate after 18- days starving at 17±1°C fell by 33%, while in *M. leidyi* it decreased almost 2 fold at 8-days starvation at 12.4°C, i. e. *B. ovata* tolerate to nutrition conditions and is able to survive without any food for more long time.

4.3.5 Reproduction

In the Black Sea *B. ovata* starts to produce eggs at length about 30–35 mm. Clutch size is related with ctenophore length and increases from 40 eggs in small to 5000–7000 eggs in 80–120 mm ctenophores (Arashkevich *et al*., 2001). Besides it is related with feeding intensity and it is a good index of food supply of animals in the sea. At once after its appearance in plankton when *M. leidyi* is in abundance, *B. ovata* starts reproduction. This time *B. ovata* has maximum fecundity and all 100% of adult ctenophores reproduce (Table. 4.4). As early as in two weeks after fast exhausting of food (sharp decrease of *M. leidyi* abundance due to *B. ovata* consumption) the share of reproduced adult ctenophores in the population reduces to 50%, and egg number in a clutch decreases in two orders. Although hatching success is high, it is lower of it in the beginning of reproductive period. Attenuation of reproduction continues till October – November.

Thus, experimental data show that *B. ovata* in the conditions of high food supply can realize their food requirements, reach maximum growth rate and increase the population due to high fecundity.

Table 4.4. Dynamics of the reproductive indices of *B. ovata* in September 2003 (unpublished data of Finenko and Arashkevich)

Date	% of ctenophore reproduced	Egg number in a clutch	Hatching success (%)
12 – 14. 09	100	4498 ± 2652	96 ± 4
18 – 20. 09	86	1684 ± 543	95 ± 1
21 – 23. 09	67	525 ± 386	87 ± 15
24 – 26. 09	50	57 ± 17	83 ± 10

4.3.6 Feeding, respiration, growth and reproduction of *B. ovata* under low salinity.

We have studied an effect of lower salinity on survival and ecological-physiological characteristics of *B. ovata* in connection with the problem of its possible introduction to other seas, in particular to the Caspian Sea with its lower salinity.

As our experiments showed *B. ovata* larvae transferred from salinity of 18 to 6‰ without preliminary acclimation died immediately. When salinity was decreased step by step they were alive at 6‰ about a day. Survival and growth rate of larvae in the salinity range of 9–18‰ raised as salinity increased. Thus, survival time of 50% of larvae abundance was 3 days at 9‰, 5 days at 12‰ and 8 days at 18‰ at 24°C (Fig. 4.11). Growth rate (µm day^{-1}) at 1 ‰ was 1.5 times lower than at 18‰ but daily ration values were the same at both salinities (Table 4.5).

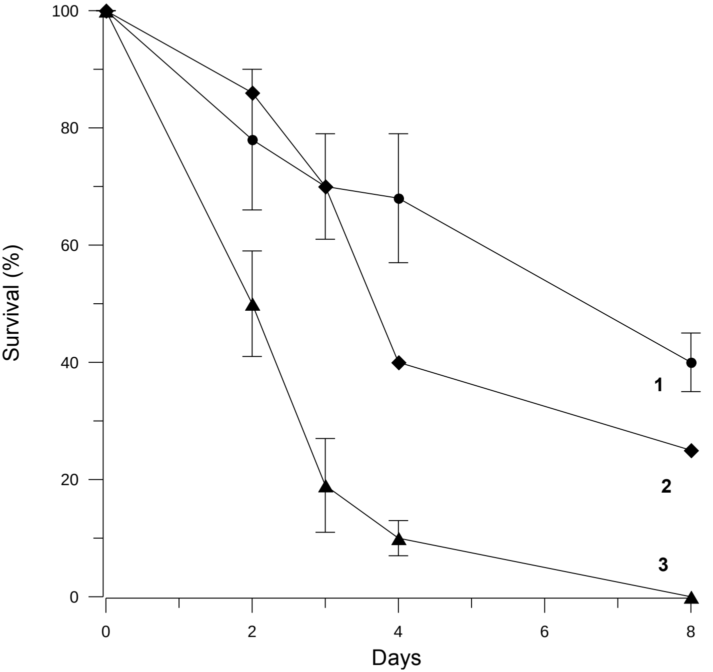

Figure 4.11. Survival of *B. ovata* larvae at different salinities (9, 12, 18‰)

Table 4.5. Growth, ingestion and survival rates of *B. ovata* larvae at salinities of 12 and 18 ‰ (unpublished data of Finenko, Romanova and Arashkevich)

Parameters	**Salinity**	
	12‰	18‰
Initial length (mm)	0.500 ± 0.075	0.500 ± 0.070
Initial number of larvae	40	40
Length at the end of the experiment (mm)	0.750	0.887 ± 0.327
Number of larvae at the end of the experiment	2	13
Days of growth	12	12
Growth rate (mm day^{-1})	0.021	0.032
Survival (%)	5.0	32.5
Daily ration (prey ind^{-1}day^{-1})	5.0	7±3

Adult *B. ovata* survived in the laboratory at 9‰ only for 3 days and on the second day there was 50% mortality (Fig.4.12). Ctenophores at 9‰ were inert, stayed near the bottom, did not feed and reproduce. At 12‰ salinity *B. ovata* were more active, swam in water column**,** their survival was higher than at 9‰, but lower than at 18‰, where during 8 days all ctenophores were alive. At the same salinity (12‰) survival was maximum (91%) in the smallest adults (10–19 mm), medium (62%) in size group of 20-29 mm and the lowest in large ctenophores > 30 mm during 5 days of the experiment (Fig.4.13).

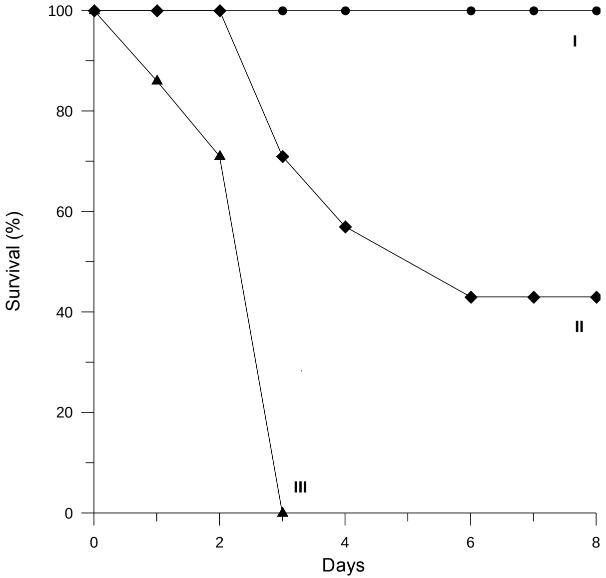

Figure 4.12. Survival of adult *B. ovata* at different salinities 18 (1), 12 (II), 9 ‰ (III)

The study of ecological-physiological indices of adult *B. ovata* that were acclimated to 12.5–13‰ salinity showed that they could feed intensively: daily ration values at high prey concentration were as high as 45–765% of wet weight, being maximum in small ctenophores (13–16 mm) (Fig. 4.14).

B. ovata growth in conditions of lower salinity was exponential during some or the largest part of life cycle ($L_t = L_o e^{k\,t}$, where L_o and L_t are initial and final length, k is growth coefficient, t is time). Daily weight specific rate of *B. ovata* at salinity of 12.5–13‰ was 0.07–0.11. These values were obtained for 30-mm ctenophores, when daily rations changed from 26 to 43% of wet weight.. The same growth rate was observed in our experiments in the Black Sea ctenophores at daily rations of 50% wet weight (Finenko *et al.*, 2003).

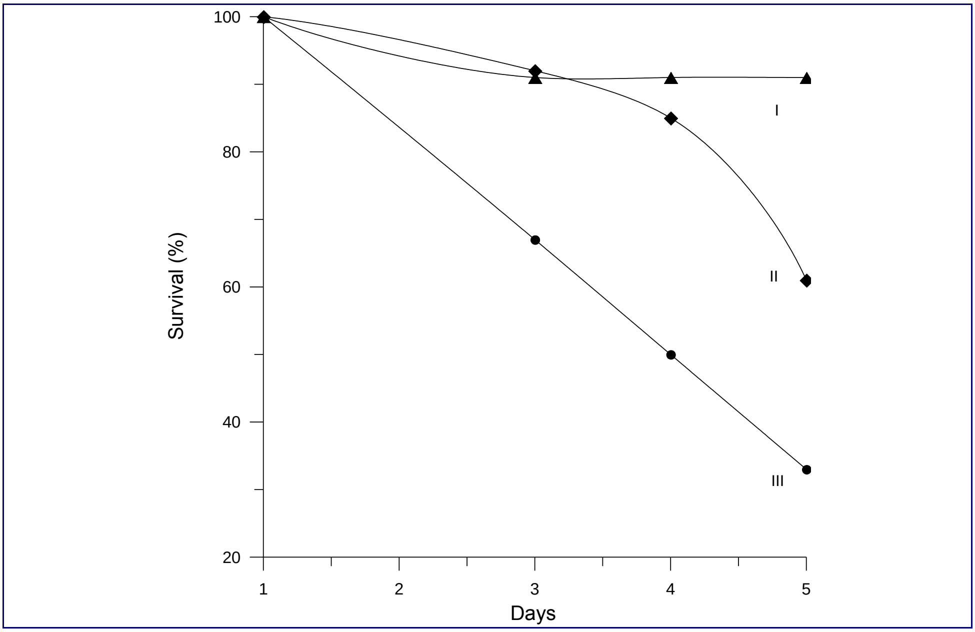

Figure 4.13. Survival of different size *B. ovata* at different salinities 12 ‰ (I – 10-19, II – 20-29, III – 30 –35 mm)

Even in conditions of lower salinity *B. ovata* assimilate food and use it for growth with high efficiency. These values calculated from ration, growth and respiration were as high as 0.72 for assimilation efficiency and 0.48 and 0.66 for efficiency of ingested and assimilated food utilization for growth, correspondingly (Table. 4.6).

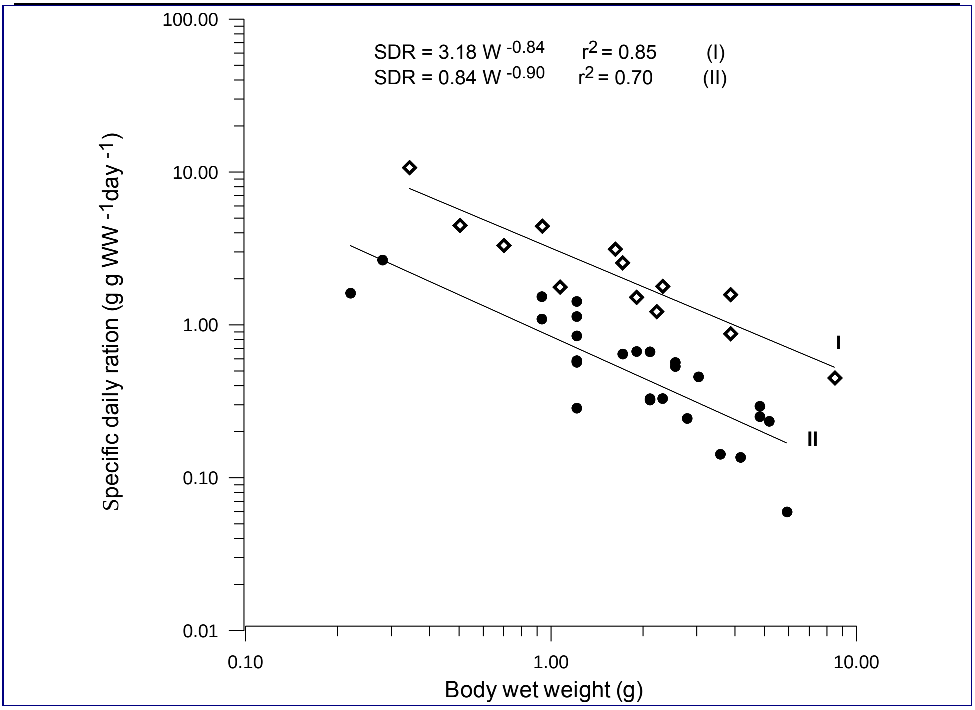

Figure 4.14. Effect of body wet weight (WW, g) on specific daily ration (SDR, g g^{-1} day^{-1}) at two food concentrations (I – 1. 66 г л $^{–1}$, SDR = $3.18W^{-0.84}$, r^2 =0.85; II – 1.00 г $л^{-1}$, SDR = $0.84W^{-0.90}$, r^2 = 0.70). t =21°C

On the whole the rates of ecological-physiological processes in adult *B. ovata* adapted to suitable salinity conditions are rather close at salinities of 18‰ and 12.5–13‰ (Table 4.7). But *B. ovata* are less tolerate to lower salinity than *M. leidyi*: lowest salinity value for *B. ovata* survival is about 7–9‰ (our data, Shiganova *et al.*, 2000) while *M. leidyi* can live at salinity from 0.1 to 38‰ (Purcell, 2005).

B. ovata eggs appeared to be the most sensitive to lower salinity. During ontogenesis the salinity range as a rule is getting wider. *B. ovata* larvae were more sensitive to lower salinity than adult ctenophores. Meantime juvenile individuals (10–19 mm) were more resistant to reduced salinity from adult *Beroe* (> 30 mm).

Table 4.6. Daily energy budget of *B. ovata* (cal ind^{-1} day^{-1})

Initial weight, g	***C***	***R***	***G***	***A***	***a***	K_1	K_2
3.17	18.06	4.73	11.27	16	0.88	0.62	0.7
3.17	10.73	2.89	4.1	6.99	0.65	0.38	0.59
3.17	17.11	3.57	7.7	11.27	0.65	0.45	0.68
Average					0.72±0.13	0.48±0.12	0.66±0.06

C = daily ration
R = respiration rate
G = growth rate
A = assimilated food
a = assimilation efficiency
K_1 = gross growth efficiency
K_2 –net growth efficiency

Table 4.7. Physiological characteristics of ctenophore *B. ovata* at the salinity of 12‰ (Caspian water) and 18‰ (the Black Sea)

Index	Caspian water (12 ‰)	The Black Sea (18 ‰)
Daily ration, % WW	14 - 380	5-460
Digestion time, h	0.5-7.5	0.5 –5.5
Respiration rate, ml O_2 g^{-1} DW h^{-1}	0.67	0.63 – 0.82

It seems possible to suppose that if to introduce *B. ovata* to water of lower salinity at the stages of early larvae the only small part of them could survive. But rather long preliminary acclimatization of juvenile animals (preferably with size of 10–20 mm) can affect upon the salinity resistance of both adult and embryos and larvae produced by them. As a result the survival range can be shifted towards lower salinity.

4. 4 State of Ctenophore invader populations after *B. ovata* introduction

4.4.1 Seasonal dynamics

Extensive monitoring studies of the seasonal and interannual dynamics of ctenophores during recent decade after *B. ovata* introduction to the Black Sea have been conducting in the coastal regions off the Crimean Coast (Sevastopol Bay and adjacent waters) and in the northeastern Black Sea (Blue Bay near Gelendzhik) (Finenko *et al.*, 2001, 2003, 2006a; Shiganova *et al.*, 2000, 2003; Vinogradov *et al.*, 2000, 2005, 2006).

The typical seasonal dynamics of both alien species in both areas is the same and we will examine it as exemplified by the ctenophores in Sevastopol Bay.

In winter - early spring (January – April) *M. leidyi* is not abundant (0.2–3.6 ind·m^{-2} and 1–10 g·m^{-2}) and is represented by large individuals of an oral – aboral length of 25–55 mm and individual wet weight of 5–35 g (Figs. 4.15, 4.16). In spring (April – May) the wintered population started its reproduction (at temperature about 16°C) and in plankton adult animals (30–55 mm), as well as larvae (0.25–0.3 mm) and eggs are presented simultaneously. From this time, owing to the recruitment of the juveniles of the new generation, the population gradually, though insignificantly, increases its abundance but biomass is still low and ranges from 7 to 20 g·m^{-2}. At the end of May – early June, the grown juveniles of the new generation of 0.3–1.25 mm length form the most population (80% of total abundance). Mainly reproduction takes place in bays and inshore waters where share of larvae (< 10 mm) in the time of maximum reproduction can exceed 90% of the total population abundance. In deeper waters (>100 m depth) there are no the individuals < 10 mm often, and large *M. leidyi* with length > 50 mm make up 20–30% of population (Vinogradov *et al.*, 2005). In the whole population size range is greater in open and shallow waters when compared to bay. Thus, in the bay *M. leidyi* maximum length is about 40 mm, and in adjacent shallow waters it is as long as 80 mm (our data).

Mnemiopsis reproduction starting in spring has been lasting over the entire summer at different rates with 2 week intervals between peaks (Vinogradov and Shushkina, 2002), that results in sharp increase of abundance in July – August at surface water temperature 23°C. This time the individuals of spring generation start reproduction also. The abundance dynamics changes sharply with appearance of another ctenophore – *B. ovata* – in plankton: *M leidyi* abundance and biomass decrease one order of magnitude during short period (10–12 days) (Fig.4.15a,b). With the further development of the *B. ovata* population, the population of *M. leidyi* decreases until December when no ctenophores can be found in the bay or other shallow regions. Probably in winter *M. leidyi* makes aggregations near bottom in deep waters with temperature of 8–9°C (Mutlu, 1999; Zaika, 2005). Sufficient food supply of this non – numerous wintered population results in high individual growth rate and fecundity of the ctenophores in spring, so maximum abundance and biomass in summer can reach values that were observed in the years before *B. ovata* introduction

The population of *B. ovata* starts reproduction next to the peak of *M. leidyi* and rapidly reaches the maximum abundance. In this time mean fecundity of adult *B. ovata* with length of 35–70 mm is as high as 4500±250 eggs in a clutch, all eggs are viable and they develop to larvae (Fig. 4.17). The high reproduction intensity of *B. ovata* provides a rapid increase in its abundance. The period of intensive reproduction is very short (about 2 weeks); later, the fecundity and the share of reproducing ctenophores decrease significantly. *B. ovata* is available in plankton during rather short period (2–3 months): from August – September till November – December in different years, then possibly like other *Beroe* species do in the northern seas the individuals of new generation sink to the near bottom waters and stay there until maximum of *M. leidyi* develops (Sieferd and Conover, 1992; Falkenhaug, 1996). As shown above, *Beroe* is tolerant to a fluctuating food supply and can survive without any food for relatively long periods of time (Anninsky *et al*., 2005). However, in winter, it may use another ctenophore species, *P. Pileus*, as its food source.

This seasonal dynamics of ctenophores – invaders is typical for all shallow regions. Thus, in the northeastern Black Sea ctenophore development had the same pattern in the whole shallow zone up to region of continental slope but with some temporal shift in separate years particularly pronounced in 2004 (Shiganova *et al.*, 2004; Shushkina *et al.*, 2004; Vinogradov and Shushkina, 2002; Vinogradov *et al.*, 2005, 2006)**.**

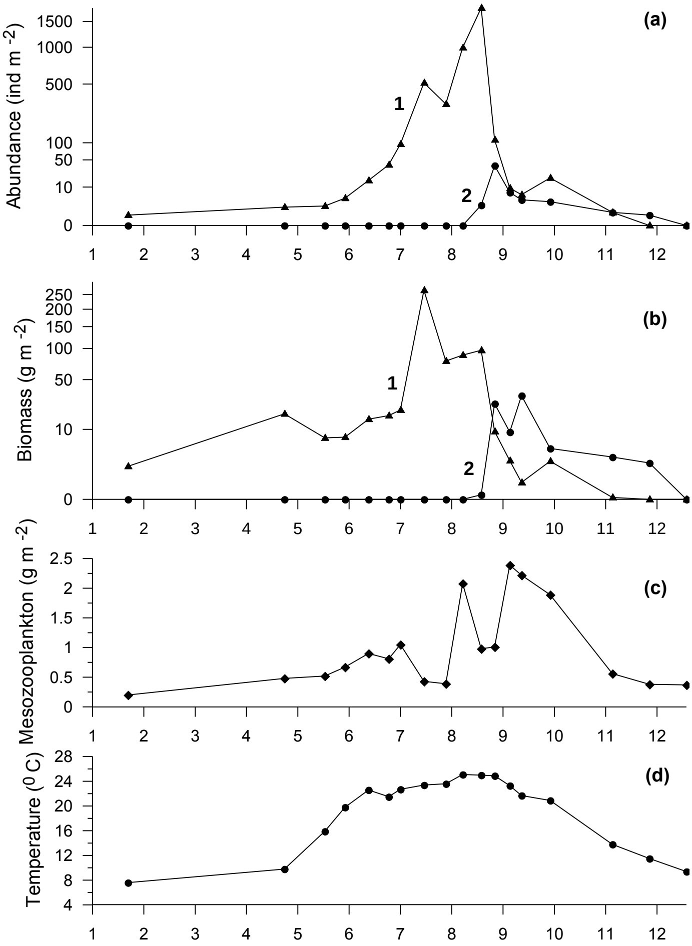

Figure 4.15. Abundance (a), biomass (b) of ctenophores *M. leidyi (1)* and *B. ovata (2)*, mesozooplankton biomass (c) and water temperature (d) in Sevastopol Bay in 2003

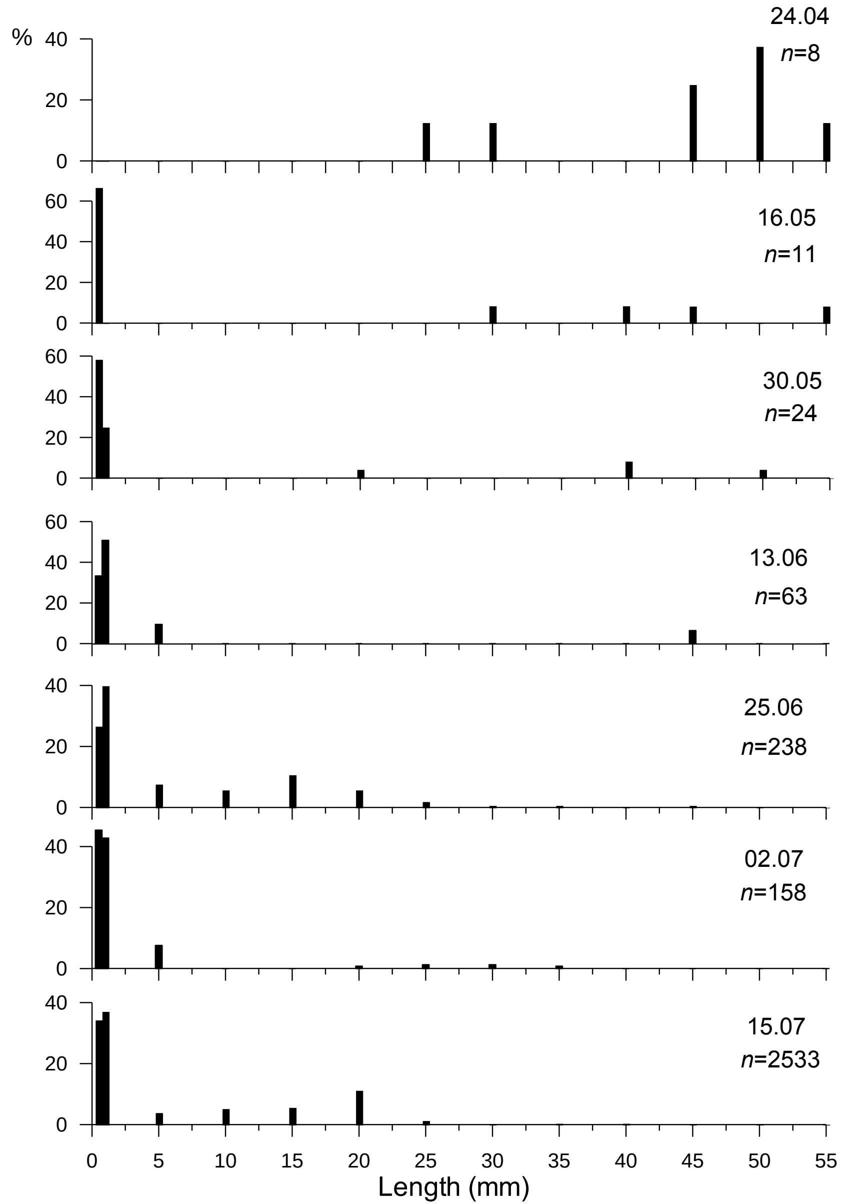

Figure 4.16. Population size structure of *M. leidyi* in Sevastopol Bay in 2003

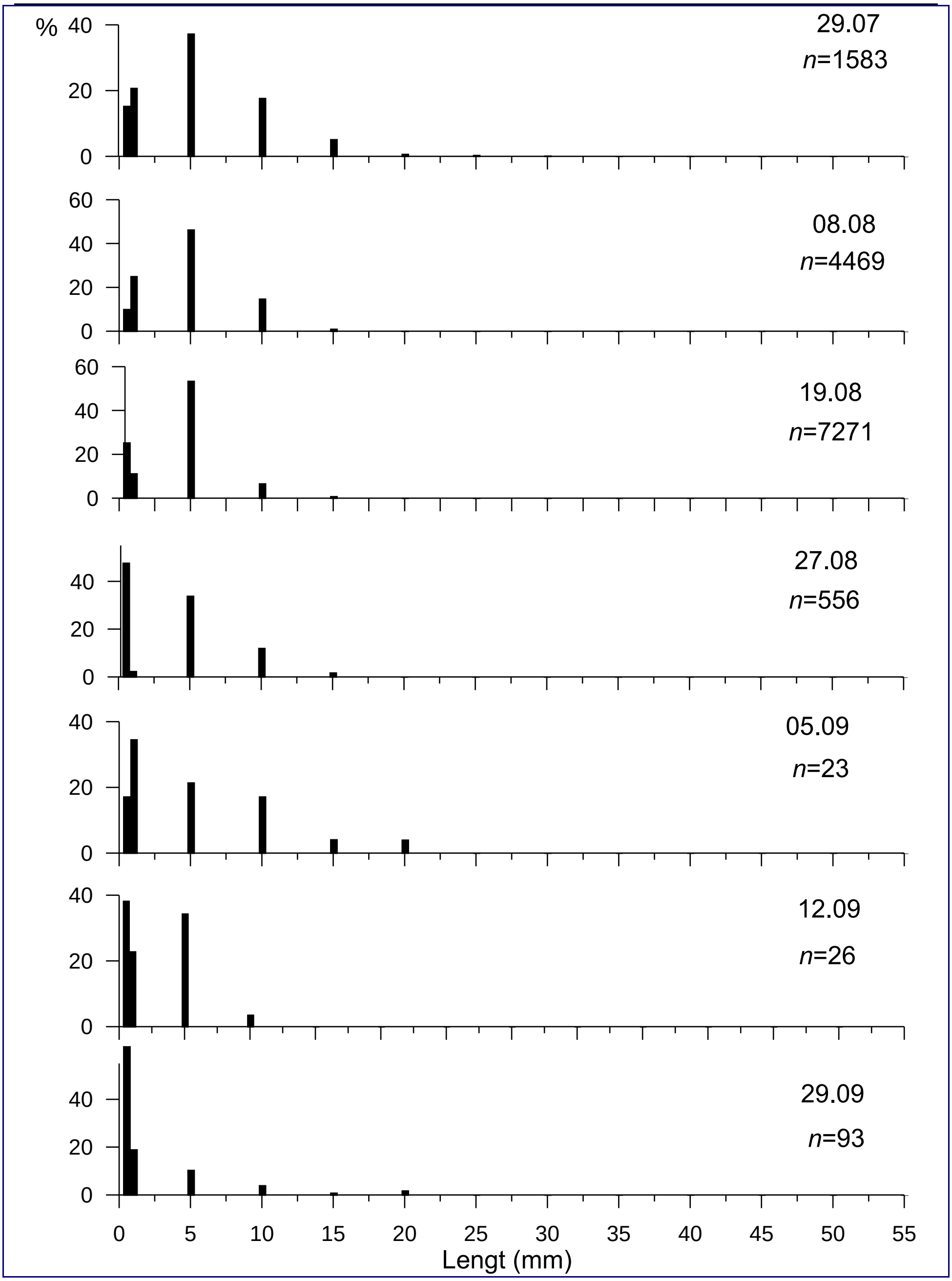

Figure 4.16 (cont'd). **Population size structure of *M. leidyi* in Sevastopol Bay in 2003**

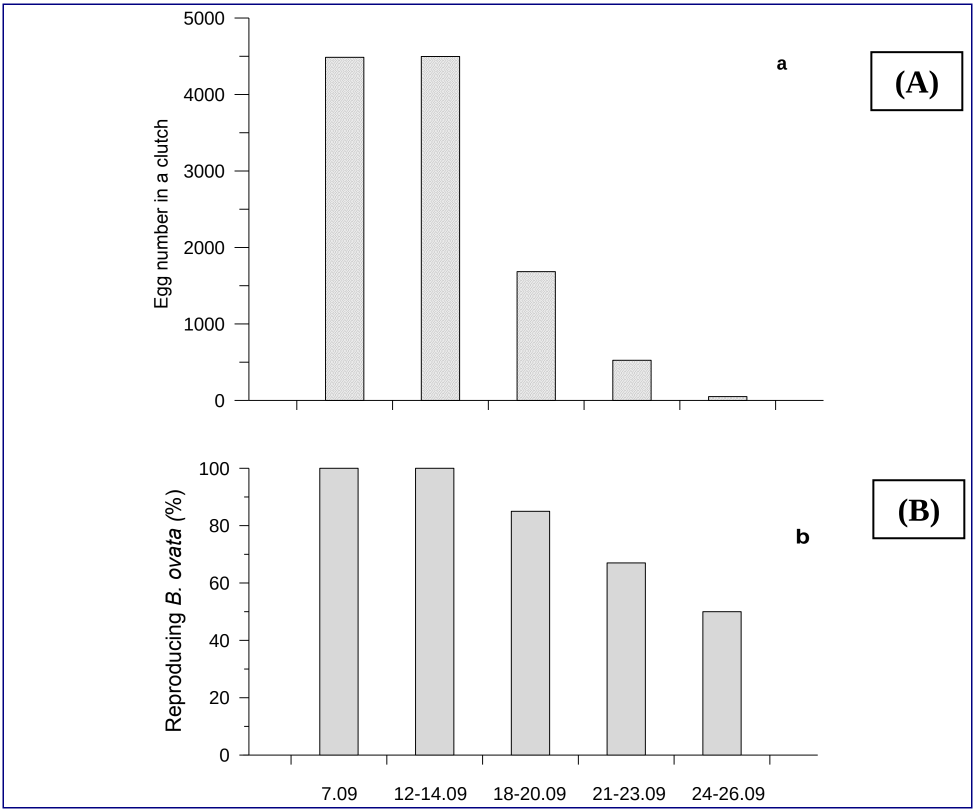

Figure 4.17. Clutch size of *Beroe* (A) and percentage of ctenophore reproduced (B) in September 2003

4.4.2 Interannual dynamics of the ctenophore invaders in different regions of the Black Sea.

During the entire study period after *B. ovata* appearance the patterns of population dynamics of both species are similar, though the timing of mass development and abundance differed in different years (Fig. 4.18). For example, the timing of maximum abundance of *M. leidyi* in the bay can be shifted for a month or more and it is governed by summer temperature. Thus, in 2000 at the lowest temperature in July for the recent 5 years (21.2°C) maximum abundance was observed in

September (temperature 23.5°C), and opposite, in 2002 it was in July at 25°C As it was shown above, *M. leidyi* maximum reproduction rate in the Black Sea occurs at 23°C, and it determines timing of seasonal maximum abundance in different years and in different sea areas as well. Maximum abundance value probably is a function of food conditions. As a rule, two – three weeks later of *M. leidyi* peak a maximum of *B. ovata* is observed followed by a subsequent decrease in its abundance. *M. leidyi* is very scarce over the whole autumn (0.4–6 ind m^{-2} or <0.1–2.4 g m^{-2} in different years in inshore areas). The peaks of abundance and biomass of both species do not coincide in time, since the main contribution to the biomass is made by large adult ctenophores, while high abundance values are provided by larvae (for *M. leidyi*) and juvenile individuals (for *B. ovata*).

The interannual differences in the development of the populations of both ctenophore species consisted in their quantitative parameters: the maximum biomass of *M. leidyi* in Sevastopol Bay (1100 $g{\cdot}m^{-2}$) was observed in 2001, while during the other years it was almost constant (160–230 $g{\cdot}m^{-2}$). Comparison between the values of the mean biomass of fodder zooplankton during the two – month period prior to maximum *M. leidyi* development showed that in 2001 it was almost twice as high as in other years (1.42 against 0.8–0.88 $g{\cdot}m^{-2}$); this was probably caused by the high summer temperatures and sufficient food supply. These factors provided a high fecundity and, correspondingly, very high abundance of ctenophores.

B. ovata reached its maximum abundance and biomass values in Sevastopol Bay also in 2001 (140 ind m^{-2} and 53 $g{\cdot}m^{-2}$); in 2000 and 2002 it was not higher than14 $g{\cdot}m^{-2}$.

The first *B. ovata* appearance in Sevastopol Bay coincided with its appearance in other regions of the Black Sea (the northeastern and southern parts of the sea) (Finenko *et al.*, 2000; Shiganova *et al.*, 2000; Vinogradov *et al.*, 2000; Kideys and Romanova, 2001). Its further development in the regions studied was similar. The seasonal dynamics of the abundance of both species in the northeastern Black Sea off the Caucasian coast (Blue Bay and adjacent regions) in the years 1999–2004 featured the same patterns with certain time shifts in some years; this was especially obvious in 2004 (Shiganova *et al*., 2001, 2003; Vostokov *et al*., 2001; Vinogradov *et al*., 2002, 2005; Shushkina *et al*., 2004). Here, similarly to Sevastopol Bay, the maximum *M. leidyi* biomass was observed in 2001 and was comparable with the values in the years before *B. ovata* invasion.

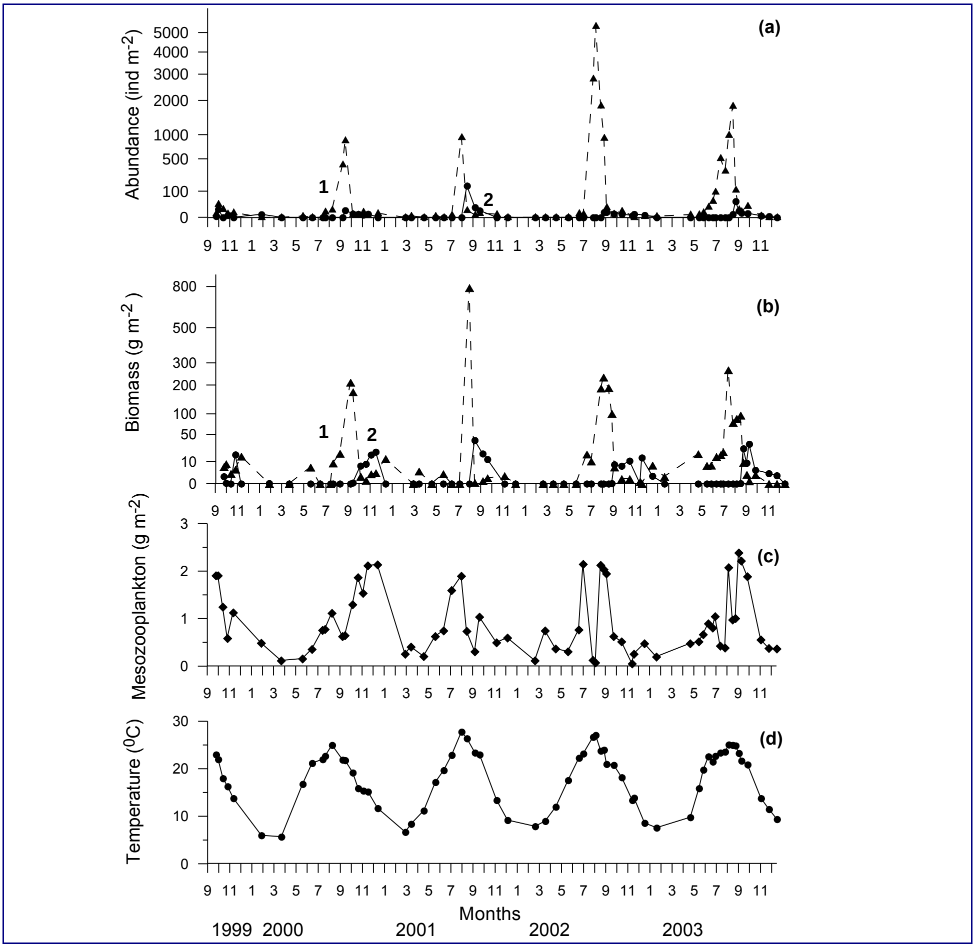

Figure 4.18. Abundance (a), biomass (b) of *M. leidyi (1)* and *B. ovata (2)*, biomass of mesozooplankton (c) and water temperature (d) in Sevastopol Bay in 1999 – 2003.

The abundance and biomass of *B. ovata* in Blue Bay were higher than those in Sevastopol Bay; in August 2001 they reached 700–800 ind m^{-2} and 500 g m^{-2}, respectively. In Sevastopol Bay during all the years the abundance and biomass of *B. ovata* did not exceed 140 ind m^{-2} and 39 g m^{-2} (August 2001). Such high difference between abundance and biomass values in different regions possibly is due not only their real distinctions but also to different methods of the calculations. We have not introduced correction coefficient for the net catch ability but in the studies of the Shirshov Institute of Oceanology in Blue Bay the authors applied various coefficients (2–4) for different size groups of ctenophores.

In the northwestern Black Sea off the Bulgarian coast, *B. ovata* was first founded in 1997 (two years before of the other regions) (Konsulov and Kamburska, 1998). During the surveys in 1998 and 1999 (in late September) *B. ovata* presented in plankton with abundance of 40–80 ind m^{-2} but in the beginning of September 2000 and August 2001 it was absent in both the coastal and the open sea waters (Kamburska, 2004). About 90% of the *M. leidyi* population at that time was comprised by juvenile individuals, which suggested a reproduction peak that preceded *B. ovata* appearance. The absence of *B. ovata* seems to be related to the time of the observations. Indeed, in these years in Sevastopol Bay we first observed *B. ovata* somewhat later – in the middle September in 2000 and in the middle August in 2001. In our opinion, the interannual variations in timing of development of two ctenophore populations and the rapid (avalanche -like) change in their abundance require frequent observations to estimate correctly the dynamics of both populations. Such kind of the research allows to obtain more valuable information even as compared to oceanographic cruises, when sampling is performed only a few times in an year**.**

So, while in the first years after *B. ovata* invasion, the issue of its ability for acclimatization under the conditions of the Black Sea was urgent, the observations of subsequent years made it clear that this species found its place in the composition of the Black Sea macrozooplankton, occupied its niche, started to control *M. leidyi* abundance and reduced its pressure on other constituents of the ecosystem. It is worth noting that *M. leidyi* maximum abundance and biomass in some years can reach rather high values**.** Probably the better food provision of this over-wintering not numerous population produces fast growth and high fecundity so that in early summer the maximum abundance and biomass values of *M. leidyi* exceeded those in the years prior to the appearance of *B. ovata*. Density of the *M leidyi* population appeared to be a function of food abundance and the role of *B. ovata* consisted in a reduction of prey biomass in autumn but not in a limitation of the maximum biomass**.**

In recent years the *M. leidyi* mass development has been limited to a significantly shorter period (1–2 months) as compare to preceding years (5–6 months).

So *B. ovata* role in planktonic community of the Black Sea consists in sharp suppression of *M. leidyi* abundance and as a result shortening of the period of mass presence in the plankton.

4. 5 Quantification of trophic relations in food chain of *B. ovata* – *M. leidyi* – meso – (micro) zooplankton

4.5.1 Predatory pressure of ctenophores on zooplankton community

In a series of previous studies, an inverse correlation was established between Copepoda density and the biomass of lobate ctenophore, which made the authors suggest that they control the copepod biomass. Meanwhile, gelatinous predators, which consume less than 10% of the zooplankton biomass per day, cannot reduce their abundance and biomass (Burrell and Van Engel, 1976; Kremer, 1979; Larson, 1987; Purcell, 1994). Higher consumption rates (more than 20% of zooplankton biomass per day) result in a sharp reduction of the prey abundance (Deason, 1982; Matsakis and Conover, 1991).

In Sevastopol Bay in June – August 1995 and in the open waters of the Black Sea in September 1996, six – seven years after the *M. leidyi* “outburst” the daily values of predatory impact were as high as 30–40%

of zooplankton biomass (Finenko and Romanova, 2000). From July to September in the years 2000–2003, after *B. ovata* appearance, *M. leidyi* pressure on zooplankton decreased significantly*:* in the period of its maximum development, the population consumed from 6.0±0.4 to 13.2±57% of zooplankton biomass per day (Table 4.8). In other periods of the year, consumption of mesozooplankton by ctenophores was insignificant (less than 1% biomass per day). The population rations in the periods of *M. leidyi* maximum changed from 0.4 to 9.2% of its energy content (Table 4.9). While the minimum food requirements, or sustaining daily rations calculated from population respiration rate data (Abolmasova, 2002) ranged from 3.3 to 6.4% of the population energy content. So, there are some periods when the *M. leidyi* population in Sevastopol Bay experiences a lack of food. This usually occurs when the forage mesozooplankton biomass is lower than 0.1 g m^{-3}.

Assuming that the daily consumption rate by the *M. leidyi* population should not exceed 10% of the zooplankton biomass and that the mean clearance rate is about 1.5 l ind^{-1} day^{-1} the calculated critical biomass of ctenophores that does not affect mesozooplankton abundance should not be greater than 4 g·m^{-3} or 120 g m^{-2} (if the greater part of population dwells in the upper 30-m layer) (Mutlu, 1999; Vinogradov *et al*,. 2005). As it follows from our observations, in recent years, after *B. ovata* invasion in the inshore regions of the Black Sea overcritical values of *M. leidyi* biomass have been observed only during short periods and only during these periods does the *M. leidyi* population control the zooplankton community.

Table 4.8. Predatory impact of the *M. leidyi* population on mesozooplankton biomass (% loss per day) in 1995 – 2003

Month/year	1995	2000	2001	2002	2003
June-August	21.3 ± 15.8	0.2 ± 0.05	6.0 ± 6.1	13.2 ± 5.9	7.1 ± 2.9
September-November	9.0 ± 3.3	1.2 ±0.9	0.01	0.06 ± 0.04	0.5 ± 0.4

M. leidyi larvae and juvenile can consume microzooplankton along with mesozooplankton (Stoecker, 1987; Sullivan and Gifford, 2004). In the period of maximum abundance larvae of <10 mm length make up to

90% of the total population numerical abundance in inshore waters. The potential maximum grazing of microzooplankton by *M. leidyi* larvae calculated from our experimental clearance rate data, ranged from 1.9 to 25% of prey biomass daily in Sevastopol Bay in July – September 2000–2005 while in adjusted water regions it was much less (0.3–5.1%) (Table 4.10). Meantime, even at such high grazing rate of *M. leidyi* larvae in the bay microzooplankton biomass have not only decreased but, in contrast, had had a trend to increase. Along with high growth rate of infusoria, that have been the main component of microzooplankton in the bay. One possible reason for this could be their mass development on mucus released by the ctenophores.

Table 4.9. Abundance, biomass, zooplankton grazing, respiration rate and daily ration of the *M. leidyi* population in Sevastopol Bay during July –September 2000

Date	*T*, °C	*N*, ind m^{-3}	*B*, g m^{-3}	*B*, cal m^{-3}	*W*, g	*ZB*, g m^{-3}	*PI*, %	*Q*, cal day^{-1}	R, cal day^{-1}	*Q/B*, %	*R*/*B*, %	*Q*/*R*
06.09.2000	21.9	40.7	21.1	266.5	0.52	0.063	1.6	9.51	4.29	3.3	1.6	2.0
03.09.2000	21.8	86.6	17.3	218.1	0.2	0.065	2.2	6.66	4.86	2.8	2.2	1.3
01.08.2001	27.8	93.8	79.0	995.7	0.842	0.19	27.0	26.6	41.6	2.5	4.2	0.6
30.07.2002	26.7	281.2	18.8	236.5	0.067	0.013	14.3	8.05	1.48	3.2	0.7	4.6
06.08.2002	27.1	535.7	23.3	294.0	0.044	0.008	20.3	10.01	1.30	3.2	0.4	8.0
20.08.2002	23.8	182.5	18.9	238.0	0.104	0.213	12.5	8.89	21.38	3.5	9.0	0.39
29.08.2002	24.0	92.2	10.1	127.5	0.11	0.20	6.6	4.81	10.75	3.5	5.4	0.65
15.07.2003.	23.4	51.2	26.6	335.4	0.512	0.043	10.7	10.83	3.68	2.2	1.1	2.94
29.07.2003	23.4	31.7	7.7	96.9	0.243	0.039	3.9	6.69	1.22	6.4	1.2	5.3
08.08.2003	25.1	99.3	8.7	110.1	0.088	0.208	6.1	4.17	10.13	3.5	9.2	0.38
19.08.2003	25	181.8	9.6	121.5	0.053	0.098	7.8	7.63	6.15	5.8	5.1	1.14

T = surface water temperature
N = abundance
B = biomass (wet weight and energy content)
W = mean wet weight of *M. leidyi* in the population
ZB = zooplankton biomass
PI = zooplankton grazing rate
Q = respiration rate
R = population daily ration

Table 4.10. Microzooplankton grazing by *M. leidyi* larvae in inshore waters of the Black Sea

Date	N (larvae m^{-3})	W_{avr} (mg)	CR (l day^{-1})	Grazing (% biomass)
Shelf				
06.09.00	20.8	251.2	21.75	2.17
01.08.01	16.1	253.0	16.92	1.69
30.07.02	106.0	69.2	51.44	5.14
08.08.02	87.2	61.1	40.18	4.02
19.08.03	117.8	25.2	41.23	4.12
21.07.04	11.7	40.1	4.65	0.46
06.08.04	16.5	36.0	6.34	0.63
20.08.04	4.6	151.2	3.37	0.34
31.08.04	6.6	213.2	6.14	0.61
03.08.05	10.0	94.0	5.64	0.56
Sevastopol Bay				
06.09.00	89.3	69.2	43.4	4.3
01.08.01	109.4	30.2	40.0	4.0
30.07.02	412.0	41.1	164.5	16.5
08.08.02	727.7	23.5	250.3	25.0
20.08.02	196.3	75.3	98.9	9.9
19.08.03	197.5	26.4	69.8	7.0
21.07.04	64.7	4.3	18.5	1.9
06.08.04	371.2	70.2	181.3	18.1
20.08.04	125.4	92.0	69.7	7.0
31.08.04	294.1	50.4	125.6	12.6
03.08.05	417.7	22.0	142.4	14.2

Predatory impact of *B. ovata* on *M. leidyi* population is short–term but very intensive. Minimum daily food requirements of *B. ovata* population in the peak abundance period ranged from 25 to 47% of *M. leidyi* population biomass (Svetlichny *et al.*, 2004). If to take account of growth and reproduction of ctenophores daily consumption rate of *M. leidyi* by the *B. ovata* population should be even higher. During some short periods it could be well in excess of the prey biomass both off the Crimean and Caucasian coasts (Finenko *et al*., 2000; Shushkina *et al*., 2004a). As early as in 2-4 weeks after its appearance in the plankton the *B. ovata* population experiences a lack of food as its respiration demands exceed prey biomass by some 20–100 times (Abolmasova *et al*., 2002). Using the model of Geritsen and Strickler (1977) we calculated that at an *M. leidyi* biomass of <7 g m^{-2} encounters and captures of prey by *B. ovata* are so occasional that it practically can't consume *M. leidyi* (Finenko, unpublished data). Probably it enables to *M. leidyi* population to avoid full extermination. Another reason for the *M. leidyi* population preservation is spatial disconnection of the species studied: while *B. ovata* inhabits in the upper mixed layer (0–15 m), some part of *M. leidyi* population (mainly large animals of > 30 mm length) inhabits at the depths of > 40–50 m in sub-thermocline layer (Vinogradov *et al*., 2002). Besides, *B. ovata* horizontal distribution is much more heterogeneous than that of *M. leidyi*, and some of the depths inhabited by *Mnemiopsis* may not be occupied by its predator.

4.5.2 Effect of ctenophores on mesozooplankton and ichthyoplankton abundance

B. ovata introduction to the Black Sea resulted in the structural changes into zooplankton community. Decrease of *M. leidyi* abundance as a consequence of *B. ovata* invasion has induced mesozoolankton biomass increasing especially the warm water species that have suffered greatly during the first years of *M. leidyi* introduction (Lebedeva *et al*., 2003; Shiganova *et al*., 2004; Shushkina *et al*., 2004; Hubareva *et al*., 2005).

In inshore waters of the northeastern Black Sea the mean annual biomass of forage zooplankton in 1999-200 was five times higher than in 1992–1998 (Lebedeva *et al*., 2003). Effect of *M. leidyi* abundance reduction was most evident in biomass values of *Acartia sp.*, *Paracalanus parvus* and Cladocera. The average *P. parvus* and Cladocera biomasses in 1999–2001 were higher 10 times of that in the years of *B. ovata* absence (1992–1998). In Sevastopol Bay average numerical abundance of planktonic Crustacea in spring – autumn 2000–2003 increased about 4 times compared to 1998 (Fig. 4.19), and Copepoda abundance raised ten – fold compared to 1995 The average *Mnemiopsis* biomass fell that time from 119.5±41.5 to 21.9±16.9 g m^{-2}. Abundance and biomass of warm water Copepoda *Centropages ponticus* and Cladocera *Penilia avirostris* and *Pleopis polyphemoides* that have been affected by *M. leidyi* most strongly after *B. ovata* introduction increased from 6 to 20% and from 12 to 39% of total forage zooplankton abundance, respectively. In separate years (August 2003) Cladocera biomass especially *P. avirostris* reached up to 80% of total forage zooplankton biomass. However, it is worth noting that besides effect of *Beroe* climatic forcing could be a factor governing zooplankton development. In July – August 1999 – 2002 the highest water temperature on the sea surface and into Cold Intermediate Layer was observed for the last 13 years (Shushkina *et al*., 2004), that stimulated development of warm water species with their short life cycle.

It was noted increasing of biomass of *Sagitta setosa*, food competitor of *Mnemiopsis* that have disappeared almost completely in the years of *M. leidyi* bloom. *S. setosa* maximum biomass in September – October 1999-2001 in the northeastern Black Sea near – shore waters was 8–9 mg m^{-3}, in waters off Crimea coast its biomass raised up to 14 mg m^{-3} in September 2003. In autumn of 2000–2002 substantial share of mesozooplankton biomass (up to 16%) in the northeastern region and in Sevastopol Bay was formed by appendicularian *Oikopleura dioica* (Hubareva *et al*., 2004).

Along with the general trend to increase forage zooplankton biomass after *B. ovata* introduction there are some inter annual differences that are caused by timing of *B. ovata* appearance in plankton and its food pressure on *M. leidyi*. As example, mean forage zooplankton biomass value in 2004 was lower than in previous years as in Sevastopol Bay as in the northeastern part of the sea that was a result of long presence of *M. leidyi* in plankton and later appearance of *B. ovata* (Vinogradov *et al*., 2005).

B. ovata introduction did affect on neither *Oithona similis* no *Pseudocalanus elongatus* abundances that are low so far (Fig. 20). Effect of *M. leidyi* on mesozooplankton structure is the result of difference in ecology of the species. Thus, *O. similis* and *P. elongatus* being the cold water species with reproduction peak at. 7-15°C are disconnected with *M. leidyi* in space and time (Gubanova *et al*., 2002); that is a reason of no apparent effect on their abundance in the years after *B. ovata* introduction. In contrast, abundance of warm- water and eurythermal Copepod species – *C. ponticus*, *A. clausi* that have a reproductive peak in July – September, in the period of *M. leidyi* reproduction and growth, revealed clear relation with *M. ovata* abundance.

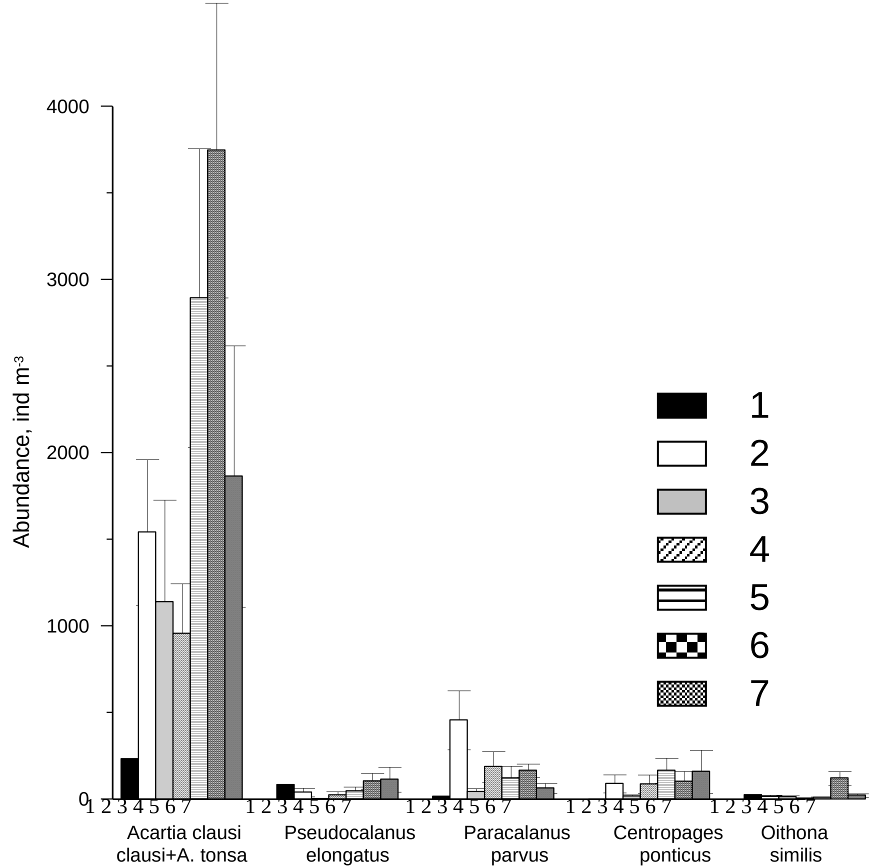

Figure 4.19. Inter annual dynamics of Copepod species abundance (ind m^{-3}) in Sevastopol Bay during 1995- 2003 (bar is a standard error): 1-th column is 1995 –1996 (Gubanova *et al.*, 2002; 2, 3, 4 – 2000, 2001, 2002 - (Hubareva *et al.*, 2004), 5,6,7 – 2003, 2004, 2005 (our unpublished data).

In inshore waters off Crimea coast as well as in the northeastern Black Sea *Mnemiopsis* biomass decrease resulted also in some changes of meroplankton abundance: in recent years (2003–2005) in inshore waters off Crimea coast its share in total biomass of forage zooplankton decreased down to 14–28% of average annual values as compared with about 40% in 1999–2002 (Fig. 4.20). However while in the northeastern inshore waters some changes in species composition in meroplankton occurred – Bivalvia larvae became dominant constituent instead of Cirripedia nauplii in previous years in waters off Crimea coast the latter remains dominant.

In summer 2000–2001 a trend to increase species number, egg and larvae fish abundance in different areas of sea was shown (Shiganova *et al*., 2004; Gordina *et al*., 2005). In inshore waters near Sevastopol eggs and larvae of the Mediterranean Sea migrates Bonito and Bluefish have been found, that testifies to favourite conditions for their spawning and feeding**.** While in the years of before *B. ovata* introduction the percentage of larvae of summer – spawning fish without any food in their stomachs was as high as 80–100% (Tkach, 1993; Tkach *et al*., 1998) in 2000–2001 54.5% of anchovy larvae and 73% of Blennidae and Gobiidae larvae had food in the stomach (Gordina *et al*., 2005).

In late August 1999, the first year of *B. ovata* appearance off the Caucasian coast ichthyoplankton consisted of eggs and larvae of 24 species of summer – spawning fish. Maximum eggs (323 ind m^{-2}) were observed for anchovy *Eugraulis encrasicolis ponticus,* abundances of *Trachurus mediterraneus ponticus*, *Mugil saliens* and *Diplodus annularis* eggs were about 1.2 ind m^{-2}. Such high abundance of anchovy and *T. mediterraneus* was no found since 1988 after *M. leidyi* introduction (Shiganova *et al*., 2003). High abundances of early stages of these species were noted in 2001 also, the year with hot summer and earlier *B. ovata* appearance in plankton.

However, predatory impact of *M. leidyi* on micro– and mesozooplankton can be considerable in short period of its peak in summer. When this period synchronizes with fish larvae development in plankton, its negative effect can be resulted in insufficient food supply and low survival of fish larvae, as it was observed in 2004, when *M. leidyi* was abundant for a long time due to later appearance of *B. ovata* (Vdodovich *et al*., 2007).

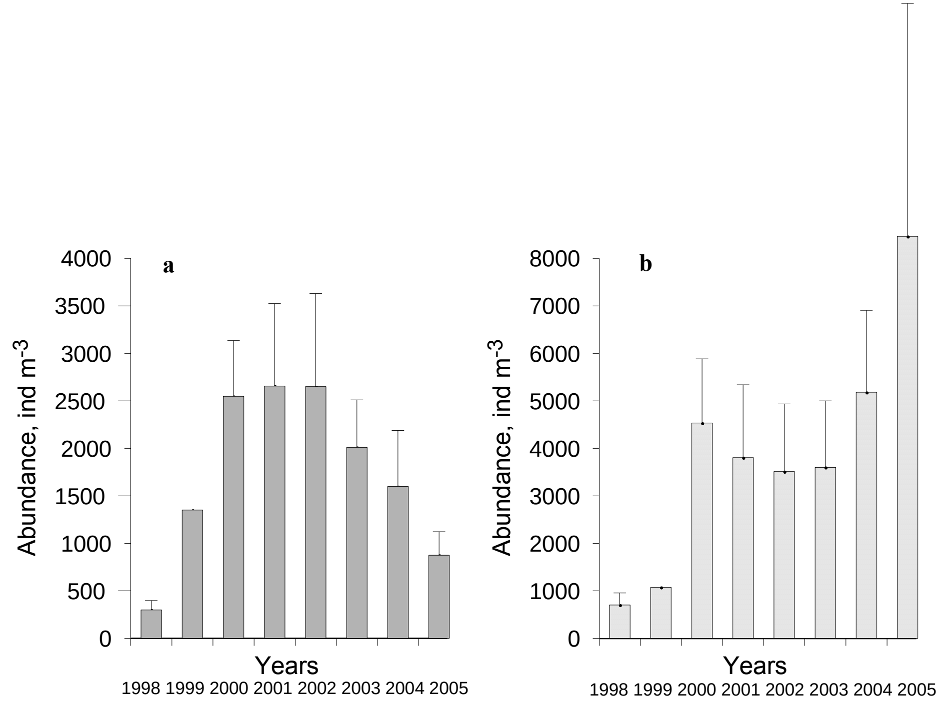

Figure 4.20. Average abundance (ind m^{-3}) of meroplankton (A) and planktonic Crustacea (B) during spring – autumn period (May – September) of 1998 – (Gubanova *et al*., 2002,) 1999 – (Gordina *et al*., 2003), 2000 –2002 (Hubareva *et al*., 2004)

Thus, after *B. ovata* introduction some features of recovery of the inshore Black Sea plankton community were evident: duration and predatory impact of *M. leidyi* on mesozooplankton have been decreased, biodiversity and abundance of which have raised and food supply of planktivorous fish larvae improved. Meantime, mesozooplankton concentration remains lower than during the 1970s–80s. *B. ovata* impact does not result in absolute suppression of *M. leidyi* and its pressure on forage zooplankton and fish larvae but essentially shortens this pressure.

Probably now as the Black Sea planktonic community as the ecosystem of inshore regions transferred to new dynamic equilibrium, that depends much on interrelations between the ctenophore-invaders.

References

Abolmasova G.I., 2001. Energy metabolism in *Mnemiopsis leidyi* A. Agassiz in relation to temperature and food conditions. *Hydrobiol. Journal*., 37 (2), 90─95 (in Russian).

Abolmasova G.I., Romanova Z.A., Finenko G.A., 2002. Energy requirements of *Mnemiopsis leidyi* and *Beroe ovata* populations in relation to nutrition conditions in Sevastopol Bay. *In* Second Intern. Conf. Oceanography of the Eastern Mediterranean and Black Sea. Similarities and differences of two interconnected basins (14–18 Oct. 2002, Ankara, Turkey). Ankara, 146–147.

Anninsky B.E., Gubanova A., 1998. Chemical composition and state of ctenophore *Mnemiopsis mccradyi* (Mayer) in the Black Sea. *Hydrobiol. Journ*., 34 (3), 36–43 (in Russian).

Anninsky B.E., Romanova Z.A., Abolmasova G.I., Gücü A.C., Kideys A. E. 1998. Ecological and physiological state of the ctenophore *Mnemiopsis leidyi* (Agassiz) in the Black Sea in autumn 1996. *In* Ivanov L & Oguz T. (eds.). Ecosystem Modelling as a Management Tool for the Black Sea. Symposium on Scientific Results. Klüwer Academic Publishers, Dordrecht/Boston/London. 1, 249─262.

Anninsky B.E., Finenko G.A., Abolmasova G.I., Hubareva E.S., Svetlichny L.S., Bat L., Kideys A., 2005. Effect of starvation on the biochemical composition and respiration rates of ctenophores *Mnemiopsis leidyi* and *Beroe ovata* in the Black Sea. *J. Mar. Biol. Ass. U.K*. 85,. 549─561.

Arashkevich E.G., Anokhina L.L., Vostokov S.V. *et al*., 2001. Reproductive strategy of *Beroe ovata* (Ctenophora, Atentaculata)

Beroida) – a new invader to the Black Sea. *Okeanologiya* 41 (1), 116‒120 (in Russian).

Baker L.D., Reeve M.R., 1974. Laboratory culture of the lobate Ctenophore *Mnemiopsis mccradyi* with Notes on feeding and fecundity. *Mar. Biol.* 26 (1), 57‒62.

Bilio M., Niermann U., 2004 Is the comb jelly really to blame for it all? *Mnemiopsis leidyi* and the ecological concerns about the Caspian Sea. *Mar. Ecol. Prog. Ser.* 269, 173‒183.

Bishop J.W., 1968. A comparative study of feeding rates of tentaculate ctenophores. *Ecology.* 49, 996‒997.

Bogdanova D.P., Konsulov A.S., 1993. On the distribution of the new ctenophore species *Mnemiopsis mccradyi* in the Black Sea along the Bulgarian coastline in the summer of 1990. *Dokl Bulgarian Akad Nauk.* 46, 71‒74.

Burrell V.G.,Van Engel W.A., 1976. Predation by and distribution of a Ctenophore, *Mnemiopsis leidyi* A. Agassiz. in the York River Estuary. *Estuar Coast Mar. Sci.* 4., 235‒242.

Caddy, J. F., Griffiths, R. C., 1990. A perspective on recent fishery-related events in the Black Sea. In: Recent trends in the fisheries and environment in the general fisheries for the Mediterranean (GFCM) area. *GFCM Stud. Rev.*, 63, 43-71.

Deason E.E., 1982. *Mnemiopsis leidyi* (Ctenophora) in Narragansett Bay, 1975-1979: abundance, size composition and estimation of grazing. *Estuar Coast Shelf Sci.* 1, 121‒134.

Falkenhaug T., 1996. Distributional and seasonal patterns of ctenophores in Malamgen, northern Norway. *Mar. Ecol. Prog. Ser.* 140, 59‒70.

Finenko G.A., Abolmasova G.I., Romanova Z.A., 1995. Intensity of the nutrition, respiration, and growth of *Mnemiopsis mccradyi* in relation to food conditions. *Marine Biology* (Vladivostok) 21 (5), 315‒320 (in Russian).

Finenko G.A., Anninsky B.E, Romanova Z.A., Abolmasova G. I., Kideys A. E., 2001 Chemical composition, respiration and feeding rates of the new alien ctenophore, *Beroe ovata*, in the Black Sea. *Hydrobiologia* 451, 177–186.

Finenko G.A., Romanova Z.A., 2000. Population dynamics and energetic of the ctenophore *Mnemiopsis leidyi. Okeanologiya* 40 (5), 720–728 (in Russian).

Finenko G.A., Romanova Z.A., Abolmasova G. I., 2000. A new invader to the Black Sea – ctenophore *Beroe ovata. Ekologiya Moray* 50, 21–25 (in Russian).

Finenko G.A., Romanova Z.A., Abolmasova G.I., Anninsky B.E., Svetlichny L.S., Hubareva E.S., Bat L., Kideys A. E., 2003. Population dynamics, ingestion, growth and reproduction rates of the invader *Beroe ovata* and its impact on plankton community in Sevastopol Bay, the Black Sea. *J. Plankton Res.* 25 (5), 539–549.

Finenko G.A., Romanova Z.A., Abolmasova G.I., Anninsky B.E., Hubareva E.S., Bat L., Kideys A., 2005. Effect of food conditions on ingestion rate and digestion time in lobate ctenophore *Mnemiopsis leidyi. Morskoi ecologic. journal.* 4 (1), 75–83 (in Russian).

Finenko G.A., Kideys A.E., Anninsky B.E., Shiganova T. A., Roohi A., Tabari M, Rostami H. , Bagheri S., 2006a. Invasive ctenophore *Mnemiopsis leidyi* in the Caspian Sea: feeding, respiration, reproduction and impact on zooplankton community. *Mar. Ecol. Prog. Ser*. 314, 171–185.

Finenko G.A., Romanova Z.A., Abolmasova G.I., Anninsky B.E., Bat L., Kideys A., 2006b. Ctenophores – invaders and their role in the trophic dynamics of the planktonic community in the coastal regions off the Crimean Coasts of the Black Sea (Sevastopol Bay). *Okeanologiya*. 46 (4), 472–482 (in Russian).

Geritsen J., Strickler J., 1977. Encounter probabilities and community structure in zooplankton: a mathematical model. *J. Fish. Res. Board. Can*. 34, 73–82.

Gibbson M.I., Painting S.J. 1992. The effects and implications of container volume on clearance rates of the ambush entangling predator Pleurobrachia pileus (Ctenophora: Tentaculata). J. *Exp. Mar. Bio*l. 163 (2), 199─208.

Gordina A.D., Zagorodnyaya JU. A., Kideys A.E. *et al.* , 2005. Summer ichthyoplankton, food supply of fish larvae and impact of invasive ctenophores on the nutrition of fish larvae in the Black Sea during 2000 and 2001. *J. Mar. Biol. Ass. UK*. 85, 537─548.

Gubanova A.D., Polikarpov I.G., Saburova M.A., Prusova I.Yu., 2002. Long -term dynamics of mesozooplankton community in Sevastopol Bay (1976–1996) as exemplified by Copepoda. *Okeanologiya.* 42 (4), 537–545 (in Russian).

Gücü A.C., 1997. Role of fishing in the Black Sea ecosystem. *In*: Orzoy E. and Mikaelyan A (eds.). Sensitivity in Changes: Black Sea, Baltic Sea and North Sea. Klüwer Academic Publishers. The Netherlands, pp. 149─162.

Gücü A.C. 2002. Can overfishing be responsible for the successful establishment of *Mnemiopsis leidyi* in the Black Sea? *Estuar., Coast. and Shelf Science* 54, 439–451.

Gücü A.C., Oguz T., 1998. Modelling trophic interrelationships in the Black Sea. *In*: Ivanov L. and Oguz T. (eds.). Ecosystem Modelling as a Management Tool for the Black Sea. 2, 359─371.

Hubareva E.S., Svetlichny L.S., Romanova Z.A., Abolmasova G.I., Anninsky B.E., Finenko G.A., Bat L., Kideys A.., 2004. Zooplankton community state in Sevastopol Bay after invasion of the ctenophore *Beroe ovata* into the Black Sea. *Mar. Ecological Journal* 3 (1), 39─46 (in Russian).

Kamburska L., 2004. Effects of *Beroe cf ovata* on gelatinous and other zooplankton along the Bulgarian Black Sea Coast. In: H. Dumont, T.A. Shiganova, U. Nierman (eds.) Aquatic Invasions in the Black, Caspian, and Mediterranean Seas. NATO Science Series. IV. Earth and Environmental Sciences. 35. Klüwer Academic Publishers, 137─154.

Kideys A., 1994. Recent dramatic changes in the Black Sea ecosystem. The reason for the sharp decline in Turkish anchovy fisheries. *J. Mar. Systems* 5, 171─181.

Kideys A.E., 2002. Fall and rise of the Black Sea ecosystem. *Science* 297, 1482─1484.

Kideys A.E., Romanova Z.A., 2001. Distribution of gelatinous macrozooplankton in the southern Black Sea during 1996–1999. *Mar. Biol.* 139, 535─547.

Kideys A.E., Finenko G.A., Anninsky B.E, Shiganova T. A., Roohi A., Tabari M, Youseffyan M, Rostamian M., Rostami H, Negarestan H. 2004. Physiological characteristics of the *Beroe ovata* in Caspian Sea water. *Mar. Ecol. Prog. Ser.* 266, 111–121.

Kideys, A. E., Roohi, A., Bagneri, S., Finenko, G., Kamburska, L., 2005. Impacts of Invasive ctenophores on the fisheries of the Black Sea and Caspian Sea. *Oceanography*. 18 (2), 76-85.

Konsulov A., Kamburska L., 1998. Ecological determination of the new ctenophore *Beroe ovata* invasion in the Black Sea. *Oceanology*, IO BAS. 2, 195─198.

Kovalev A.V., Gubanova A.D., Kideys A.E., Melnikov V.V., Nierman U., Ostrovskaya N.A., Prusova I.Yu., Skryabin V.A., Uysal Z., Zagorodnyaya J., 1998. Long-term changes in the biomass and composition of fodder zooplankton in coastal regions of the Black Sea during the period 1957–1996. *In*: Ivanov L.I., Oguz T. (eds). Ecosystem Modelling as a Management Tool for the Black Sea. Klüwer Academic Publishers. Dordrecht/Boston/London. 1, 209–221.

Kremer P., 1979 Predation by the Ctenophore *Mnemiopsis leidyi* in Narragansett Bay, Rhode Island. *Estuaries* 2, 97─105.

Larson R J. 1979. *In situ* feeding rates of the Ctenophore *Mnemiopsis mccradyi*. *Estuaries* 10 (2), 87─91.

Lebedeva L.P., Shushkina E.A., Vinogradov M.E. *et al*., 2003. Long – term transformation of mesoplankton community structure in the Northeastern Black Sea under impact of the alien ctenophores. *Okeanologiya* 43 (5), 710‒715 (In Russian).

Matsakis S., Conover R.J., 1991. Abundance and feeding of medusae and their potential impact as predators on other zooplankton in Bedford Basin (Nova Scotia, Canada) during spring. *Can J Fish Aquat Sci*. 48, 1419‒1430.

Mutlu E., 1999. Distribution and abundance of ctenophores and their zooplankton food in the Black Sea. II. *Mnemiopsis leidyi*. *Mar Biol*. 135, 603‒613.

Niermann U., Bingel F., Gorban A. *et al*., 1994 Distribution of anchovy eggs and larvae (*Engraulis enchrasicolus* Cuv.) in the Black Sea in 19991 and 1992 in comparison to former surveys. *ICES J. Mar. Sci*. 51, 395‒406.

Niermann U., Kideys A., Kovalev A.V. *et al*., 1999. Fluctuation of pelagic species of the open Black Sea during 1980-1995 and possible teleconnection. *In*: Besiktepe S.T. *et al*.(eds). NATO Science Series. Environmental degradation of the Black Sea: challenges and remedies. Resulting from ARW, Constanta–Mamaya (Romania). 1997, Klüwer Acad. Publ., 143–147.

Oguz T., 2005. Black Sea ecosystem response to climatic variations. *Oceanography*. *Black Sea special Issue*. 18, 122‒133.

Pereladov M.V., 1988. Some observations on biocenosis dynamics in Sudak Gulf of the Black Sea. III All- Union Conference in Marine Biology., 1. (Abstracts, Sevastopol, 18–20 October 1988). Kiev. Publishers IBSS. 1, 237‒238 (in Russian).

Purcell J.E., White J.R., Roman M.R., 1994. Predation by gelatinous zooplankton and resource limitation as potential controls of *Acartia tonsa* copepod populations in Chesapeake Bay. *Limnol. Oceanogr*. 39, 263‒278.

Purcell J.E., 2005. Climatic effects on formation of jellyfish and ctenophore blooms: a review. *J. Mar. Biol. Ass. U.K.* 85, 461‒476.

Reeve M.R., 1980. Comparative experimental studies on the feeding of Chaetognaths and Ctenophores .*J. Plankton Res*. 2 (4), 381–393.

Reeve M.R., Walter M.A., Ikeda T., 1978. Laboratory studies of ingestion and food utilization in lobate and tentaculate Ctenophores. *Limnol. Oceanogr*. 23 (4), 40–51.

Romanova Z.A., Abolmasova G.I., Finenko G.A., 2005. Reproductive strategy of *Mnemiopsis leidyi* in inshore waters of the Black Sea. *Proceedings of Volodimir Gnatyuk Ternopol National teacher's training University. Biology issue*. 4 (27), 197–198 (in Russian).

Shiganova T., 2005. Changes in appendicularian *Oikopleura dioica* abundance caused by invasion of alien ctenophores in the Black Sea. *J. Mar. Biol. Ass. U.K*. 85, 477–494.

Shiganova T.A., Bulgakova Yu.V., Sorokin P.Yu., Lukashev Yu.F., 2000. Results of study of *Beroe ovata*, a new invader to the Black Sea. *Proceedings of RAS*. 2, 581–590 (in Russian).

Shiganova T.A., Bulgakova Yu.V., Volovik S.P. *et al*., 2001. The new invader *Beroe ovata* Mayer 1912 and its effect on the ecosystem in the north – eastern Black Sea. *Hydrobiologia* 451, 187–197.

Shiganova T.A., Musaeva E.I., Bulgakova Yu.V., Mirzoyan Z.A., Martynyuk M.L., 2003. New settlers comb jellies *Mnemiopsis leidyi* (A. Agassiz) and *Beroe ovata* Mayer 1912 and their influence on the pelagic system of northeastern Black Sea. *Proceedings of RAS. Issue Biology*. 2, 225–235 (in Russian).

Shiganova T.A., Dumont H.J., Mikaelyan *A. et al*., 2004. Interactions between the invading Ctenophores *Mnemiopsis leidyi* (A. Agassiz) and *Beroe ovata* Mayer 1912, and their influence on the pelagic ecosystem of the northeastern Black Sea. *In*: H. Dumont, T.A. Shiganova, U. Nierman (eds). Aquatic Invasions in the Black, Caspian, and Mediterranean Seas. NATO Science Series. IV. Earth and Environmental Sciences. 35. Klüwer Academic Publishers, 33–70.

Shushkina E.A., Nikolaeva G.G., Lukasheva T.A., 1990. Changes in the Black Sea planktonic community structure under mass occurrence of *Mnemiopsis leidyi. Journal of General Biology* 51 (1), 54─60 (in Russian).

Shushkina E.A., Vinogradov M.E., 1991. Long–term dynamics of planktonic biomass in the open Black Sea. *Okeanologiya* 31 (6), 973–980 (in Russian).

Shushkina E.A., Musaeva E.I., Anokhina L.L., Lukasheva T.A., 2000. Role of the gelatinous macroplankton: jellyfish *Aurelia*, ctenophores *Mnemiopsis* and *Beroe* in the planktonic communities in the Black Sea. *Okeanologiya*. 40 (6), 859─866 (in Russian).

Shushkina E.A., Vinogradov M.E., Lebedeva L.P., Lukasheva T.A., 2004. Zooplankton distribution on the shelf of the northeast Black Sea in the warm climatic period of 2000 –2002. *Okeanologiya*. 44 (4), 524─537 (in Russian).

Shushkina E.A., Vinogradov M.E., Lebedeva L.P., Lukasheva T. A., 2004a. The structure of the coastal plankton communities at the beginning of the cooling cycle (2003) in the northeastern part of the Black Sea. *Okeanologiya* 44 (6), 892 – 900 (in Russian).

Sieferd T.D., Conover R.J., 1992. An opening – closing plankton net for horizontal sampling under polar sea – ice. *Sarsia*. 76, 273─277.

Stoecker D.K., Verity P.G., Michaels A.E., Davis L.H., 1987. Feeding by larval and post–larval ctenophores on microzooplankton. *J. Plankton Res*. 9, 667─683.

Sullivan L.J., Gifford D.J., 2004. Diet of the larval ctenophore *Mnemiopsis leidyi* A. Agassiz (Ctenophora, Lobata). *J. Plankton. Res.* 26 (4), 417─431.

Svetlichny L.S., Abolmasova G.I., Hubareva E.S., Finenko G.A., Bat L., Kideys A., 2004. Respiration rates of *Beroe ovata* in the Black Sea. *Mar. Biol*. 145, 585─593.

Tkach A.V., 1996. Feeding of the Black Sea fish larvae. *In*: Konovalov S. M. (ed.). Recent state of the Black Sea ichthyofauna.– IBSS NASU, 153─168 (in Russian).

Tkach A.V., Gordina A.D., Niermann U., Kideys A.E., Zaika V.E., 1998. Changes in the larvae nutrition of the Black Sea fishes with respect to plankton. *In*: Ivanov, L. and Oguz, T. (eds.). Ecosystem Modelling as a Management Tool for the Black Sea. Klüwer Academic Publishers, Dordrecht/Boston/London. 1, 171─188.

Tsikhon–Lukanina E.A., Reznichenko O.G., Lukasheva T.A., 1995. Feeding of ctenophore *Mnemiopsis*. *Fish Industry*. No 4, 46─49 (in Russian).

Tsikhon–Lukanina E.A., Reznichenko O.G, Lukasheva T.A., 1993. Predation rates on fish larvae by the ctenophore *Mnemiopsis* in the Black Sea inshore waters. *Okeanologiya*. 33 (6), 895–900 (in Russian).

Vdodovich I.V., Gordina A.D., Pavlovskaya T.V., Finenko G.A., Klimova T.N, Abolmasova G.I., Romanova Z.A., Polikarpov I. G., 2007. Specific features of the feeding of larval fish of the families Blenniidae and Gobiidae in relation to changes in the coastal plankton community of the Black Sea. *Voprosy ichthyologii*, 47 (4), 542–554.

Vereshchaka A.L., 2002. Microscale distribution pattern of gelatinous macroplankton in the Black Sea off the Gelendgik Coasts in August 2002. *Okeanologiya*. 42 (1), 91─98 (in Russian).

Vinogradov, M. E., Sapozhnikov, V. V., Shushkina, E. A., 1992. Ecosystem of the Black Sea Nauka, Moscow, 112 pp. (in Russian).

Vinogradov M.E., Shushkina E.A., Musaeva E.I., Sorokin P.Yu., 1989. A new invader to the Black Sea – ctenophore *Mnemiopsis leidyi* (A. Agassiz) (Ctenophora: Lobata) *Okeanologiya* 29 (2), 293─299 (in Russian).

Vinogradov M.E., Sapoznikov V.V., Shushkina E.A., 1992. The Black Sea ecosystems. M. Nauka. 112 pp. (in Russian).

Vinogradov M.E., Shushkina E.A., 2002. Dynamics of age structure of ctenophores Mnemiopsis leidyi and Beroe ovata populations off the Caucasus Coast of the Black Sea. *In*: Zatsepin A.G., Flint M.V. (eds.) Comprehensive studies of the Northeastern Black Sea. M. Nauka, 272─288 (in Russian).

Vinogradov M.E., Shushkina E.A., Anokhina L.L., Vostokov S.V., Kucheruk N.V., Lukashova T.A., 2000. Dense aggregations of the ctenophore *Beroe ovata* (Eschscholtz) near the north-eastern shore of the Black Sea. *Okeanologiya* 40 (1), 52─55 (in Russian).

Vinogradov M.E., Lebedeva L.P., Vinogradov G.M *et al*., 2005. Monitoring of the pelagic communities of the northeastern Black Sea in 2004: macro and mesoplankton. *Okeanologiya* 45 (3), (in Russian).

Vinogradov M.E., Vinogradov G.M., Lebedeva L.P., Lukasheva T.A., Zasko D.N., 2006. Patterns of ctenophore populations in the north-eastern part of the Black Sea in 2005. *Okeanologiya* 46 (3), 406–414 (in Russian).

Vostokov S.V., Arashkevich E.G., Drits A.V., Lukashev Yu.F., 2001. Ecological and physiological characteristics of the ctenophore *Beroe ovata* in the coastal waters of the Black Sea: quantity, biomass, size distribution, hunting behavior, feeding and metabolism. *Okeanologiya* 41 (1), 109─115 (in Russian).

Waggett R., Castello T.H., 1999. Capture mechanisms used by the lobate ctenophore, *Mnemiopsis leidyi*, preying on the copepod *Acartia tonsa*. *J Plankton Res*. 21, 2037–2052.

Zaika V.E., 1994. The drop in anchovy stock in the Black Sea: Result of biological pollution? Report of the Second Technical Consultation on Stock Assessment in the Black Sea. Ankara, Turkey, 15-19 February 1993. Rome, Italy, FAO. No 495, 78─83.

Zaika V.E., 2005. Where and how the Black Sea population of the ctenophore *Mnemiopsis leidyi* is wintering? *Marine Ecological Journal*. 4 (1), P. 51–54 (in Russian).

Zaika V.E., Revkov N.K., 1994. Anatomy of gonads and reproduction pattern of ctenophore *Mnemiopsis* sp. in the Black Sea. *Zool. Journ*. 73, (3), 5–11 (in Russian).

Zaika V.E., Revkov N.K., 1998. Food of the Black Sea ctenophore *Mnemiopsis* in relation to zooplankton community composition. *Hydrobiol. Journal*. 34 (3), 29–35 (in Russian).

Zaitsev Yu.P., Alexandrov B.G., 1997. Recent man-made changes in the Black Sea ecosystem. In: Örsoy E. and Mikaelyan A. (eds.) Sensitivity to Change: Black Sea, Baltic Sea and North Sea. Klüwer Academic Publishers, Dordrecht/Boston/London, 25–31.

5. FOOD SUPPLY OF THE BLACK SEA SMALL PELAGIC FISH

5.1. Introduction to the problem

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The condition of marine fish stocks is usually characterized using data on abundance, biomass, catches, length – weight and age composition, sex ratio, distribution etc. (Ivanov and Beverton, 1985; Caddy and Griffiths, 1990; Laevastu, 1993; Caddy and Oliver, 1994; Prodanov *et al.*, 1997; Chashchin, 1998; Sinovcic, 2000; Daskalov, 2002; Alheit, 2007; Grishin *et al.*, 2007). However it is sorrow that relatively few reference is made to the nutritional condition and food supply (or provision), despite the fact that food is one of the major channel connecting organisms with their environment. Nutritional condition and food supply are characteristics connected closely each other. First one is indicator of consumed food effect on organism condition; second one is indicator of quantity and quality of consumed food. In investigations these indicators are often distinguished difficulty just as characteristics of organism and its environment are differed difficulty too. Famous Russian scientists use “food supply” only in their fundamental publications; so it is sufficiently for description of this characteristic (Karzinkin, 1952; Ivlev, 1955; Vinberg, 1956; Nikolsky, 1963). This one is often considered too extensively. But it is very important to descript it with exact quantitative criteria.

Food supply of organisms or populations (Fs) could be described as the ratio between food consumed (Fc) and required (Fr):

$$F_s = F_c \,/\, F_r \quad (5.1)$$

The direct determination of these characteristics in field conditions is very difficult. The indirect determination of organism and population food supply is also confronted with serious problems since supply is dependent on many factors including some that cannot be defined:

$$F_s = f\,(A_b\ , A_{bv}\ , A_{ba}\ , A_{c1}\ , ..., A_{cn}\ , T, L\ , ..., X_1\ , ..., X_n\ )\ , \quad (5.2)$$

where A_b is the biomass of fodder organisms, A_{bv} is their nutritional value, A_{ba} is their availability, A_{c1}, ..., A_{cn} is the biomass of consumers of own species as well as competitors of other species, T is temperature, L is illumination (which is especially important for aquatic organisms) and X_1, ... , X_n are undefined factors.

Nevertheless, there is a rather effective way of estimating food supply, which is the determination of one of the most important result of the feeding process which reflects nutritional condition, such as weight increment (ΔW) and accumulation of energy reserves (ΔE):

$$\Delta W, \Delta E = f\,(F_s). \qquad (5.3)$$

Weight increment and growth is clearly defined by protein retention; energy accumulation (for many marine fishes) by forming of fat stores (fats are neutral lipids - triacylglycerols mainly). Determination the weight (protein) increment for adult fishes is not an easy task. Though it used now frequently RNA / DNA ratio or RNA content in tissues (better transport RNA) for estimation “immediate” rate of protein biosynthesis and consequently growth rate of larvae and fry fish (Bulow *et al.*, 1981; Thorpe *et al.*, 1982; Nakano *et al.*, 1985; Varnavsky *et al.*, 1991; Clemmensen *et al.*, 1997; Bergeron, 2000; Drazen, 2002; Gwak *et al.*, 2003; Smith *et al.*, 2003).

On the contrary, estimation fat content is an easy and useful method for evaluating the condition of fish. This process of energy accumulation begins every year almost from “zero” following the spawning period and reaches a peak at the end of the intense feeding period. The content of reserve lipids at the time of feeding completion is, therefore, an integral indicator of conditions under which fish stocks or populations consumed food.

$$FAT = \int_{t_1}^{t_2} f(F_s)dt \qquad (5.4)$$

Determination of fat (neutral lipids) content is widely used for evaluating fish condition (see reviews by Shulman, 1960; Lovern, 1964; Love, 1970, 1980; Ackman, 1980; Shatunovsky, 1980; Kreps, 1981; Sidorov, 1983; Minyuk *et al.*, 1997; Morris and Culkin, 2000, etc.). Unfortunately, however, most of these investigations did not look at the problem from a food supply point of view. The few papers that have been published on the characterization of the feeding history of fish in which the food supply was estimated using lipid parameters devoted larvae of Atlantic sardine *Sardina pilchardus* (Frazer *et al.*, 1988), Californian anchovy *Engraulis mordax* (Hakanson, 1989 a, b, 1993), the North Sea sprat *Sprattus sprattus* (Hakanson and Coombs, 1994), Atlantic cod *Gadus morhua* (Lochman *et al.*, 1995). Last time interesting data on relationship between lipid content and nutritional factors devoted adult benthic fishes of Mediterranean were published by Levi *et al.* (2005) and Lloret *et al.* (2005) as well as North Atlantic cod (Jonsson, 1996; Lambert *et al.*, 2000; Yaragina and Marshall, 2000; Orlova *et al.*, 2000) and capelin *Mallotus villosus* (Orlova *et al.*, 2002). Good food supply of fishes through high lipid content affects on maturation. Improving of lipid content in eggs and larvae and promotes high survival and replenishment of populations (Lasker and Theilacker, 1962; Nikolsky, 1965; Guisande *et al.*, 1998; Marshall *et al.*, 1999; Sogard and Olla, 2000; Zenitani, 2001).

Here it is appropriately to make one considerable depression. Favourite indicator for ichthyologists is “condition factor” that is used very widely for estimation of fish condition. This one bases on relation between fish weight and length. Although its determination is very easy and simple it is far from significant characteristics of physiological condition of fish (Shulman, 1974; Gershanovich *et al.*, 1984; Sutton *et al.*, 2002; Lambert and Dutil, 2000; Dutil, 2003). We showed that dynamics of this index does not expressive enough and often distorts the features of processes of energy accumulation or expenditure processes (Shulman, 1974; Shulman and Shchepkina, 1983; Shulman and Love, 1999). Is the reason in less specific gravity of lipids than water? But just last one consists main mass of living organism. Thus lipid accumulation in fish body due to this reason is often accompanied by decrease of water content and body mass and quite the reverse. Besides this it is difficult to compare "conditional factor" for fishes of different size, species, and body forms. Liver index of fishes that accumulate fat in this organ (Gadidae, Gobiidae, Scorpaena etc.) is only exclusion as increasing of liver mass in these fishes is carried out by fat accumulation.

Assessment of the food supply of several marine fish populations by determination of accumulated fat stores (fat content) have been carried out by some authors since the second part of last century. These investigations devote to Pacific sardine *Sardina pilchardus* in California current (Lasker, 1970), Californian anchovy (Garcia–Frankco *et al*., 1999), Atlantic cod (Marshall *et al*., 1999, Orlova *et al*., 1999), Azov and Black Sea subspecies of European anchovy *Engraulis encrasicholis maeoticus* and *E.e. ponticus* (Shulman, 1963; Shulman and Dobrovolov, 1979), Black Sea sprat *Sprattus sprattus phalericus* (Shulman *et al*., 1994, 2005; Minyuk *et al*., 1997; Nikolsky and Shulman, 2005; Nikolsky *et al*., 2007).

Anchovy and sprat are the two main small pelagic fish species of the Black Sea and have significant effect on the ecosystem. Nutritional spectrum of both species (copepods) is rather close. Sprat is a cold – tolerant, planktivorous fish, spawning in winter and forming local populations. It feeds intensively in spring and in start of summer (Svetovidov, 1964) and reaches considerable fat stores about 10 – 15% of body mass. On the contrary anchovy is a warm – tolerant fish, spawning in summer, carries out long migrations and feeds intensively preparing for wintering migration in last of summer and first two months of autumn. Anchovy as well as sprat accumulates fat about 10 – 15% of body mass but to the end of the autumn (Fig. 1). So determining fat content in sprat populations at the end of feeding in the summer period and in anchovy populations in November – December we can estimate "degree of well-being" / nutritional condition of small pelagic fishes in the Black Sea at the most part of the year. Moreover, sprat and anchovy condition could be an indicator of mesozooplankton biomass (the main food of pelagic fishes) in the Black Sea in whole. The monitoring of nutritional condition of both species undertaken in the Black Sea is based on this situation. It has been carried out on sprat almost every year since 1960; but not on anchovy until more recently. However, the relationship between fat content level at the end of autumn feeding and features of the wintering migration of the Azov (Shulman, 1974, 2002) and Black Sea subspecies (Chashchin and Axelev, 1990) of European anchovy have been studied. Surely, this relationship also is caused by food supply. We are aware of only a few investigations in which the long-term fat content of fish populations has been monitored. There are investigations of Lasker (1970) on Californian sardine, Garcia-Franko *et al*. (1999) on anchovy from the same region, Marshall *et al*. (1999) and Orlova *et al*. (2000) on Atlantic cod.

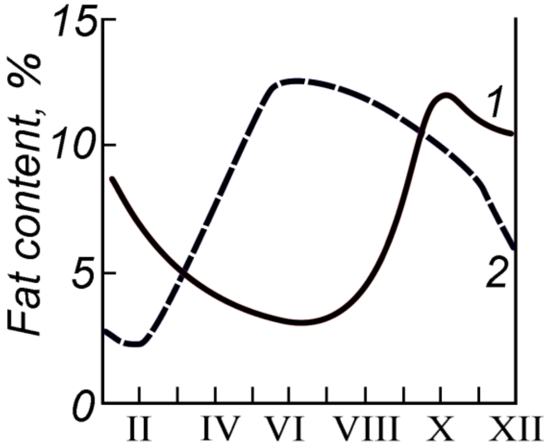

Figure 5.1. Annual dynamics of fat content in anchovy (1) and sprat (2) (Shulman, 1974)

Necessity of food supply monitoring of Black Sea mass pelagic fishes is significant not only for estimation of their condition but, may be in more degree, for estimation of pelagic ecosystem condition in whole. We hope to show in next chapters that just this monitoring may clarify complicated processes in the Black Sea ecosystem which cannot be revealed without definition the final result of food consumption by sprat and anchovy. It is known that the Black Sea ecosystem has been affected by many factors since at least the 1970s (Vinogradov *et al.*, 1992; Zaitsev, 1993; Sorokin, 2002; Kideys, 2002; Eremeev and Efimov, 2003). These include eutrophication, pollution, invasion of ctenophore aliens, over-fishing and climate change. The data on dynamics of fat content in sprat and anchovy populations must make significant contribution to study the Black Sea ecosystem condition.

Of course, study of fish food supply by lipid characteristics must not be limited indices of fat content (neutral lipids or triacylglycerols) only. Significant information for this problem is obtained in comparison of fractional and especially fatty acid content of fish lipids and lipids of their food (Lovern, 1964; Sargent, 1976; Sargent and Henderson, 1989). The final chapter of this monograph is devoted to this.

So the aim of our investigation is to trace the long – term changes in food supply of the Black Sea sprat and anchovy stocks based on determination of their fat content dynamics. To this end, the objectives of investigation are to review how the characteristics studied are connected with (1) dynamics of sprat and anchovy stock biomass, (2) dynamics of biomass of the lower trophic levels (i.e. zoo – and phytoplankton) and (3) climate (temperature) changes in the Black Sea.

5.2 Interannual changes of lipid content in the Black Sea sprat

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The sprat *Sprattus sprattus phalericus* (Risso) is one of the most abundant and commercially important fish species in the Black Sea. It also plays a significant role in pelagic ecosystem as a link between the plankton community and top predators (dolphins, spiny dogfish, mackerel, whiting, turbot etc). Its early life stages are components in diets of horse mackerel and predatory zooplankton (Golenchenko, 1940; Chayanova, 1958; Prodanov *et al*., 1997). As a boreal species, the Black Sea sprat prefers the coldest waters and inhabits mainly the CIL layer below the thermocline (Aslanova, 1954; Yur'ev, 1979; Sorokin, 2002). It has a life span up to 5 years, reaches sexual maturity at one year old and reproduces mainly during cold months between November and March (Pavlovskaya, 1952; Stoyanov, 1953; Aslanova, 1954; Gorbunova, 1958, Dekhik, 1973; Avşar and Bingel, 1994.). The sprat feeds on zooplankton (mainly Copepoda) the hole year round, but more intensively after reproduction (Lipskaya, 1960). Increasing growth of the fish occurs just in spring as well as intensive accumulation of lipids in its body. The level of lipid reserves that fish accumulate to the end of their feeding period is an integrated indicator of their food supply (Shulman, 1974).

The Black Sea sprat starts to accumulate lipids since February–March when a new annual production cycle begins in the sea. Then the lipid accumulation becomes slower and usually achieves its maximal values in June–July (see Fig. 5.1). Later, during its spawning period in winter, sprat utilizes almost completely the lipid reserves accumulated during summer. Further, this cycle repeats again. Therefore, the lipid content in sprat body in June–July characterizes its food supply conditions in a current year (within all the feeding period). The degree of lipid content in abundant fish species (as sprat is in the Black Sea) can serve also as an indicator of ecosystem condition (Shulman and Love, 1999). Therefore, estimating the quantity of the lipid accumulated by sprat during its feeding period, it is possible to characterize not only the present state of this species but also a condition of the Black Sea pelagic ecosystem as a whole. The indicator of lipid content in sprat can be used also to the forecasting its stock conditions and school formations (Minyuk *et al.*, 1997). The results of monitoring of lipid content in Black Sea sprat that were carried out more than 45 years, allowed us to interpret its variability in connection to environmental changes (Shulman *et al.*, 1994, 2005).

The aim of the study was to compile the long-term data set of the food supply indicator (*FSI*) and analyze its fluctuations in connection with long-term variability of some characteristics of the Black Sea pelagic ecosystem.

It is widely recognized that there are two main goals of the analysis of time-series data. They are: (a) identifying the nature of the phenomenon represented by the data obtained, and (b) forecasting, i.e. predicting future values of the time-series variable. First of all, these goals require input data to be reliable. An application of any quantitative indicators to a forecast needs reliable input data reflecting adequately the quantitative aspect of the investigated phenomenon. This assumes that the data uncertainty should be estimated and probable errors should be considered when subsequent calculations and conclusions are performed.

The objectives of our investigation were to answer three questions:

i. Whether observed interannual variations of the *FSI* reflect adequately the natural variability of sprat food supply (i.e. do our very "noisy" data contain useful information concerning interannual variability of sprat food conditions)?
ii. To what degree are the observed interannual variations in the *FSI* connected to long-term environmental variability?
iii. Can we predict the interannual variation of the sprat fatness (i.e. food supply) using available data?

For this purpose, the data of long-term monitoring of lipid content in Black Sea sprat have been subjected to the statistical analysis.

5.2.1 Material and methods

The techniques of sampling and determination of lipid content in fish have been explicitly described earlier in several publications (Shulman *et al.*, 1994; Minyuk *et al.*, 1997). Fish were caught with trawling either by research or commercial vessels. A mean randomized sample of fish (not less than 100 individuals) was selected from each catch. Fish were sized on 5 mm intervals, and then every size group was separately homogenized. About 10–15 g of homogenate was dried in oven at 100–105°C to a steady weight. Lipid content (fatness) in dry matter was determined gravimetrically with diethyl ether extraction in a Soxhlet apparatus (Ackman, 1980) between 1960 and 1984. On the basis of the results obtained by ether extraction, the linear regression between fat (*FC*) and dry matter (*DM*) content in sprat body was calculated (Minyuk *et al.*, 1997):

$$FC = 0.84\,DM - 13.28, \qquad (5.5)$$

where *FC* and *DM* are expressed in % wet mass ($n = 300$; $r = 0.9$). Since 1985, fish fatness has been estimated by a calculation method based on the data for dry matter content. Such approach for estimation lipid content of many fish species has a wide application in monitoring investigation (Iles and Wood, 1965; Hunter and Leong, 1981; Minyuk *et al.*, 1997; Shulman *et al.*, 2005). Recently, we make direct determination of total lipids in wet fish mass by chloroform-methanol extraction (Folch *et al.*, 1957) but the calculation method allows us to compare recent results with a considerable massive data on sprat fatness obtained in the past, when only diethyl the ether extraction method was widely used.

To characterize the lipid content in fish for each sample, average weighted values were calculated by equation:

$$X = \frac{1}{N}\sum_{1}^{k} n_i x_i\ , \qquad (5.6)$$

where n_{i} is number of fish in size group *i*, y_i is lipid content in size group *i*, *k* is number of size groups, and *N* is number of all fish analyzed. Then the food supply indicator (*FSI*) was calculated as simple arithmetic mean of some *X* for the period June–July.

The lipid contents of Black Sea sprat were determined annually between 1960 and 2007 except for 1966, 1973, 1975 and 1993. To make long-term time series of the *FSI*, the data sampled from the late June to the end of July (when the lipid content reaches its maximum) have been selected. In some cases (1985, 1990, 1994-96, and 2001) the data sampled during early August have been also involved because of lack of data for June–July or, if a lipid content in August were no less than mean values for June–July (the *t*-test was applied).

Samples have been collected from three main fishing grounds of this species (Fig. 5.2): off the western Crimea coast (1), along the shelf off Romania and Bulgaria (2), and off the Caucasus coast (3). Those rare data from locations close to the main areas also were used if they no found out significant distinctions (the *t*-test) from average values for the nearest main area. Since 1994, fish were sampled only in area 1 where more than 56 % of all data were collected. In total, the data from 226 catches from three main areas (128, 56, and 42 catches from areas 1, 2, and 3, respectively) had been selected (Table 5.1).

To analyse spatial and temporal variability of the sprat fatness, the two-sample *t*-test and the single factor analysis of variance (ANOVA) were applied (Afifi and Azen, 1979; Zar, 1984). The data compiled were tested for normality using the Kolmogorov-Smirnov test and then the multiple regression method was applied to make a linear model predicting the *FSI*. Some missing values in the *FSI* time-series (for 1966, 1973 and 1993) were replaced by series' mean while regressions were calculated.

We also compared the compiled data series to other available variables, including the North Atlantic Oscillation Index (NAO) index (Jones *et al.*, 1997; Osborn, 2006), sea-level air temperature from coastal stations (NASA GISS, downloaded at http://data.giss.nasa.gov/gistemp/, surface water temperatures (SST) southward of Crimea from archives of Marine Hydro-physical Institute and Institute and Marine Branch of Ukrainian Hydro-meteorological Institute (Nikolsky and Shulman, 2005). The AVHRR Oceans Pathfinder SST data were obtained from the Physical Oceanography Distributed Active Archive Center (PO.DAAC) at the NASA Jet Propulsion Laboratory, Pasadena, CA. (http://podaac.jpl.nasa.gov). Data on phytoplankton and zooplankton were collected by the Southern Institute of Marine Fisheries and

Oceanography (YugNIRO) and Institute of Biology of the Southern Seas (IBSS). They were compiled from (Bryantseva *et al*., 1996; Mikaelyan, 1997) and zooplankton (Prodanov *et al*., 1997; Kovalev *et al*., 1998). Annual sprat biomass as well as catch data were reported by Prodanov *et al*. (1997) and Chashchin (1998). The data set on annual catch of the Black Sea sprat for the 1970–2005 are also available from the FISHSTAT PLUS database (FAO, 2007).

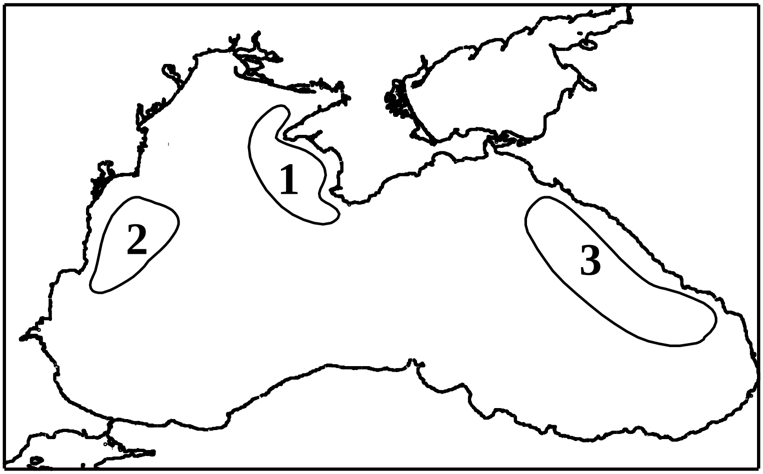

Figure 5.2. Sprat sampling locations in the Black Sea.1: northwestern part of the sea (off the eastern Crimea); 2: western part of the sea (off Romania and Bulgaria); 3: eastern part of the sea (off Caucasus) (Shulman *et al*., 2005).

Table 5.1. Number of analysed samples

Year	Region (sample location)	No. of Trawls	No. of samples	**Year**	Region (sample location)	No. of trawls	No. of samples
1960	1,2,3	12	34	1985	1,2	14	53
1961	1,2,3	11	24	1986	2,3	12	54
1962	1,2,3	11	34	1987	1,2,3	15	57
1963	3	3	7	1988	1,2	3	15
1964	3	1	2	1989	1,2	4	22

Year	Region (sample location)	No. of Trawls	No. of samples	**Year**	Region (sample location)	No. of trawls	No. of samples
1965	3	1	3	1990	1,3	5	28
1967	1,3	2	10	1991	1,3	3	13
1968	1,3	2	10	1992	1,3	4	14
1969	1,3	2	10	1994	1	3	10
1970	1,3	2	10	1995	1	3	13
1971	1,3	2	10	1996	1	4	8
1972	1,3	2	10	1997	1	5	15
1974	2	1	5	1998	1	5	20
1976	2	1	3	1999	1	5	15
1977	1,2	8	29	2000	1	5	15
1978	2	1	7	2001	1	5	20
1979	2	2	7	2002	1	2	7
1980	2	2	4	2003	1	3	11
1981	1,2	10	44	2004	1	6	12
1982	1,2	6	27	2005	1	9	44
1983	1,2,3	5	18	2006	1	12	67
1984	1,2	6	22	2007	1	6	44
				Total		226	887

5.2.2 Results

Spatial variability of the sprat food supply indicator

The sprat does not make long-distance migrations in the Black Sea; it moves from the open sea toward the coasts in spring and in opposite direction in autumn. Therefore, various fish shoals feed in some local feeding grounds with different forage capabilities. Thus, sprat taken from the local area around island Zmeiniy is usually fatter than sprat caught from the other nearest areas. Some years, the lipid content in sprat off the Caucasus coast is higher than off the western coast of Crimea. Nevertheless, any stable and statistically significant differences in sprat fatness have not been revealed between different regions

(Minyuk *et al.*, 1997) with the exception of Gulf of Varna where sprat fatness is always much higher (up to 20–22 %) than in areas close to the Bulgarian shelf. That was the reason that we had to examine the spatial variability of the *FSI* before studying its temporal (interannual) variability. Spatial variability of the *FSI* was estimated for those years there were data from compared areas. Observations with sufficient number for statistical comparisons had been carried out in all three main areas only within four years (1960, 1961, 1962 and 1987), additionally, within seven years there were data collected at the same time from two areas (Table 5.2).

Statistical analyses (Table 5.3) had not shown any significant spatial distinctions of the *FSI* from various areas within the same years (calculated value $F > F_{0.05}$ in all cases without exception). This fact allowed us to use all data obtained from different areas to estimate average value of the indicator in each year and to investigate its interannual variability. Therefore, the data from main areas were combined into a single pool and further analysed as a common temporal data set.

Table 5.2. Sprat FSI values, % wet mass (mean ± SE) from different regions

Year	Region			Average
	1	2	3	
1960	12.32 ± 0.35	11.50 ± 0.64	12.53 ± 0.44	12.31 ± 0.37
1961	10.77 ± 1.31	11.81 ± 0.71	11.97 ± 1.16	11.47 ± 0.39
1962	12.97 ± 0.95	12.74 ± 1.16	12.17 ± 0.77	12.71 ± 0.39
1967	10.38 −		10.20 −	10.29 ± 0.91
1968	8.36 −		8.72 −	8.54 ± 0.91
1969	10.00 −		9.62 −	9.81 ± 0.91
1970	9.40 −		9.34 −	9.37 ± 0.91
1971	8.52 −		9.00 −	8.76 ± 0.91
1972	9.24 −		8.84 −	9.04 ± 0.91
1977	12.31 ± 0.70	14.57 ± 0.39		13.16 ± 0.45
1981	15.67 ± 0.25	15.71 ± 0.36		15.69 ± 0.41
1982	13.22 ± 0.49	12.56 ± 0.32		13.00 ± 0.52

Year	Region			Average
	1	2	3	
1983	9.11 ± 0.31	11.85 ± 0.37	10.37 –	10.46 ± 0.57
1984	12.26 ± 0.34	10.77 ± 0.19		11.76 ± 0.52
1985	11.96 ± 0.14	12.84 ± 0.28		12.72 ± 0.34
1986		10.77 ± 0.56	11.69 ± 0.24	11.54 ± 0.37
1987	9.91 ± 0.21	9.85 ± 0.26	9.92 ± 0.85	9.88 ± 0.33
1988	13.75 –	12.28 ± 0.84		12.77 ± 0.74
1989	10.24 –	12.84 ± 0.76		12.19 ± 0.64
1990	9.88 ± 0.30		13.62 –	10.63 ± 0.57
1991	13.39 ± –		12.10 ± 0.58	12.53 ± 0.74
1992	14.57 ± 0.62		15.13 –	14.71 ± 0.64
Overall average ± *SE*				11.74 ± 0.28

Interannual variability of the sprat food supply indicator

Being applied to the all long-term data, the ANOVA has resulted that the *FSI* interannual variability exceeds significantly ($F < F_{0.001}$) its spatial variability making 56 % of a total variance (Table 5.4). This implies that interannual deviations of the *FSI* cannot be casual; i.e. despite of considerable variation within each year, the analysed long-term data set contains useful information on interannual variability of the *FSI*.

Based on the average square of variation within groups (years), standard deviation (*SD*) for a single observation can be found as $SD = \sqrt{1.83} \approx 1.3$ (%) and the standard error (*SE*) of the mean from n observations would be calculated as $\mathrm{SE} = \sqrt{1.83/n}$. For instance, standard error for mean of 5 observations amounts to 0.6 (%), inversely, to obtain a mean value of the *FSI* with accuracy ± 0.5 (%), we should make at least 7–8 observations within peak of sprat feeding period ($n = 1.83 / 0.5^2 = 7.32$).

Table 5.3. Summary of spatial variability of the *FSI* (ANOVA was used)

Years	Areas	No of samples	Variances		*F*	*F*–critical	*P*–value
			between areas	within areas			
1960	1,2,3	12	0.83	1.38	0.61	4.26	0.567
1961	1,2,3	11	1.59	5.94	0.27	4.46	0.772
1962	1,2,3	11	0.65	5.41	0.12	4.56	0.889
1977	1,2	8	9.65	2.30	4.20	5.99	0.086
1981	1,2	10	0.003	0.72	0.005	5.32	0.948
1982	1,2	6	0.59	1.06	0.55	7.71	0.498
1983	1,2	4	7.47	0.47	15.83	18.51	0.058
1984	1,2	6	2.98	0.50	5.96	7.71	0.072
1985	1,2	14	1.35	0.98	1.38	4.75	0.263
1986	2,3	12	1.40	0.68	2.06	4.97	0.182
1987	1,2,3	15	0.006	0.91	0.007	3.89	0.993

Table 5.4. Summary of interannual variability of the *FSI* (ANOVA was used)

Source of variation	*SS*	*df*	*MS*	*F*	*F* critical	*P* value
Between groups	331.13	24	13.80	7.53	1.59	< 0.001
Within groups	260.28	142	1.83			
Total	591.41	166				

The compiled data series, its long-term mean value (11.43%), and standard deviations of observed data from its mean long-term value (± 1.81%) are shown in Fig. 5.3. As the various amounts of observations have been made in various years, standard errors of the data differ so considerably. Nevertheless, the lipid data reveal well-pronounced changes in the food supply condition of sprat from 1960 to 2007. According to the *FSI* dynamics, they were favourable for sprat in early 1960s but became significantly worse in 1965-1972. From 1975 to 1981 the food supply remarkably improved, and abnormal high fatness was recorded in 1980, 1981, and 1992. The definite decreasing of the *FSI* during last decade suggests deterioration of sprat food supply condition: in 2006, the lowest *FSI* was recorded for the period of our investigations.

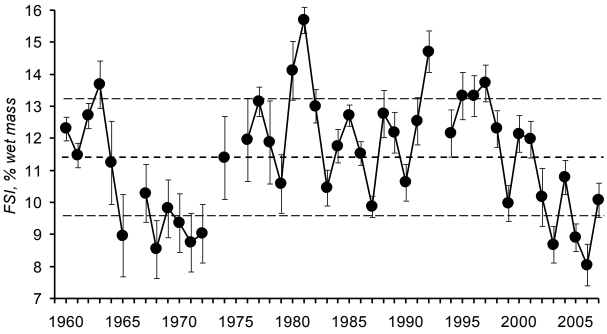

Figure 5.3. Long-term variability of the sprat food supply indicator (mean ± *SE*) from 1960 to 2007. Broken lines show the overall long-term mean value ± *SD*.

Long-term variability of sprat fat content in connection to long-term environmental changes in the Black Sea

We compared the *FSI* long-term pattern to the first (*PC*1) and second (*PC*2) principal components of variability hydro-climatic, biological, and anthropogenic variables of the Black Sea ecosystem for the period 1960–1993 reported by Daskalov (2003). There were no found correlations between *FSI* and *PC*1 although more complex relations between them would be probable. On the contrary, the *FSI* variation found out similarity to dynamics of the *PC*2, and the correlation coefficient calculated based on all *FSI* data was approximately 0.55 (Fig. 5.4). It is important to note that the correlation had increased up to 0.87 ($P < 0.05$) when the data with number of observations less than 5 were removed from the data series. This phenomenon emphasizes an importance to assess errors of measurements and it may be an example that it is better to have a few data but more reliable.

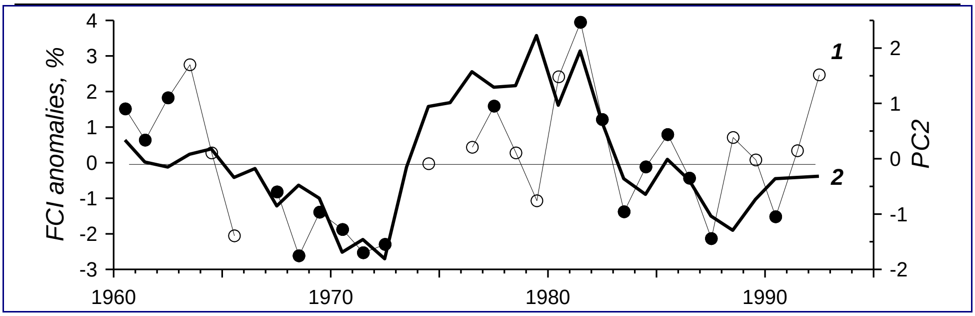

Figure. 5.4. Detrended *FSI* (1) compared to the second principal component *PC2* (2) describing variability of environmental variables (according to Daskaslov, 2003) Statistically significant *FSI* values are specified as black circles (Nikolsky and Shulman, 2005).

It also allows us to draw a conclusion that long-term variability of the *FSI* depends actually on ecological variables because according to Daskalov, the PC2 positively correlated with variability of atmospheric sea level pressure, total run-off, inorganic phosphorus loading from Danube, phytoplankton biomass and recruitment abundance of whiting, anchovy and horse mackerel; and negatively correlated with biomass of zooplankton (in eastern part of the sea) and *Pleurobrachia pileus*, phytoplankton concentration during blooms etc. Although relations between the sprat fat content and environmental variables are complicated and not very clear yet, nevertheless, these results provide an argument to consider the *FSI* as a suitable indicator to investigate ecosystem behaviour and also to predict a response of sprat population to environmental changes.

We have also compared the obtained data series to other available variables, including winter NAO index (Fig. 5.5), mean annual sea-level air temperature (Fig. 5.6), annual surface water temperatures southward of Crimea (Fig. 5.7), phytoplankton (Fig. 5.8) and zooplankton biomass (Fig. 5.9), biomass and catch of sprat (Fig. 5.10) etc and in some cases, significant correlations (r > 0.5) have been calculated (Table 5.5). Apparently, such correlations seem to be not very poor because biological time-series data are rarely examined for their reliability and generally, they contain substantial “random noise”.

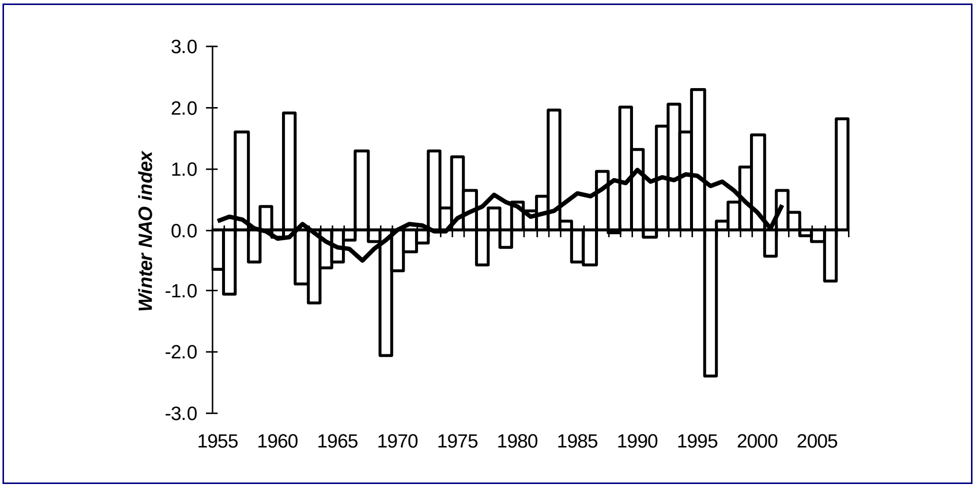

Figure 5.5. Winter (November to March) North Atlantic Oscillation Index (bars) from 1955 to 2007; broken line shows the same data smoothed by 11-year filter. Data downloaded from

http://www.cru.uea.ac.uk/cru/data/nao.htm

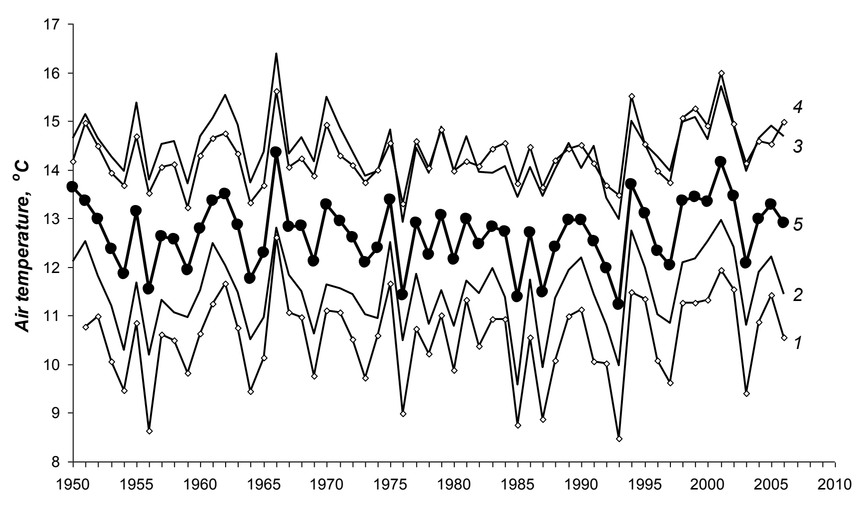

Figure 5.6. Annual sea-level air temperature from coast station: Simferopol (1), Sulina (2), Istanbul (3), Samsun (4), and its mean values (5). Data downloaded from NASA GISS http://data.giss.nasa.gov/gistemp/

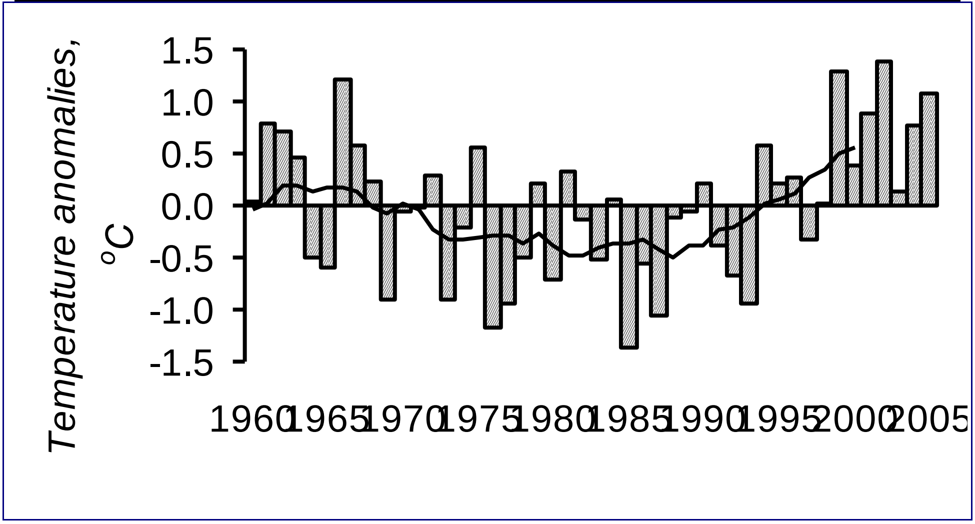

Figure 5.7. Annual sea surface water temperature anomalies off South Crimea (bars); broken line shows the same data smoothed by 11-year filter. Data obtained by the Marine Hydrophysical Institute and Marine Branch of Ukrainian Hydrometeorological Institute (Nikolsky and Shulman, 2005)

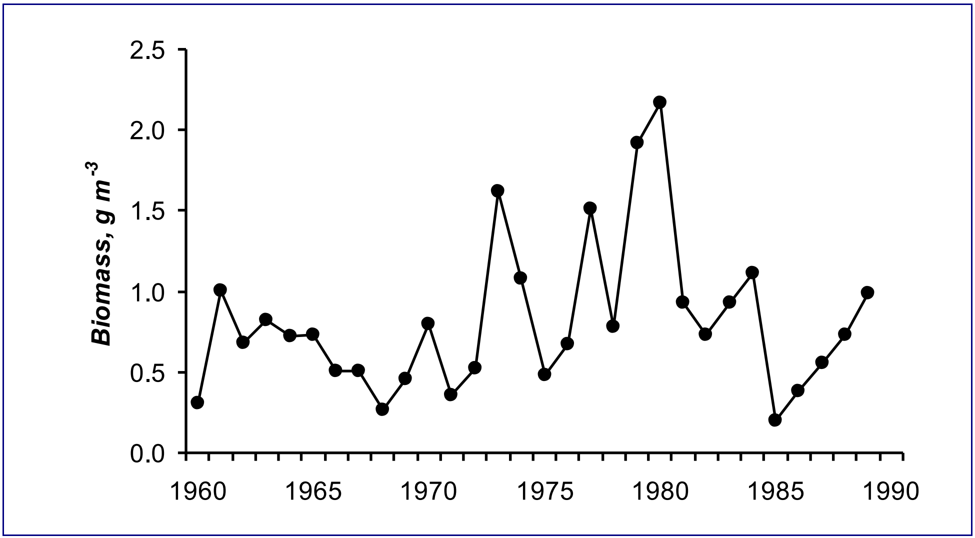

Figure 5.8. Annual phytoplankton biomass in the Northwest part of the Black Sea during 1960–1989 (Bryantseva *et al.*, 1996)

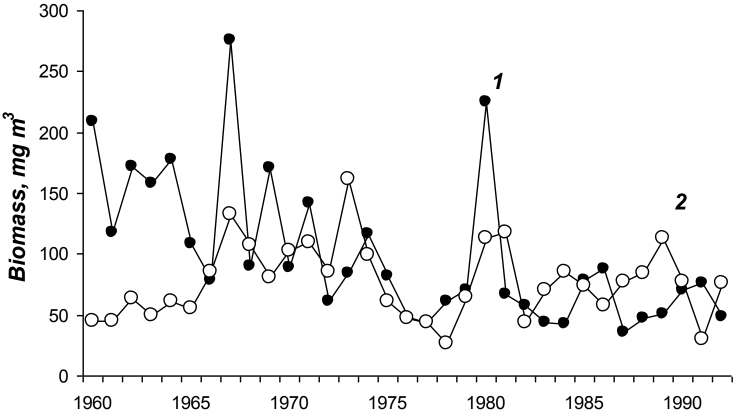

Figure 5.9. Long-term changes in the fodder zooplankton biomass in the Northwest (1) and Northeast (2) parts of the Black Sea during 1960–1992 (data of YugNiro, after Prodanov *et al.*, 1997; Kovalev *et al.*, 1998)

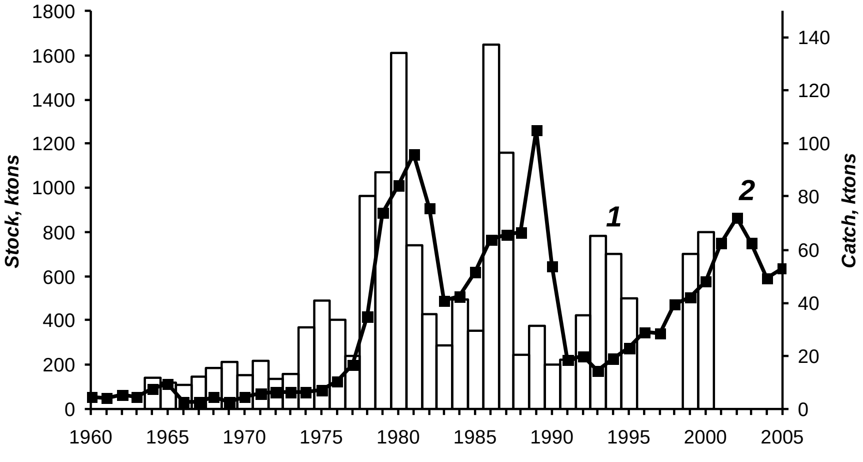

Figure 5.10. Long-term variations of sprat stock biomass (1), and catch (2) during 1960–2005 (Prodanov *et al.*, 1997)

Table 5.5. Summary of correlation tests between sprat fat content (*FSI*) and some other variables. Only significant correlation coefficients ($P < 0.05$) are presented

Variable and time lag	Correlation coefficient	Period	Source of data
Winter NAO index (t-2)	0.45	1958–2007	Osborn, 2006
Annual air temperature (t-4)	0.52	1956-2006	NASA GISS
Mean annual SST(t-4)	– 0.63	1960–1999	MHI/UHI archive
Mean annual SST(t-4)	– 0.69	1985–2004	NOAA/ NASA archive
Mean winter SST(t-2)	– 0.31	1960–1999	MHI/UHI archive
Phytoplankton NW (t-1)	0.61	1957–1989	Bryantseva *et al.*, 1996
Phytoplankton NW (t-2)	0.51	1957–1989	Bryantseva *et al.*, 1996
Phytoplankton E (t-4)	0.57	1960–1990	Mikaelyan, 1997
Sprat biomass (t-1)	0.39	1960–1991	Chashchin, 1998
Sprat biomass (t-2)	0.39	1960–1991	Chashchin, 1998
Sprat biomass (t-3)	0.38	1960–1991	Chashchin, 1998
Sprat fat content (t-1)	0.51	1960–2001	Shulman *et al.*, 2005
Sprat fat content (t-3)	0.41	1960–2001	Shulman *et al.*, 2005

There is a feature of the data series that we have to examine here. The time-series data studied have found out significant autocorrelation with temporal lags 1 and 3 years (Fig. 5.11). It still demands the explanation yet; nevertheless, regardless of the deep of our understanding and validity of our interpretations of the phenomenon, we can use the identified feature to predict future events. The Table 5.5 shows those correlations between the *FSI* and other variables tested which we could use for forecasting the *FSI* behaviour if those data were available for the previous years.

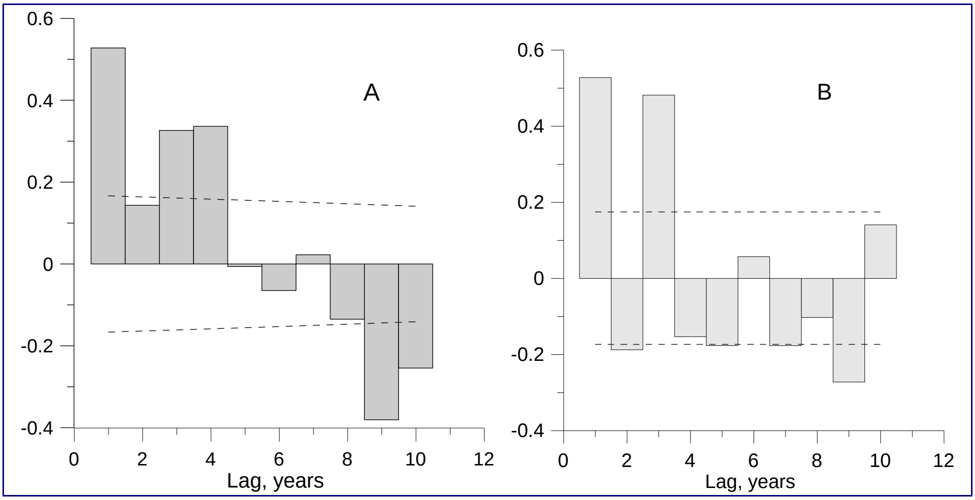

Figure 5.11. Summary of autocorrelation test: autocorrelation function (A) and partial autocorrelation function (B) of the *FSI*. Broken lines indicate levels of significance.

$$FSI(t) = a_0 + a_1\, FSI(t\text{-}1) + a_2\, FSI(t\text{-}3) + a_3\, SST\,(t\text{-}4), \quad (5.7)$$

First steps to a forecast of *FSI*

As an example of possibility to predict the sprat food supply condition, we calculated very simple linear regression model that included only 2 predictors: *FSI* for the preceding year and with time lag –3 years and the sea surface temperature (*SST*) with time lag –4 years (Nikolsky and Shulman, 2005):

where a_0 … a_3 are coefficients recalculated yearly while new data are available.

In spite of it was a very ordinary regression, it explained about 50% of total variability of studied indicator (coefficient of determination r^2 =

0.48). Certainly, such simple model could not predict those extraordinary magnitudes of sprat fat content occurred in 1965, 1968, 1981, 1983, 1987, 1992 and 1999 but in some cases it predicted correctly the tendency of future changes (Fig. 5.12).

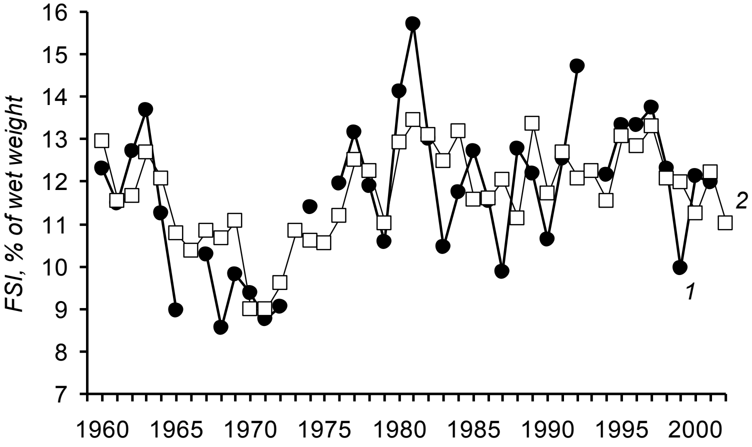

Figure 5.12. Observed (1) and predicted (2) values of sprat food supply indicator (*FSI*) for the period 1960-2001 and the forecast for 2002. Missing data has been replaced to its long-term value for the prediction model (Nikolsky and Shulman, 2005)

There was a hypothesis about behaviour of the *FSI* in near future. After enormous regime shifts occurred in the Black Sea ecosystem since 70th, it was believed the *FSI* to be returned to its stable (equilibrium) state about its long-term mean value. But the model predicted decreasing *FSI* for the 2002, and in fact, it was even lower (Fig. 5.13). For the next year (2003), the predicted value was much lower, and really, the *FSI* decreased to the lowest level that was not found from the late 60th. For the next year (2004), the model predicted increasing *FSI*, and it had really increased. As it was mentioned above, the lowest values of *FSI* since 1960s was recorded in 2006. That very low value was rather well predicted by the model. What *FSI* value do we expect in 2008? According to the model, it should remain under its long-term mean value (see Fig. 5.13).

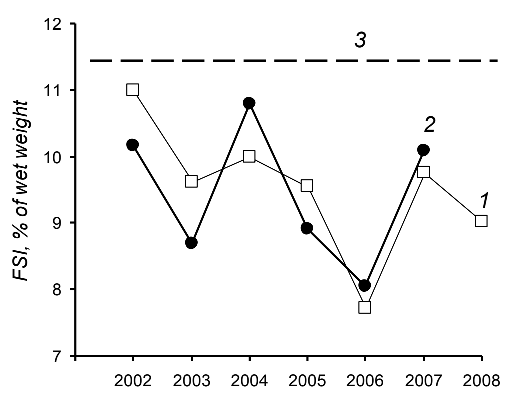

Figure 5.13. Predicted (1), observed (2) *FSI* values, and long-term mean (3) for the period 2002–2008

Somewhat better result can be approached if the regression is calculated using the data on the sea surface temperatures obtained from Earth observing satellites (the AVHRR Oceans Pathfinder data, available throw Internet). In that case, the model explains 74% of total variance of the *FSI* but the *SST* time series is not so long: it has been started since 1985 (Fig. 5.14).

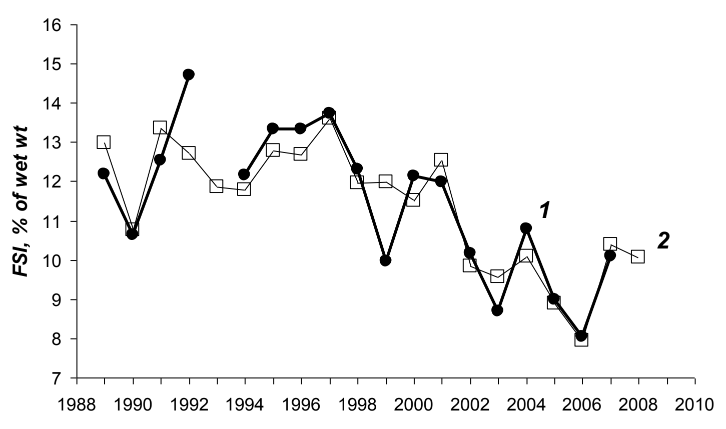

Figure 5.14. Observed (1) and predicted (2) values of the sprat food supply indicator (*FSI*) for the period 1988 to 2007 (simple linear model, using SST data from space observations)

As a conclusion for the section, we can positively answer those three questions asked previously: 1) the compiled time-series data reflect natural interannual dynamics of sprat fatness; 2) the interannual variability of sprat fatness is related to the variability of environmental variables; 3) in some cases, behaviour of sprat fatness (and hence, its food supply condition) can be predicted based on available ecological variables.

5.3 Lipid composition of the Black Sea sprat

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Black Sea sprat feeding and lipid accumulation have both strongly pronounced seasonal dynamics (Svetovidov, 1964; Shulman, 1974), which closely relate to seasonal phytoplankton and zooplankton biomass changes. Phytoplankton seasonality in the Black sea has been monitored and maximum chlorophyll is usually observed in spring (Yunev *et al.*, 2002) followed by an active period of zooplankton reproduction (Sazhina, 1987). Maximum zooplankton biomass in the Black sea was generally recorded in late spring and early summer (Sorokin, 2002). Summer and early autumn months (July to October), with density stratification of water column, were characterized in the Black Sea by low biomass of phytoplankton (Yunev *et al.*, 2002) and low to intermediate concentration of mesozooplankton (Sorokin, 2002). Last two decades the condition of mesozooplankton community in the Black Sea during summer additionally strongly depressed by jellies (Kideys *et al.*, 2005; Finenko *et al.*, 2006). In autumn when jellies predator pressure drop, increase of mesozooplankton biomass followed mixing of water and phytoplankton bloom.

Coldwater Black Sea sprat inhabits around the year at the temperature 6-13°C. During cold months sprat distribute in water column and during period of seawater density stratification since May till October situated in layers under the thermocline where feeding (Svetovidov, 1964). Sprat feed the most intensively since April-May till July during mesozooplankton development. The main food items of sprat during feeding period are the elder stages of coldwater copepods such as *Calanus euxinus, Pseudocalanus elongatus, Oethona nana* etc. (Svetovidov, 1964). In summer, sprat move to shallower waters and warm-water zooplankton species (copepods, meroplankton etc.) may be additional food for them (Svetovidov, 1964; Sirotenko and Sorokalit, 1979). In spring sprat begins to accumulate lipids in the body and complete it in early summer (Shulman, 1974). In 60-80th seasonal dynamic of sprat lipid content had pronounce maximum in June-July (Fig. 5.1, after Shulman, 1974; Minyuk *et al*., 1997). The maximal level of lipid accumulation in June-July was recommended as indicator for estimation annual sprat food supply (Shulman, 1974). Lipid reserved by sprat declined in autumn and winter during maturation and spawning, which culminate in January-February (Svetovidov, 1964). These months Black Sea sprat as well as sprat from other temperate basins reduces to feed (Last, 1987).

Last decade events of cardinal changes in composition of the Black Sea sprat food species were recorded: during feeding period of 2000-2003, the slimmer small warm-water copepods such as *Acartia sp., Paracalanus parvus, etc.,* and earlier stages of *C. euxinus* and *Ps. elongatus* dominated in stomachs (Glushchenko *et al*., 2005). Changes in food composition compare with period of 1953-1993 when during feeding period content of cold-water copepods in sprat food consisted about 90-100% (Sirotenko and Sorokalit, 1979; Gapishko *et al*., 1985) coincided with sharp decrease of stomach fullness. In May-June 2000-2003 the mean indexes of stomach fullness of sprat with size about 80 mm dominated in population, ranged from 18 to $30\%_{oo}$ and were much less compare with mean data for 70-80th ($60 - 110\%_{oo}$) (Sirotenko and Sorokalit, 1979; Yur’ev, 1979; Glushchenko *et al*., 2005). Remarkably that soon after 2000 lipid content in sprat body tended to decrease and reached minimum value compare with average long-term annual value for period since 1960 till 2000. Last years were noted also that sprat grows smaller and its catches declined (Zuev *et al*., 2004). There is no evidence that these finding were due to overfishing rather they are associated with changes in fish food supply. Earlier it was shown that low lipid accumulation by sprat, which associated with slow growth

(Shulman, 1974) negatively, influenced on fish schooling behavior (Minyuk *et al*., 1997) and may cause declining of catches.

The main components of lipids are fatty acids. Fatty acid composition of reserve lipids (triacylglycerols, TAG) mainly and in lesser degree structural lipids (phospholipids, PL) depend on food composition. When lipid assimilation much exceeds the expenditure, fatty acids of predator reserve lipids is the most approach to the fatty acid composition of its diet (Sargent *et al*., 1988). Sargent group was among first to show that dietary fatty acids are incorporated into marine animal lipids with little or no modification original structure and some of fatty acids may be markers of species consumed by fish (e.g. Sargent *et al*., 1988; Parrish *et al*., 2000). The markers of cold-water *Calanus sp*., including *C. euxinus* in the Black sea, in fish lipids are the long-chain monounsaturated fatty acids 20:1n-9 and 22:1n-11 (Yuneva *et al*., 1998, 1999), which correspond to the fatty alcohols of the wax esters lay down by the *Calanus* feeding on algae (Sargent and Henderson, 1986; Lee *et al*., 2006). Content of these fatty acids is significantly less or they practically absent in lipids of warm water copepods (Lee *et al*., 2006). In the temperate waters, the increasing 20:1n-9 and 22:1n-11 in fish lipids shortly after mass development of calanoid copepods because of spring and autumn algae blooms was recorded (Sargent *et al*., 1989; Yuneva *et al*., 1990). The study 20:1n-9 and 22:1n-11 content in sprat reserve lipids may be useful for evaluation *C. euxinus* input in diet especially if shift in food composition from cold water to warm water species presuppose.

In addition to trophic markers, essential polyunsaturated fatty acids (EFA) such as docosagexaenoic (DHA, 22:6n-3) and eicosapentaenoic (EPA, 20:5-n3) acids play crucial role in realization many biochemical and physiological functions and might indicate fish fitness (Masuda, 2003). These fatty acids, which not biosynthesized effectively by animals, originate mainly from phytoplankton and transform through food web to higher trophic level. Although the wild diet of fish generally contains essential fatty acids, they can fluctuate dramatically in food during algae and zooplankton succession (Parrish *et al*., 2000) and be deficient in some cases (Bell and Sargent, 1996).

Well known, that DHA and EPA in animal lipids are the main components of phospholipids in all biomembranes and precursors for eicosanoids that fulfil a variety of metabolic functions. They positively influence on development nervous system, modulate many behavior reactions and may be very important not only for adult fish and in great extend determine the fate of recruitment (Bell and Sargent, 1996; Sargent *et al.*, 1999). There is close relationship between DHA and EPA concentrations in the parental body and survival of fish larvae and fries (Sargent, 1995; Yuneva *et al.*, 1990). Deficiency of PUFA (especially DHA) in lipids of mother fish during maturing drive to loss of sight of its posterity, abnormalities in behavior and increasing of predator elimination (Masuda, 2003). Undoubtedly, monitoring of essential fatty acids in TAG and PL allows not only estimate condition of adult fish but predict wellbeing of recruitment.

For estimation sprat nutritional condition, science spring- summer 2006 till autumn 2007, we measured dynamic of lipid content and composition, fatty acids as trophic indicators and markers in TAG and PL during feeding period. Along with lipids, we analyzed fullness of sprat stomachs and inspected zooplankton food in it. Unlike lipid accumulation which indicate fish food supply during relatively long time (total feeding period duration of months), analyses of stomachs allow to estimate food supply immediately before catching.

5.3.1 Material and methods

Samples of sprat were obtained in the Northern Black Sea. Cohort of 100 fishes (anchovy as well as sprat) from the catch was measured to the nearest 0.5 cm and divided to 5-7 size groups. Fishes from each size group was weighted and homogenised as a whole in home processor. Homogenate of 500 mg was used to extract lipids by chloroform-methanol (2:1) (Folch *et al.*, 1957). After washing and drying extracts in vacuum evaporator, lipids were determined by gravimetric method (Kates, 1975).

Lipid classes were separated using unidimentional thin-layer chromatography (TLC) on Silufoll UW254 plates (Kavalier Ltd, Czech

Republic) with solvents of different polarity. Chromatograms were visualized by heating at 110°C and quantified with ERS densitometer (Karl Zeiss, Germany). The lipid classes: (phospholipids: PL, sterols: S, free fatty acids: FFA, triacylglycerols: TAG and sterol esters: SE) were identified against a suitable of lipid standards (Sigma Co). The percentage of each component was determined by the triangulation. The fatty acid compositions of the triacylgycerols, TAG and phospholipids, PL analyzed with gas-liquid chromatography (GLC). The fatty acid components converted to their methyl esters derivatives by transesterification in methanol containing 3% concentrated sulphuric acid at 80°C for 4 h. After extraction with hexane, fatty acid methyl ester (FAME) composition was determined with a PYE-101 (Model 24) gas chromatograph equipped with hydrogen flame ionization detector, on a glass column (3 mm i.d. and 2 m length) packed with 10% diethylene glycol succinate (DEGS) on acid-washed Chromosorb W silanised, 80-100 mesh. The column temperature was 185–190°C. Helium used as carrier gas with flow rate 200 ml per min. Individual methyl ester peaks were identified:

(a) by comparison of the retention time with those of commercially available standards, secondary standard (cod liver oil fatty acids) according to the data of Ackman (1986);

(b) plotting the logarithm of the relative retention times versus the number of carbon atoms in the molecule (Ackman, 1986).

The percentage of each component was determined by the triangulation. The accuracy of determination was >5% in the case of major fatty acids and no more than 20% in the case of minor ones.

Fish of modal groups (no less than 20 individuals from each catches) used to study gut fullness and stages of maturity. Total length and weight of each fish were measured. The entire digestive tract removed, cut open, weighted and examined in a Petri dish under dissecting microscope. Prey items were identified down to species level whenever possible but the level of identification depended on completeness of food organism and its condition. Soft-body preys were particularly difficult to identify to species level because they were rarely in good condition. Thus, such evaluation may be underestimates.

The stomach fullness index (FI) was calculated from:

FI = stomach content weight / fish body weight * 10000 (Pravdin, 1966).

Gonads were dissected and weighted. The gonad somatic index (GSI) was calculated such that:

GSI (%) = Gonad weight/Gutted fish weight *100 (Pravdin, 1966)

5.3.2 Results and discussion

Seasonal dynamics of total lipid content and composition in sprat body.

In 2006 and 2007 small sprat with body length 6.5-7.5 cm and with body weight about three grams dominated in catches. At the end of May until August 2006, lipid content in the sprat body was extremely low (6.2 ± 0.3%WW) (Fig. 5.15): almost twice less compare with long-term mean annual value (11.7%, Shulman *et al*., 2005) and unexpectedly did not changed up to the end of October 2006. Decrease in lipid content was observed since November to January because not only maturation and spawning (Fig. 5.16) but due to shortage of feeding at the winter (Fig. 5.17). Fatness of sprat started to increase gradually science 3.6% in the middle of March until 7.5% at the end of April 2007 (Fig. 5.15). That period the rate of lipid accumulation had maximum values and averaged about 0.1% per day. During next month, the rate decreased twice, but TL continued to rise. In June-July 2007 TL in sprat body was 9.4 ± 0.3%WW. Up to the late of October 2007, it decreased insignificantly (8.7 ± 0.6%WW) possibly because start of gonad maturation. Thus, during feeding period of 2007, sprat fatness was the 1.5 times higher than in 2006 and obviously, food supply was better. Remarkable, that during feeding period (June – July) of 2005 sprat fatness was the same as in 2007 (9.6%WW).

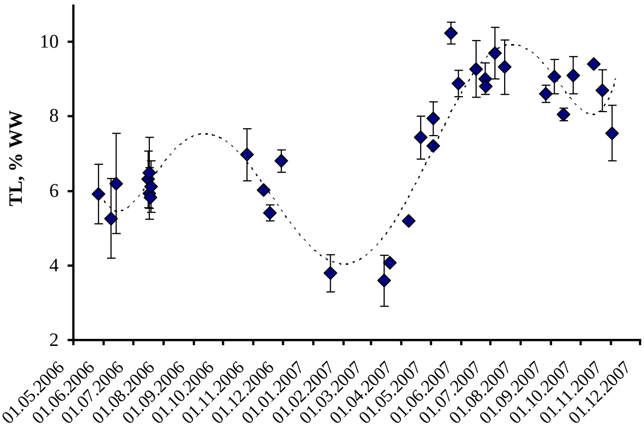

Figure 5.15. ***Sprattus sprattus*** **Variability of the lipid content (%WW) for the period of investigation in 2006/2007**

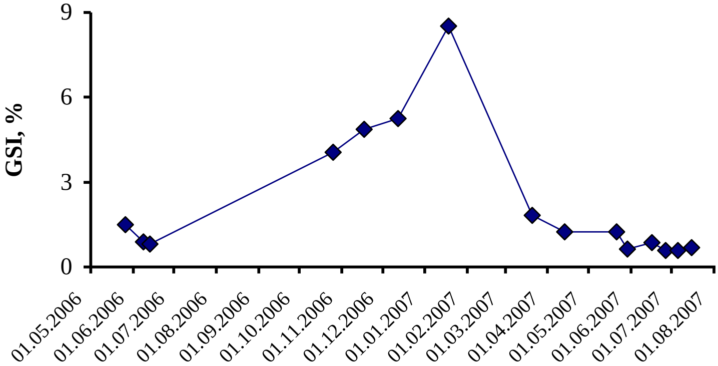

Figure 5.16. ***Sprattus sprattus.*** **Dynamics of the gonadosomatic index (GSI) for the period of investigation in 2006/2007**

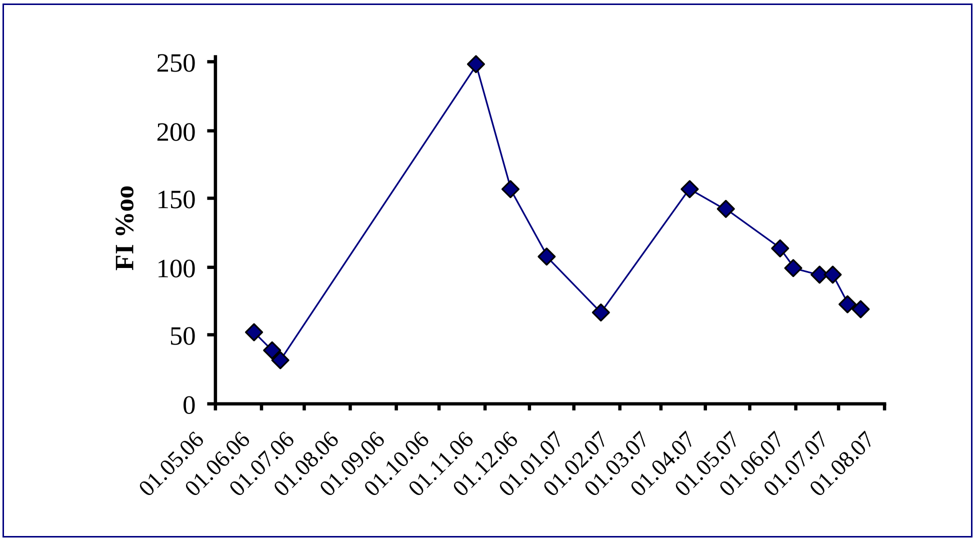

Figure 5.17. ***Sprattus sprattus*** **Dynamics of stomach fullness (FI) for the period of investigation in 2006/2007**

Seasonal changes in lipid accumulation in 2006 had no strongly pronounced maximum during summer months (Fig. 5.15). On the first glance, such finding might relate with peculiarity of zooplankton food consumed by sprat. Really, in 2006 during summer feeding period gut fullness of sprat was very low (Fig. 5.17) and the main items of sprat food were mainly slim small copepods, as far as in autumn gut fullness were great and there were extremely high abundance of fat *C. euxinus* 5 copepodites and females (Fig. 5.18). Overall, during the most part of 2007, fullness of stomachs was bigger and almost all this period coldwater copepods (mainly *Calanus* older stages) were predominant in gut content (Fig. 5.18).

However, in 2007 when cold-water copepods prevail among food items, seasonal dynamic of TL in sprat body again has no pronounced maximum in summer months (Fig. 5.15). In autumn the TL was relatively high, almost the same as in summer in spite of sprat expenditures on maturation. We can speculate that such rather strange dynamic in both years compare with period of 70-80th, may related with predator pressure on zooplankton (especially warm water copepods) by jellies (*Mnemiopsis* and *Aurelia*) which were the competitors of planktivorous fish during summer in shallow shelf waters last two decades (Kideys *et al*., 2005). Although to date, jellies impact on zooplankton decline it may be significant in short period of *Mnemiopsis* and *Aurelia* mass development in the middle of summer when its abundance increase sharply (Finenko *et al*., 2006). In the middle of October, beginning of November 2007 after fall water mixing and drop of jellies pressure on zooplankton, fullness of fish stomachs was very high and along with *Calanus* older stages, a lots of small copepods such as *Acartia*, *Paracalanus*, *Pseudocalanus* were founded out in it (Romanova, personal communication). Most likely, plenty feeding in autumn could be compensation of malnutrition in summer and allowed sprat to support appropriate level of fatness for maturation.

Significant changes in lipid concentration noted since summer 2006 until autumn 2007 were conditioned by reserve lipid (TAG) changes mainly (Fig. 5.19), which percentage in total lipids during maximum of fattening was less in summer 2006 than in 2007 and made up 63% and 70%, accordingly (Fig. 5.20). In contrast, concentration of structural lipids (phospholipids, PL and sterols, S) science May 2006 until November 2007 changed in lesser degree (Fig. 5.19). Content of PL and S consisted of 10-15% and 8-10% of total lipids, accordingly (Fig. 5.20). Their maximum concentration was in summer and minimum in winter during spawning when not only reserve lipids but some components of structural lipids utilize (Shatunovsky, 1980).

Fatty acid composition TAG and PL in sprat body during feeding period 2006 and 2007

The fatty acid composition in reserve lipids (TAG) of fish first depend on quantity and quality zooplankton food (Sargent *et al*., 1989). During feeding period of 2006 and 2007 in sprat TAG more than 20 fatty acids with 14-22 carbons and 0-6 double chains were distinguished and identified (Fig. 5.21). Quantity of eight of them made up 80% of total fatty acids. Saturated fatty acids (SFA) averaged about 30%, monounsaturated fatty acids (MUFA) dominated and made up about 40-50% and polyunsaturated fatty acids (PUFA) were about 20-30%. Among SFA 14:0 (7-9%) and 16:0 (20%) were prevailed. The main MUFA were 16:1n-7, 18:1n-9, 20:1n-9 and 22:1n-11: 8-9%, 14-16, 11-

13%, 10-13%, accordingly. Two last of them originated mainly from lipids of *C. euxinus* consumed by sprat (Yuneva *et al.*, 1998). The relatively high content of *Calanus* markers was recorded in sprat TAG not only in summer 2007 when cold-waters copepods were plenty among food items but in summer 2006 when feeding was rather poor and only small copepods were registered in sprat stomachs (Fig 5.17, 5.18).

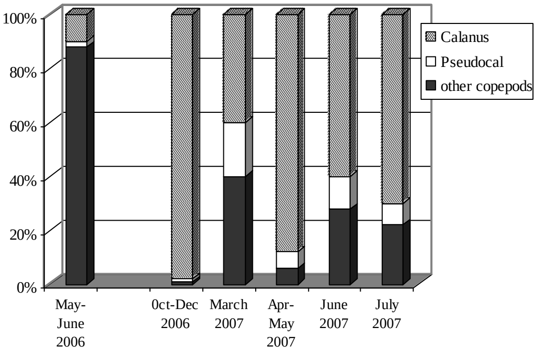

Figure 5.18. ***Sprattus sprattus*. Percentage of different copepod species in stomachs for period of investigation in 2006/2007**

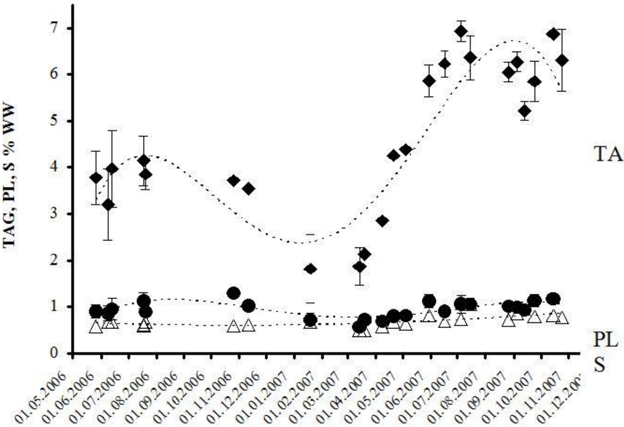

Figure 5.19. ***Sprattus sprattus*. Variability of triacyglycerol (TAG), phospholipid (PL) and sterol (S) content (%WW) in the body for the period of investigation in 2006 and 2007**

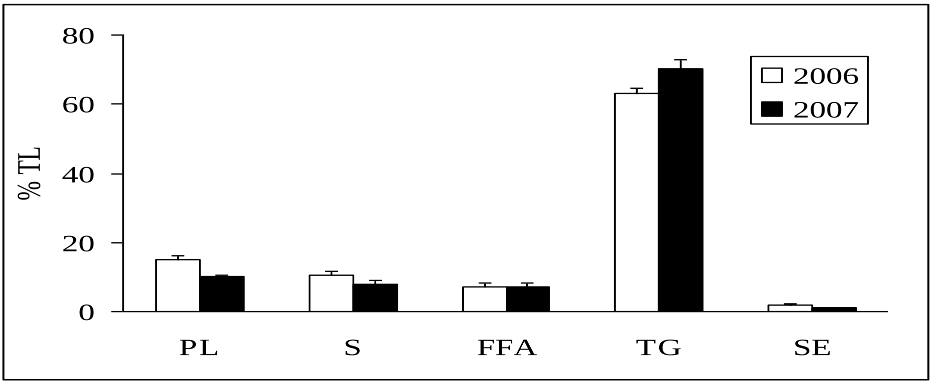

Figure 5.20. ***Sprattus sprattus.*** **Content of lipid classes (% TL) during feeding period (June-July) of 2006 and 2007. Phospholipids: PL, Sterols: S, Free fatty acids: FFA, Triacylglycerols: TAG, Sterol ethers: SE. Mean ± std**

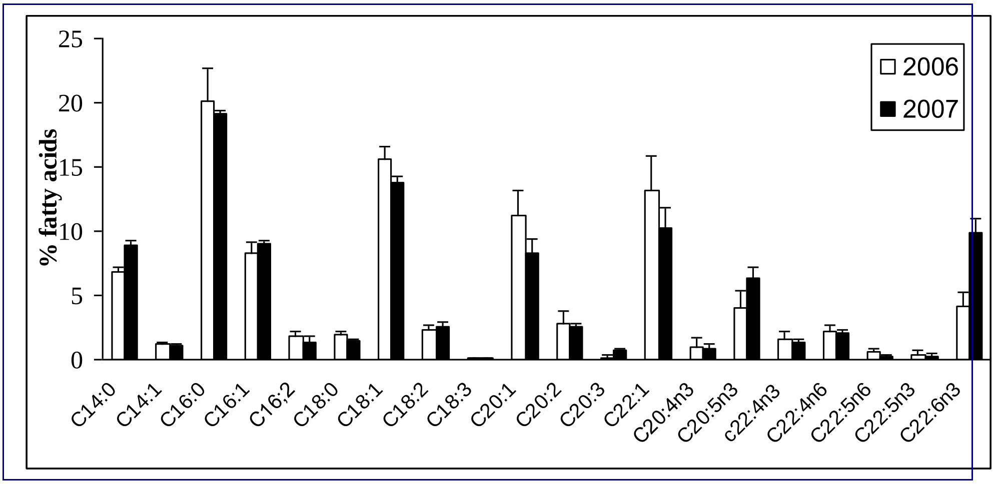

Figure 5.21. ***Sprattus sprattus*****. Triacylglycerol fatty acid composition (% total fatty acids) during feeding period (June – July) 2006-2007. Mean ±std**

It is possible to assume that 20:1n-9 and 22:1n-11 high concentrations in both years were consequence of lipid accumulation at sprat predator on *Calanus* in spring. Poor feeding during summer 2006 because of prevailing inappropriate food and insufficient it quantity may be reason that positive balance in lipid accumulation was absent until autumn when after water mixing many 5 copepodites and adult *Calanus* in plankton appeared and jellies pressures became weaker. The above assumption may illustrated by data of Figure 5.22, where fatty acid concentration in May, June, July and October 2006 and in May, June, July 2007 calculated in absolute term as percentage of wet body weight (%WW), taking into account differences between years in TL and TAG contents in them. It was shown that concentration of main fatty acids did not change since May until July 2006 and then increase of 20:1n-9 and 22:1n-11 took place in October (Fig. 5.22B) with appearance large abundance *Calanus* in plankton. Since May until July 2007, when *Calanus* was presented in stomachs during spring as well summer months and nutritional condition was much better the concentrations of all fatty acids including markers of *Calanus* increased gradually since May until July (Fig. 5.22).

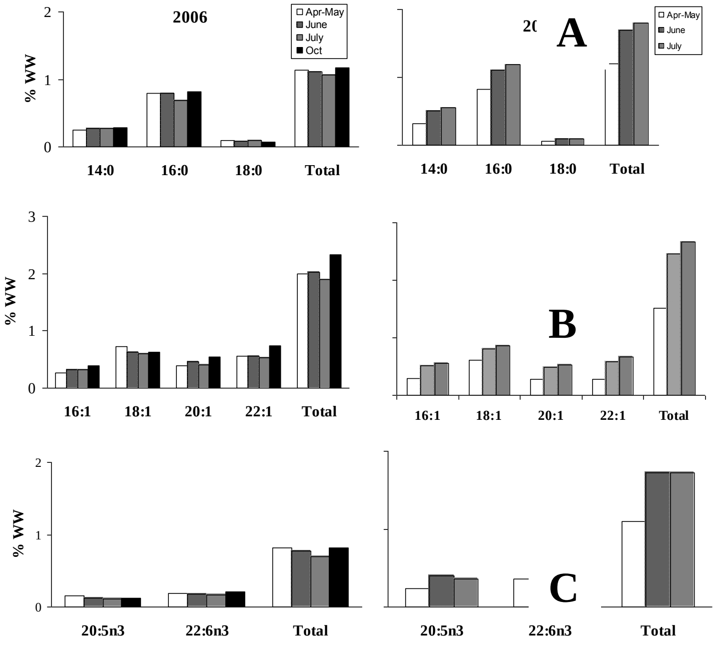

Figure 5.22. ***Sprattus sprattus*. Mean fatty acid content (% WW) in TAG during spring, summer and autumn months of 2006 and spring-summer of 2007. A – saturated fatty acids, B – monoenic fatty acids, C - polyunsaturated fatty acids**

Among PUFA in sprat TAG, 20:5n-3 and 22:6n-3 predominated (Fig. 5.21).

20:5n-3 and 22:6n-3 content in 2006 constituted about 4% of total fatty acids, each and in 2007 about 6% and 10% respectively. Thus during feeding period of 2006 the percentage of 20:5n-3 and 22:6n-3 in TAG was in 1.5 and 2.2 times less respectively then in 2007. The positive relationship between 22:6n-3 and 20:5n-3 content (% fatty acids) and TAG, % WW (as well as lipid content) in sprat body (Fig. 5.23) may also confirm that these fatty acids are accumulated in lipid reserves as much as lipid accumulated because they are much more conservative compared with SFA and MUFA and not used for energy purpose.

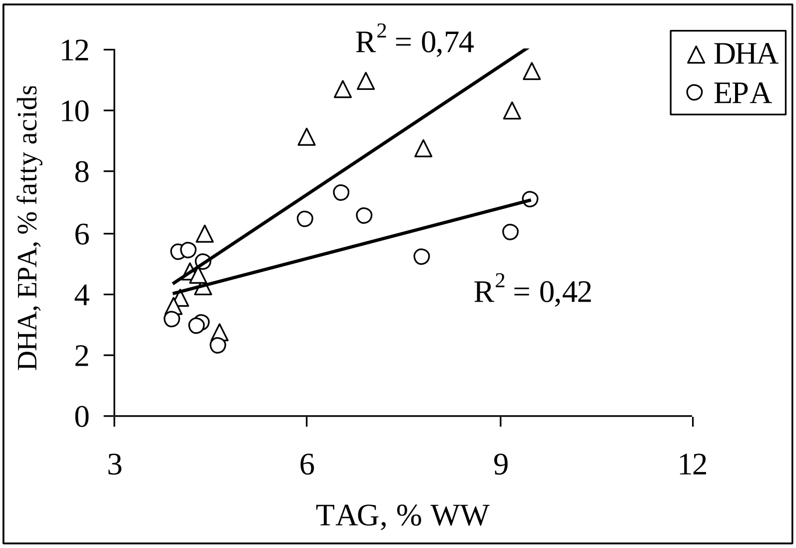

Figure 5.23. ***Sprattus sprattus*****. Relation between EPA (20:5n-3) and DHA (22:6n-3) content (% fatty acids) in TAG and TL content (% WW) in sprat body**

If fatty acid content is calculated in absolute terms (%WW), the differences in concentration of these essential fatty acids between years would be much more pronounced (Fig. 5.22C). In summer of 2006 content 20:5n-3 and 22:6n-3 was about three times less than in 2007.

Moreover, the ratio between both this fatty acids changed between investigated years: it was about one in 2006 and about two in 2007.

Thus, poor food supply with prevailing warm-water versus cold-water copepods in summer 2006 displayed not only in low accumulation of lipids in the sprat body mainly due to reserve lipids (TAG) but may caused the shortage of essential fatty acid content and changes in relationship between them. In fact, there are significant differences in lipid content and composition, fatty acid composition of main lipid classes between typical cold-water *C. euxinus* and typical warm-water *A. clausi* (Yuneva and Shchepkina, 2005). Older stages of *Calanus euxinus* are as much as 5 times fatter than *Acartia clausi* (8.0% and 1.4% WW, accordingly), contain higher content of reserve lipids with prevailing energy rich monounsaturated fatty acids (MUFA) (Yuneva *et al*., 1990; Yuneva and Shchepkina, 2005), have much more essential PUFA (DHA and EPA) (Table 5.6). Moreover, the biggest Black Sea copepod *Calanus euxinus* with body weight app. 1 mg/ind. and size 3.2 mm (Sazhina, 1987) seem to be more suit and energy valuable prey for sprat than *Acartia sp*. with body weight 0.04 mg/ind. and size about 1 mm (Hubareva *et al*., 2008).

Table 5.6. DHA and EPA concentrations (mg per 100 gram of WW) in the *Calanus euxinus* and *Acartia clausi* from the open Black Sea in spring 2002. WE – wax esters, TAG – triacylglycerols, PL – phospholipids

Essential fatty acids	**C. euxinus**				**clausi**		
	WE	TAG	PL	Total	TAG	PL	Total
DHA22:6n3	69	30	239	338	10	106	116
EPA20:5n3	256	35	113	404	15	53	68
Total	325	65	352	742	25	159	184

Reserve lipids required by all animals, both for the provision of metabolic energy, generated as ATP through the oxidative metabolism of fatty acids and for the production of structural lipids especially during gonadogenesis (Sargent *et al*. 1999). Deficiency in the DHA and EPA content in adult fish reserve lipids has been identified as an important factor affecting negatively fecundity, fertilization, hatching rate and viability of produced eggs (Watanabe *et al*., 1978; Yuneva *et al*., 1990b; Sargent *et al*., 1995). The certain levels of these acids are required to obtain optimal growth and survival larvae (Watanabe *et al*, 1978; Sargent, 1989). The specific role of DHA in the development of neural tissues as brain and retina eyes has been well-documented (Bell *et al*., 1995). Maternal DHA-EPA optimal ratio also important for larvae because specific role of DHA and EPA during development are different and imbalance of EPA and DHA in larvae membranes may result in poor larval viability (Watanabe, 1993). Therefore, it is possible to assume that warm water copepods in diet with less essential fatty acids may negatively influence on quality of sprat recruitment.

Unlike TAG, PL fatty acid composition of fish and other animals predominant mainly genetically within the same species (Kreps, 1981) and depends on food consumed in lesser degree. The fatty acid profiles in PL correspond to the trends generally observed in various fish with high DHA, 16:0, 18:1 and with relatively high EPA contents compare to TAG (Ackman, 1980). In sprat PL SFA made up about 30% with predominance of 16:0, MUFA consisted about 20% with predominance of 18:1 (Fig. 5.24). PUFA dominated in sprat PL and made up 45 and 52% of total fatty acids in 2006 and 2007 accordingly. EPA consisted of 10% in both years where as DHA content was 25.9% in 2006 and 32.9% in 2007. Thus, decrease of DHA content in 2006 under unfavourable feeding regime was observed not only in sprat TAG but in PL also.

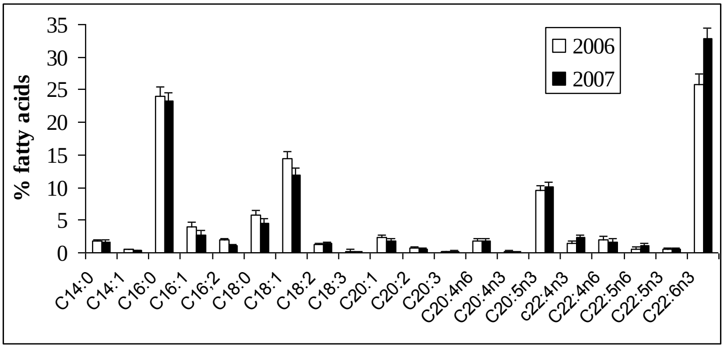

Figure. 5.24. *Sprattus sprattus*. Phospholipid fatty acid composition (% total fatty acids) during feeding period (June – July) 2006-2007. Mean ±std

Being the main component of cell membranes, phospholipids with large concentration of PUFA play important role in animal adaptation to environment changes (Kreps, 1981). As was mentioned above, PL fatty acid composition depend on composition of food in lesser degree compare with TAG, nevertheless fish capacity to adapt to any sustained or acute environmental stress may be affected by fish nutritional status (Sargent *et al.*, 2002). Dietary supplementation in DHA and EPA increased fish tolerance to temperature changes or hypoxia (Rainuzzo *et al.*, 1997).

Traditionally role of PUFA in cell membranes is connected with its low melting points and possibility to support membrane fluidity (homeoviscous regulation) (Bell *et al.*, 1986). Well-known, PUFA content in fish PL alters depending on environmental changes, mainly temperature, increasing at low temperature of water and during cold seasons (Kreps, 1981; Hazel and Williams, 1990; Farcas *et al.*, 2001). However, it is not true for several fish species, in which tissues decrease of temperature did not induce major changes in polar lipid fatty acid composition (Trigari *et al.*, 1992; Ventrella *et al.*, 1993).

Rabinovich and Rippati (1991) and Sargent *et al.* (2002) denied traditional notions about role of PUFA in biomembrane adaptation to environmental changes. They have shown that the most unsaturated DHA due to specific spatial organization allows cell membranes with high content this fatty acid to be practically insensitive to external forces (temperature, pressure, oxygen tension etc.). That is why there is a lot of DHA in neural tissues of all animals (Kreps, 1981; Ackman, 1980; Navarro *et al.*, 1992) and in PL actively swimming migrating fish and copepods, which sustain drastic changes of water temperature, pressure, oxygen tension etc. (Shulman and Yuneva, 1990; Yuneva *et al.*, 1998).

Studying seasonal dynamic fatty acids in sprat we found out that maximal DHA content in sprat PL was not in cold winter-spring season but in summer: 21 and 32% accordingly (Yuneva, 1990). This phenomenon may be explained by increasing of sprat moving activity in summer when it should feed intensively, providing lipid accumulation and growth. Sprat habits around year in cold water at temperature in the 6 to 13 degree C range with maximum in summer. Such increase of temperature should increase sprat energy metabolism and locomotion in 1.5 times (Shulman and Urdenko, 1987). Energy expenditure during summer and utilization lipids (mainly MUFA) for energy purpose may be reason for increasing of PUFA relative content in TAG and PL, especially its main component DHA because the later is very conservative compare with MUFA and SFA and even compare with another essential PUFA like EPA (Ackman, 1980). In summer when food is scarce high locomotion will improve feeding condition extending sprat-feeding area. In 2007 when sprat food supply was more favourable than in 2006, the higher DHA content in sprat PL could promote this purpose better. Unlike summer, in winter declining lipid content in sprat body during spawning take place, when along with reserve lipids, PL expenditure took place (Shatunivsky, 1980). Being very important component of fish eggs DHA mobilize during spawning from PL too.

In conclusion, should add some comments about significant of DHA and EPA (named in medicine practice as omega-3) for human health. Extensive research indicates that these fatty acids reduce inflammation and help prevent risk factors associated with chronic diseases such as heart disease, cancer and arthritis. DHA is highly concentrated in the brain and appear to be particularly important for cognitive (brain memory and performance) and behavioural function. In fact, infants who do not get enough this fatty acid from their mothers during pregnancy is at risk for developing vision and nerve problems. Symptoms of omega-3 fatty acid deficiency include extreme tiredness, poor memory, dry skin, heart problems, mood swings or depression etc. (Arts *et al*., 2001).

Mean human requirements in DHA+EPA average 1.25 grams per day which equal approximate 3-4 grams of standard fish oil (Arts *et al*., 2001; Sargent *et al*., 1999). Familiar fish species used in the production of fish oil in the World include, among others, anchovies, capelin, cod, herring. Although sprat is the most abundant (after anchovy) commercial fish species in the Northern Black Sea, it has not used as source of fish oil yet.

We compare DHA and EPA concentration (mg per 100 gram wet weight) in sprat PL and TAG during feeding period of 2006 and 2007 (Table 5.7). Both PL and TAG make up about 80% of total lipids, so analyzing fatty acid composition both lipid classes allow to estimate with certain extend content of essential fatty acids in sprat body. During relatively favourable feeding condition in 2007, total DHA and EPA content in sprat body was as much as twice higher than in 2006 and about 100 gram of sprat satisfies diurnal human needs in these acids that year.

Thus changes in quantity and quality of plankton food, which was observed during feeding period of 2006 compare with 2007, caused not only worsening of sprat nutritional condition but also declining essential fatty acid content needed for well-being of sprat population, its recruitment and important for human health.

Table 5.7. DHA and EPA concentrations (mg per 100 g of WW) in TAG and PL of the Black Sea sprat during feeding period of 2006 and 2007.

Essential fatty acids	**2006**			**2007**		
	TAG	PL	Total	TAG	PL	Total
DHA22:6n3	189	207	394	605	291	896
EPA20:5n3	123	76	199	385	90	475
Total	312	283	593	990	381	1371

5.4 Lipid content of anchovy during 2005-2007

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Anchovies are represented in the Black Sea by two forms: the Black Sea anchovy *E. encrasicolus ponticus* and the Azov anchovy *E. encrasicolus maeoticus.* The present study considers mainly first subspecies from two locations: off Crimea and Anatolian coasts although second one taken from the Sea of Azov has been also examined occasionally.

After summer spawning, warm-water anchovy feed intensively and accumulate lipid reserves. Lipid accumulation is important for surviving winter. When water temperature declined, anchovy start to migrate from feeding grounds for a wintering toward the Southeast part of the sea. Anchovy from the Sea of Azov migrate in September–October and hibernate along Northern part of the Caucasus coast and partly along the Crimea coast. It may also approach the Anatolian coast to winter. Anchovy foraging in the Northwestern part of the Black sea migrate usually in October and hibernate in the warmer waters off the Anatolian coast, off the Caucasus and off the Crimea (Svetovidov, 1964; Ivanov and Beverton, 1985; Chashchin and Akselev, 1990; Sorokin, 2002). The Black Sea and Azov Sea anchovy almost do not differ morphologically but they are distinguished genetically (Kalnin and Kalnina, 1985; Ivanova and Dobrovolov, 2006). Genetic-biochemical analyses carried out during our investigation in 2005–2006 showed that anchovy taken off the Crimea belonged to the Black sea type and it was close to the anchovy sampled off the Anatolian coast. Nevertheless, in one sample taken off Sinop, mechanical mixed populations were found in 2005 that suggested some Azov anchovy to be winter there (Bat *et al.*, 2007).

5.4.1 Material and methods

Fish for monitoring investigation had been collected weekly during their feeding periods in 2005, 2006, and 2007. Samples of anchovy were obtained mainly in October, November and December from the 43 hauls of Turkish fishing vessels and 41 hauls of Ukrainian commercial trawlers. The same techniques of sampling were applied for anchovy as well as sprat (see section 5.2).

Lipids from homogenised fish had been extracted by chloroform-methanol (2:1) (Folch *et al.*, 1957). After washing and drying extracts in vacuum evaporator, lipids were determined by gravimetric method (Kates, 1975). Using the data obtained by the direct extractive method for the period 2005 to 2007 the following relationships between dry mass content (DM, % wet mass) and total lipid (TL, % wet mass) in anchovy were computed (Fig. 5.25):

$$TL = 0.937\ DM - 18.57. \qquad (5.8)$$

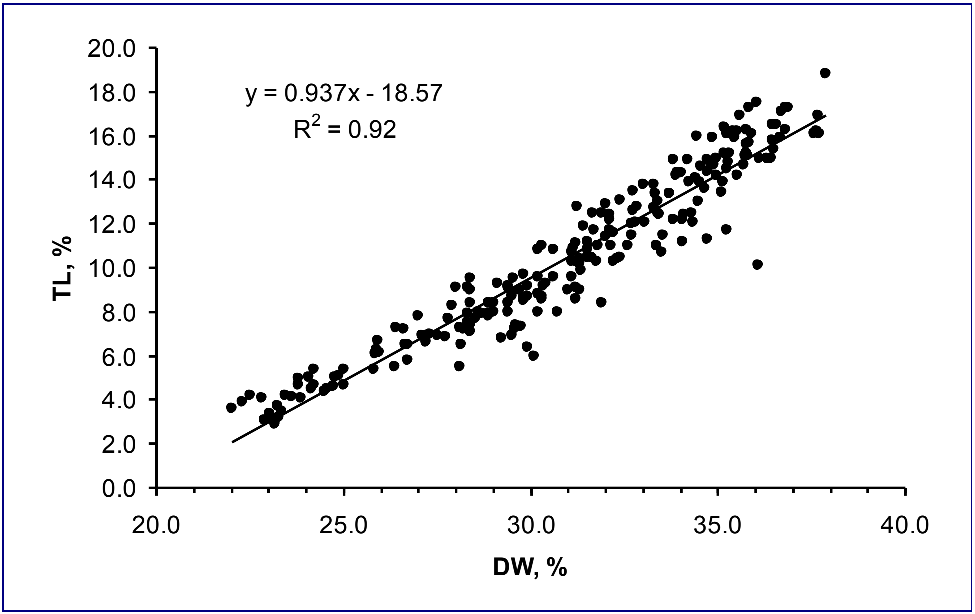

Figure 5.25. Relationship between dry mass (DM, % wet mass) and total lipid content (TL, % wet mass) in anchovy body

Afterward, the equations were applied to calculate total lipid content for each 5 mm size group of fish through its dry mass. To characterize lipid content in each sample, the average weighted values were calculated by eq. 5.6.

5.4.2 Results

In fishing season 2005/06, anchovy came to the Anatolian coast with not great lipid content (11 – 12 %) in November. Close to the Crimea, anchovy appeared later then it was generally but Ukrainian trawlers were catching them well since December to February. During winter months, the total lipid content was naturally decreasing in fish from both regions similarly but remaining higher 7 % up to February (Fig. 5.26 and 5.27).

In the 2006/07fishing season, near the Crimean coat, anchovy appeared in catches of fish traps in October. About 65 % fish had food in stomachs, and the plankton items in anchovy stomachs were copepods (*Acartia clausi, Calanus euxinus, Paracalanus parvus, Centropages* *ponticus*), cladocerans (*Penilia avirostris*), decapods, meroplankton, fish ova, phytoplankton etc. Lipid content in anchovy body increased gradually from 8–10 % in the beginning of October to 14–16% wet mass in November (Fig. 5.28).

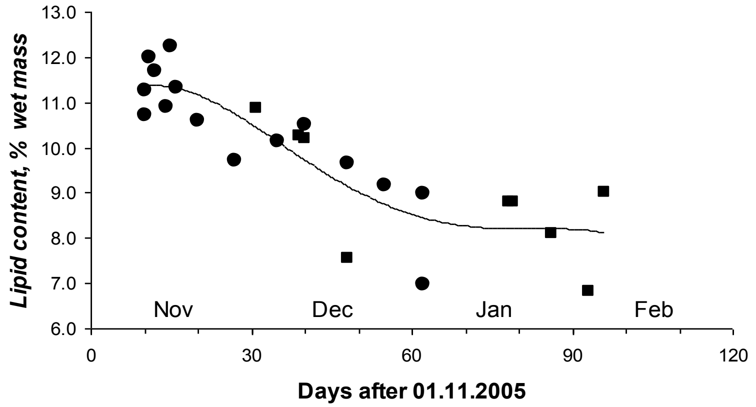

Figure 5.26. Lipid content dynamics in anchovy from Turkish (circles) and Ukrainian (squares) waters during the 2005/06 fishing season

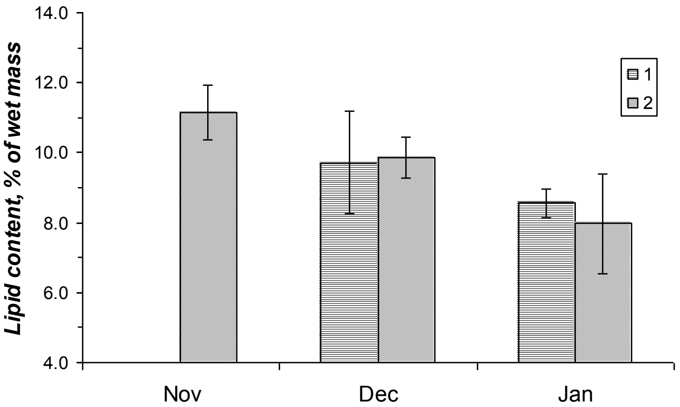

Figure 5.27. Lipid content (mean ± SD) in anchovy from Ukrainian (1) and Turkish (2) waters during the 2005/2006 fishing season

At the same time, off the Anatolian coast, anchovy did not feed: about 90% fish had empty or practically empty stomachs. Lipid content of anchovy from Turkey waters was bigger than off the Crimea. So in October, anchovy was preparing to winter in different extent: more fat ones migrated to Southern part of the sea where did not eat and wintered, the slimmer stay at the place of feeding longer. In November 2006, anchovy fishing near Crimea coast was rather poor but in Turkey it was much extensive. Fish from the waters off Turkey did not feed and share of feeding specimens near Crimea coast in November decreased approximately up to 30%. All anchovy off the Crimea coast had less fatness than off the Anatolian coast: 12.5% and 14.5%, accordingly (Fig. 5.29). Within December 2006 lipid content of anchovy off the Crimea as well as off Anatolian coast decreased due to loss of reserve lipids because of energy expenditure for wintering.

In fishing season 2007/08, anchovy appeared near Crimea and Turkish coasts in October as well. At the beginning of the month, their fatness was higher than in October 2006 and gradually increased from 9 to 14%. In November 2007 anchovy fatness in waters near Crimea and Turkey were close enough with ranges of 13.9–15.3% and 12.6–15.1%, accordingly (Fig. 5.30). In November, the fishing of anchovy was intensive in both areas.

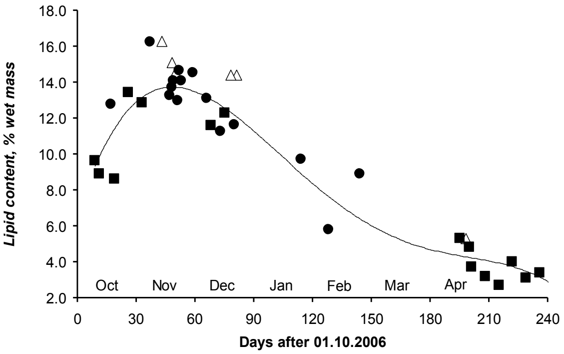

Figure 5.28. Lipid content dynamics in anchovy from Turkish (circles) and Ukrainian (squares) waters, and from the Sea of Azov (triangles) during the 2006/07 fishing season

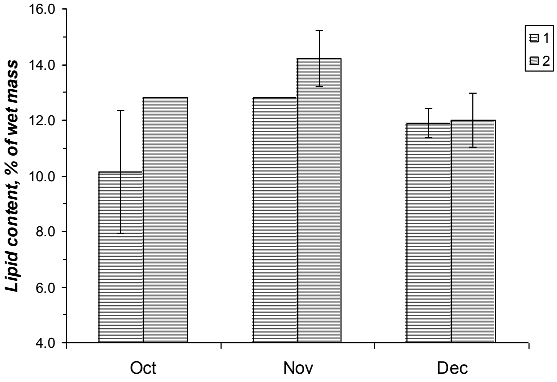

Figure 5.29. Lipid content (mean ± SD) in anchovy from Ukrainian (1) and Turkish (2) waters in the 2006/07 fishing season

In December 2007, there were large differences between anchovy fatness in the Crimea and Turkey areas. The anchovy fatness near the Crimea coast was low and varied in great extent (5–12%) while near the Turkey the fatness was big and rather constant with ranges 14–15% (Fig. 5.31). The fishing decreased in Crimea waters relatively sharply while it continued to be intensive in Turkey. The data had shown that almost all anchovy with high fatness migrated to Southern coast while fish with low fatness stayed near the Crimea coast.

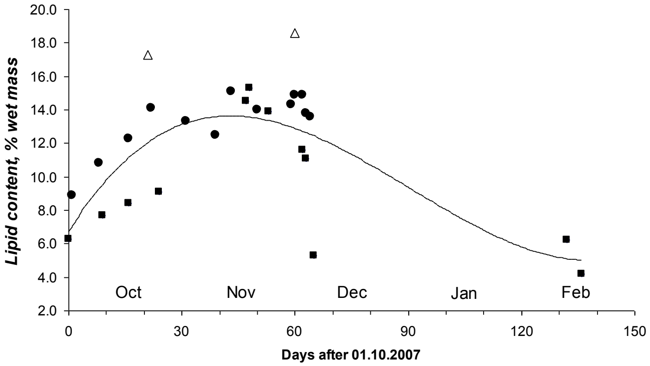

Figure 5.30. Lipid content dynamics in anchovy from Turkish (circles) and Ukrainian (squares) and waters during the 2007/08 fishing season

Thus, in 2006 and 2007, the anchovy accumulated lipids during October and completed it in November, when fatness reached maximum values about 14% wet mass in both years. In November 2005, lipid content in anchovy body was much less than in 2006 and 2007 and made up 11.2% (see Fig. 5.27).

The important question, which address fishery to science, is the question about terms of the wintering migration when anchovy fishing. The principles of anchovy wintering migration and spatial distribution were elaborated firstly on the Sea of Azov anchovy (Shulman, 1974) and then disseminated on the Black Sea anchovy as well (Chashchin and Akselev, 1990). It was shown that, there is close relationship between level of lipids in anchovy body in autumn and sea surface temperature, which triggered migration. If anchovy fatness is bigger (better nutritional condition), fish migrate to the south-eastern part of the Black sea at higher temperature (Fig. 5.32). Actually, the anchovy with worse nutritional condition stay at place of feeding and cannot migrate at all (Shulman, 1974, 2002).

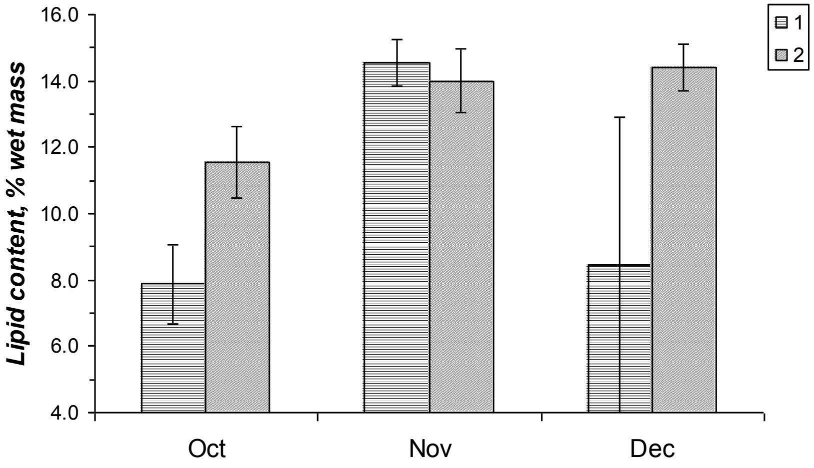

Figure 5.31. Lipid content (mean ± SD) in anchovy from Ukrainian (1) and Turkish (2) waters during the 2007/08 fishing season

As mentioned above, along with fatness, the temperature strongly influence on terms of anchovy wintering migration and stock distribution between northern and southern part of the sea (Chashchin and Akselev, 1990). The water temperature (average monthly values) in November 2005, 2006 and 2007 in areas off Crimea and Turkey differed noticeably (Fig. 5.33). Near the Crimea coast, it declined from 14.3°C in 2005 to 12.6°C in 2007. On the contrary, in waters off Turkey, it raised from 13.7 to 14.4°C. In 2005, anchovy fatness was low (11.2%) but the temperature off Crimea was higher than off Turkey. As a result, the considerable part of anchovy stock stayed to winter in warmer waters off Crimea, and fishing continued there during all winter and even spring months. On the contrary, in 2006, anchovy fatness reached 14%, and the autumn fall of water temperature became clearer in the northern part in comparison with the southern part of the sea. That situation was accompanied by absent anchovy in catches off Crimea during January, February, and March while the fishing of anchovy continued off the Anatolian coast. Anchovy returned to the Crimea again only in the middle April 2007 having fatness near 3–5% (see Fig. 5.28). In November 2007, anchovy fatness exceeded 14% and the temperature difference was even greater than in previous year. Apparently, that situation caused the majority anchovy stock migrated to the Anatolian coast providing appropriate condition for Turkish fishery in the 2007/08 fishing season.

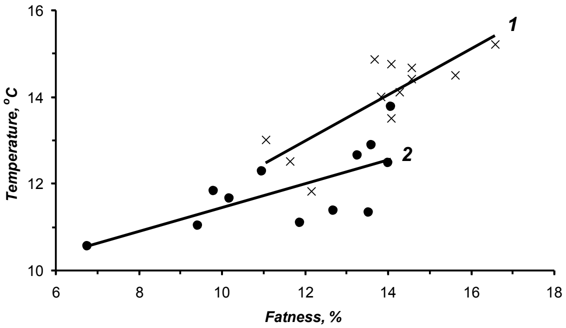

Figure 5.32. Relationship between Black Sea anchovy fatness at the end of feeding period and sea surface water temperature triggering a wintering migration: 1 – adults, 2 – juveniles (Chashchin and Akselev, 1990)

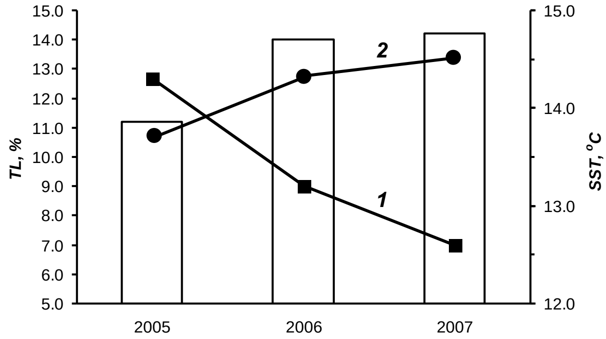

Fig.ure 5.33. Interannual changes of the lipid content (% wet mass) in anchovy body at the end of feeding period in November (bars) and sea surface temperature (average monthly for November) in Crimea (1) and Turkish (2) fishing areas. Temperatures were calculated on the basis of NASA satellite data (http://disc.sci.gsfc.nasa.gov/techlab/giovanni/)

The interannual changes of the lipid content in anchovy during three last years showed how climate variability might force distribution of anchovy stock. The brief scenario of anchovy distribution, depending of its nutritional condition and environmental changes, may be as follow. Anchovy start to accumulate lipid reserve in the northern part of the sea soon after algae bloom following fall water mixing, which stimulate development of autumn zooplankton bloom. If autumn storms start early in October and food supply is plenty, anchovy will feed actively in October and complicate lipid accumulation at least at the beginning of November. Further, the anchovy spatial distribution is strongly controlled by water temperature. If water is cooling in the Northern part of the sea due to prevailing north winds, anchovy migrate to warmer southern part of the sea to winter at the end of October beginning of November. Obviously in fishing seasons 2006 and 2007 anchovy food supply was good and its fatness (about 14% wet mass) was optimal for migration at the temperature in the northern part of the sea about 12°C and in southern part about 14°C (Chashchin and Akselev, 1990). Another situation take place when due to stagnation or weak mixing of water mass in autumn, which may continued up to November, anchovy fail to accumulate appropriate for migration lipid reserve. If water temperature is relatively high, anchovy will winter at the place of feeding manage to improve its nutritional status. The situation like above was observed in fishing season of 2005 when anchovy nutritional condition was bad (lipids content in the body was about 11% wet mass) and water temperature in Northern part of the sea in November was relatively high (about 14°C).

How do the recent data on the lipid content in anchovy correspond to anchovy food condition in the past? The data on anchovy fatness have been published for periods of 1955–1964 (Shulman, 1974), 1965–1973 (Shulman and Love, 1999) and the 1980s (Chashchin and Akselev, 1990; Shulman, 2002). Unlike sprat, the anchovy lipid data are much less regular and were interrupted at the end of the 1980s. The study on anchovy was recommenced in 2005-2007 after twenty-year interval (Shulman *et al.*, 2007). In 2005, the lipid content in anchovy body was low (11.2%) and comparable with mean values for the period 1965–1973 (Fig. 5.34) when fodder base of small pelagic fish was surely plenty. Therefore, according to the lipid data, anchovy food supply was changeable: in autumn 2006 and 2007, it was good and better than in previous 2005.

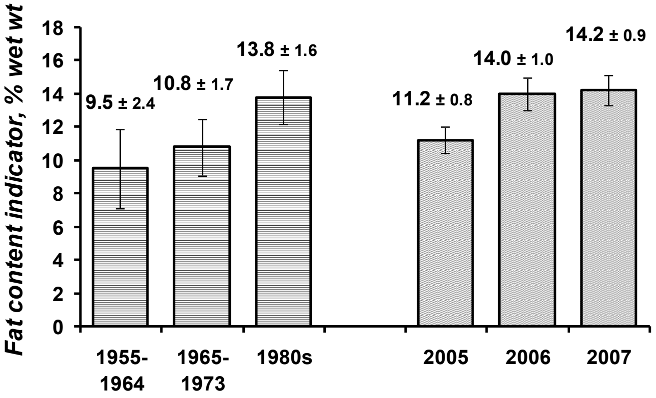

Figure 5.34. Lipid content (% wet mass) accumulated by anchovy at the end of feeding period (November) of 2005, 2006, and 2007 compare with mean values for the period 1955-1973 (Shulman, 1972; Shulman and Love, 1999) and for the 1980s (Chashchin and Akselev, 1990

5.5 Influence of global climatic changes and regional anthropogenic factors on the Black Sea sprat and anchovy condition

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Climate changes recorded last years became evident on local basins. The Black Sea ecosystem, affected during last decade's pollution, eutrophication, overfishing, introducing aliens via ships' ballast etc., seems to be very vulnerable to the climate variations (Sorokin, 2002; Zaitsev and Mamaev, 1997). The environmental changes and anthropogenic impact must influence on upper ecosystem trophic level: on stock and condition Black sea small pelagic fish. Sprat and anchovy are the most abundant among them. Having short life span, earlier maturation, high fecundity, feeding on plankton, moving actively both species integrate changes taken place on all lower trophic levels and may indicate ecosystem wellbeing degree. As was mention above, the level of energy (lipid) reserve accumulated in the body, along with abundance, is the important characteristic of fish condition (Shulman, 1974). Influence of climate changes on the Black sea small pelagic fish condition was studied on the base of long-term data of the lipid content in sprat (Shulman *et al.*, 1994; Shulman *et al.*, 2005) and anchovy populations (Chashchin and Akselev, 1990; Shulman, 1974; Shulman and Love, 1999), analyses their catches and stocks.

Between 1960 and 2007 sprat body lipid content demonstrated significant interannual variability. This correlated positively with long-term changes of sprat stock and phytoplankton biomass (see Fig. 5.3 and Table 5.5). At the same time there was no correlation between sprat fatness and mesozooplankton biomass because mesozooplankton must be not eaten by sprat only but also by other predators such as anchovy, jellyfishes, etc. The positive correlation between sprat lipid content and biomass (stock value), may witness that food influence along with lipid accumulation on the efficiency of reproduction, survival of juveniles and adult fish.

The 9-11 years fluctuations of lipid content in sprat body, biomass of phytoplankton and sea surface temperature (SST) were noted (Table 5.8). It is known that SST in the 70-90s was significantly less compare with long-term mean value, and then was increasing last years (since the 1999). High SST were also recorded in the 60s. Increasing SST during last decade coincided with declining of phytoplankton biomass (Boichenko, 2005; Velikova and Mihneva, 2005) and sprat lipid content as well.

Table 5.8. Decadal changes in the analyzed indicators for the period 1960–2005

Indicators	**Decades**				
	1960s	1970s	1980s	1990s	Beginning of the XXI century
Surface water temperature	High	Low	Low	Increasing	High
Phytoplankton biomass	Decreasing	Increasing	High	High	Low
Sprat fat content	Decreasing	Increasing	High	High	Low

Negative relations between changes of SST and ecosystem biotic parameters (phytoplankton biomass, sprat lipid content and biomass) for the period 1960 to 2007 may be explained as follow. During cold winters with stronger mixing of water column, the bigger concentration of nutrients input into the euphotic layer and promote increasing of phytoplankton biomass (Oguz *et al.*, 2006). Because of food chain “phytoplankton-mesozooplankton-fish”, the later appeared also influenced by cold weather changes with SST as an indicator. It is possible to assume that decline of SST may affect positively on cold water sprat extending its feeding area and improving fodder base due to favourable condition for cold water copepods. Presumably, the low temperature might extend also area of sprat spawning as well, and improve condition for fish recruitment. Apparently, all that favoured to sprat condition in the middle 1970s, during fall of temperatures. In contrast, the high SST in winter months might slow down of water mixing, declining level of phyto- and zooplankton. It may be reason the decreasing of sprat lipid accumulation and possibly its stock during last decade during the warming, which was recorded since 1999-2000. Naturally, the climate affects fish populations not directly, but through complex ecosystem relationships. That is why, the fish response on this forcing is not prompt and has time lag which duration related with biochemical and physiological alterations on the level of individuals, and possibly becomes apparent within next generations (Fig. 5.35).

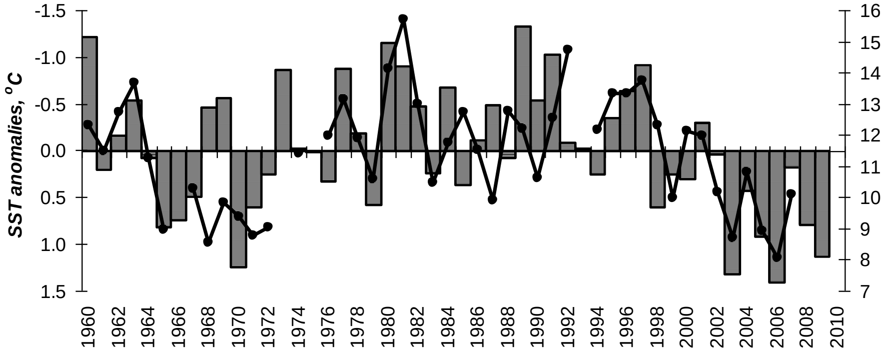

Figure 5.35. Sprat food supply indicator (*FSI*) compared to annual *SST* anomalies south of Crimea delayed for 4 years

Using the long-term data of sprat fatness and temperature changes for the period of 1960 until nowadays, we elaborated predictive linear model for estimation sprat condition on the future according to SST. However, there were significant differences between predicted and observed data in several years (1968, 1981, 1987, and 1992, see Fig. 5.12). It might be connected with sharp influence of heterogeneous environmental and anthropogenic factors on sprat during the mentioned years. At the end of the 60s, the switch in direction of temperature trend was recorded in different World Ocean regions (Lalli and Parsons, 1997). In 1981, fish fatness and biomass were extremely high because very favourable condition for sprat (and anchovy too) during eutrophication. In 1987, the fatness decreased due to strong food competition between sprat and anchovy at the highest levels of their stocks. In 1992, the fatness increased possibly while the *Mnemiopsis leidyi* predator pressure on mesozooplankton became weaker (Shulman *et al.*, 2005).

Thus, climate changes with SST as indicator influence on the Black Sea sprat condition. It seems to be due to global climate changes, because the temperature regime of the Black Sea connects closely with global Earth processes (Polonsky *et al.*, 2000).

Black Sea sprat condition depends also on local and regional factors, which strengthen or weaken climate influence. The Black sea ecosystem during the 1970-90s was under strong anthropogenic impacts. During the 1970-80s it was period of eutrophication (Sorokin, 2002; Zaitsev and Mamaev, 1997). At the beginning, eutrophication promoted to increasing of phytoplankton and mesozooplankton biomass, improving sprat food supply and as consequence increasing of sprat and anchovy stocks. The food supply for the period of eutrophication appeared also favourable for medusa *Aurelia aurita*, which was the strong competitor of fish for food. During 80s fluctuation of sprat lipid content observed which may explain by boom of medusa in the Black sea. Along with eutrophication, during the 1970s and especially the 1980s the Black Sea were strongly polluted because development of agriculture and heavy industry in the riparian countries. The eutrophication and pollution make Black Sea very vulnerable and at the end of 80s, it stressed again by comb jelly *Mnemiopsis* which arrival in the sea via ships' ballast. The food supply of small pelagic fish because predator pressure on mesozooplankton by *Mnemiopsis* worsened at the end of the 1980s. In the 1990s, after the economic crisis in former Soviet Union countries, the condition of sprat and the wider Black sea ecosystem improved.

Among anthropogenic factors, influencing on the Black sea ecosystem, overfishing is often considered the most important (Gucu, 2002). We subscribe to the opinion that short living small pelagic fish with great fecundity may recovered very quickly and the shutting down at least of 30% of fish total abundance could not affect on their stocks (Ivanov and Beverton, 1985) The above-mentioned ecological and anthropogenic factors should influence on fish population much stronger than overfishing.

Thus, Black Sea sprat condition depends on climate as well as regional ecosystem changes. For the long time (during 70s-middle of 80s), influence of both Global climatic and regional factors on sprat condition was synchronous and positive and then became negative (Fig. 5.36).

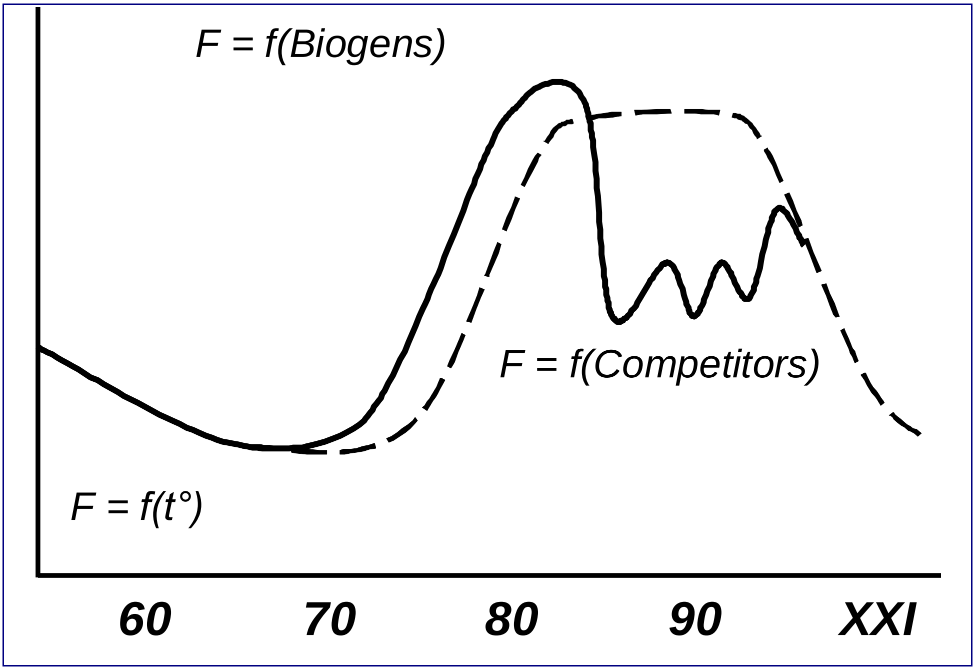

Figure 5.36. Scheme of long-term dynamics of sprat fat content: observed pattern (solid line) and probable trend caused by global climatic factors (dashed line)

Beginning in 1995, the Black Sea climate entered a warming cycle. SST increased at rate of 0.2°C per year. Due to warm winters last years, food supply of cold-water sprat apparently was getting worse and level of lipid accumulation continues to be low. From a fisheries perspective warming should negatively affect sprat stocks and catches as well.

Some words about anchovy. According to the lipid data, anchovy food supply in 2006 and 2007 was rather good and comparable with mean values for the eutrophication period during 1980s. We can suppose that climate warming during last decade does not influence significantly on warm-water anchovy and its condition rather affect local weather changes during feeding period.

Unfortunately there is no long-term data on energy (lipid) reserve in the body of small pelagic fish inhabit in other World Ocean basins where catches or stock values usually use for estimation fish condition. We compared these data with interannual dynamics of lipid content, stocks and catches of the Black Sea sprat and anchovy. There were similar trends between biomass and lipid changes of the Black Sea sprat and biomass of sprat from Adriatic Sea for the period 1976–1996 (Azzali *et al*., 2001). At the beginning all parameters increased (1970s-1980s), then significantly oscillated (1980s), declined (end of the 1980s – beginning of the 1990s), and recovered during mid-1990s. The Adriatic Sea anchovy (Bombace, 2001; Sinovcic, 2002) and anchovy *E. e. mediterraneus* from many Mediterranean basins (Arnery, 1996; Caddy and Oliver, 1994; Floentini *et al.*, 1997; Lleonart and Recasens, 1996; Lopez-Cazorla and Sanchez, 1986; Pertierra and Lleonart, 1992; Stergiou, 1988; Tudela, 1992) have shown the same tendency. Remarkably, the temperature (Caddy, 1990; Konovalov, 1995) and phytoplankton biomass (Marasovich *et al*., 1998; Vucetic, 1988) in the Adriatic sea changed at the same way as in the Black Sea. Moreover, the similar interannual stock and catch dynamics observed for many fish species from the seas of Atlantic Ocean such as Bengal *E. meridionalis* and South Africa anchovies *E. capensis* (Lluch-Belda *et al*., 1992), Iceland herring *Clupea harengus harengus* (Shelton *et al*., 1993), Iceland and Barents Sea capelin *Mallotus villosus villosus* (Bakken, 1983; Yakobsson, 1991). Additionally, the Californian (*E. mordax*, Lluch-Belda *et al*., 1992) and Peru (*E. ringens,* Klyashtorin, 2003) anchovies follow the same trends.

However, the catches of sardine *Sardina pilchardus* from the Mediterranean Sea (Bombace, 2001) and *S. sagax* from the Pacific Ocean (Klyashtorin, 2003) changed in opposite to the anchovy direction. Unexpectedly, there were no similarities in catch dynamics between

Black Sea sprat and sprats from Baltic (Grygiel and Wyszynski, 2003; Koster *et al*., 2003) and North Sea (FAO Fishery Statistics, 2007). Such asynchronous changes, most likely, related with different tolerance to temperature and peculiarity of feeding between species.

The analysis presented here is mainly qualitative and descriptive but they can suggest that climate played a crucial role in fluctuations Black Sea small pelagic fish stock and condition. The water temperature changes intensifying circulation influence on ecosystem productivity and thus food supply of small pelagic fish (Fig. 5.37). Namely, food supply governs the formation of fish stock. It controls also energy accumulation, maturation, spawning, elimination and survival both juveniles and adults (Shulman and Urdenko, 1989).

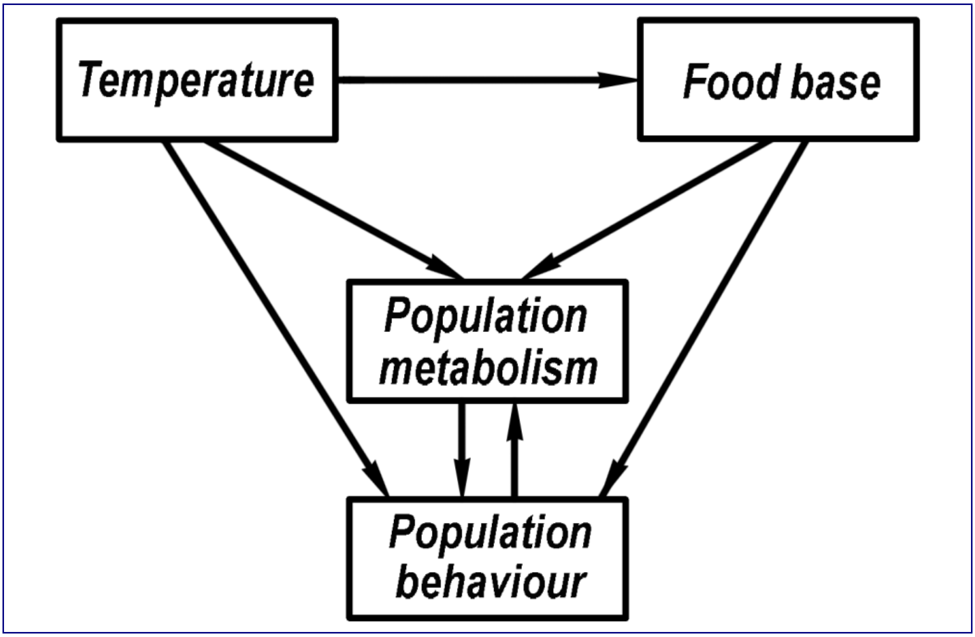

Figure 5.37. Influence temperature and food supply on small pelagic fish condition (after Shulman and Urdenko, 1989)

Examining of the long-term biotic and abiotic parameter changes during last four decades, investigators came to the conclusion that eutrophication influenced on the Black Sea ecosystem the most strongly

(Konovalov, 1995; Yunev *et al*., 2002; Mee *et al.*, 2005; Oguz and Gilbert, 2007). The eutrophication means increasing of primary production related with excess of biogenic riverine input into basin because of anthropogenic forcing.

According to dynamic of primary production parameters (phytoplankton biomass and chlorophyll-a concentration), which characterize eutrophication intensity in the Black Sea since 60s till present, the follow periods can be denoted: 1) pristine, 2) eutrophication, 3) ecological crises, 4) present state (recovery?) (Fig. 5.38). The changes of primary production influencing on all elements of food web, touched small pelagic fish. During eutrophication period, Black Sea anchovy and sprat catches changed synchronously with increasing of primary production characteristics. At the end of 80s on the background of strong eutrophication, bloom of ctenophore alien *Mnemiopsis* preyed on zooplankton, undermined fish food base. The catches decreased abruptly while phytoplankton biomass continues to increase and culminated at the beginning of the 1990s.

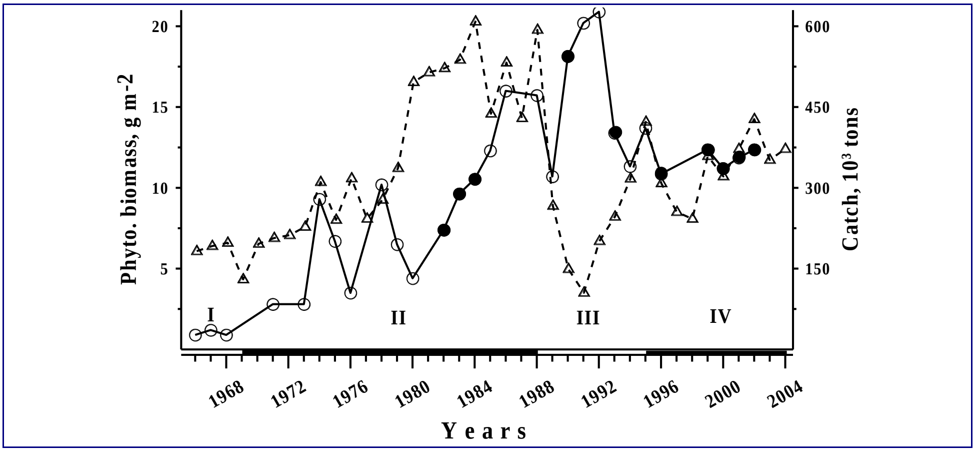

Figure 5.38. Long-term changes of phytoplankton biomass (g m^{-2}) (white circles), phytoplankton biomass recalculated through chlorophyll-*a* concentration (black circles), and annual catches of small pelagic fish (in 10^3 tons) (triangles) in the Black sea (after Mikaelyan, 1997; Yunev *et al*., 2002; FAO Fishery Statistics, 2007). The Roman numerals indicate periods: I - pristine, II - eutrophication, III - ecological crises, V - present state (recovery?)

Ecosystem crises that period may illustrate high nitrate, low silicate concentrations in the water, blooms of small algae with low trophic value and even harmful, hypoxia increasing not only in shelf but also in open sea, *Mnemiopsis* bloom, deterioration of food base of small pelagic fish and catastrophic decrease of fish biomass and catches (Kideys, 2002; Mee *et al*., 2005). During the crises synchronous observed earlier between changes of primary production characteristics and fish catches was broken.

Two-factor influence on declining of eutrophication and increasing of catches at the middle of 90s: deterioration of biogenic input because of industrial crises in Former Soviet Union countries and decreasing of Mnemiopsis abundance because of natural stabilisation. The phytoplankton biomass decreased up to level of 1982-88 and catches increased up level of 1974-1981. During present period ecosystem apparently became more stable than earlier at least small pelagic fish and phytoplankton became changed synchronously. Nevertheless, the level of small pelagic catches does not meet high trophic ecosystem level.

It is possible to assume that factor limited recovery of the small pelagic stock to maximal levels became not food quantity but it quality. Due to enduring eutrophication and ecological crises, the Black Sea pelagic ecosystem has very changed biochemical status (Yunev *et al*., 2002): high relationship between N/Si prevented to development of diatoms during the most part of the year and fish food web inverted with prevailing the small size phyto- and zooplankton species vs. diatom/copepod species. The changes of size spectrum of food items may be reason that fish food supply became much worse and it catches decline in spite of high ecosystem trophic level.

Problematically that pelagic Black Sea ecosystem could sometime to recover to the low productivity status of the quasi-pristine 1960s period. The Black sea ecosystem is eutrophicated strongly still and unstable, which do it convenient target for damaging influence of climate changes and impact of new aliens. In case of overcome of economical crises in Black Sea riparian countries one can suppose increase of biogenic riverine inflow and new wave of eutrophication with evident for ecosystem consequences.

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References

Ackman, R. G., 1980. Fish lipids. Part I. *Adv. Fish. Sci. and Technol. Pap. Jubilee*, 86-103.

Ackman, R. G., 1986. Marine lipids, fats and oils. In: Hamilton, R. J. and Rossel, J. B. (eds). Analysis of Oils and Fats. Elsevier, London, p. 137-206.

Afifi, A. A., Azen, S. P., 1979. Statistical analysis. A computer oriented approach. Academic Press, New York, San Francisco, London (translated into Russian).

Alheit, J., 2007. Teleconnection patterns of impact of climate variability on pelagic ecosystems across Europe. In “42nd European Marine Biology Symposium”, Kiel 27 – 31 august 2007. Inter Research Science Center, 10.

Arnery, E., 1996. Fisheries resources assessment and management in the Adriatic and Ionian seas. *FAO Fish. Rep*. 533 (Suppl), 7-20.

Arts, M. T., Ackman, R., Holub, B. J., 2001. “Essential fatty acids” in aquatic ecosystems: a crucial link between diet and human health and evolution. *Can. J. Aquat. Sci*. 58, 122-137.

Aslanova, N. E., 1954. The sprat of the Black Sea. *Tr. VNIRO*, 28, 75–101 (in Russian).

Avşar, D., Bingel, F. A. 1994. Preliminary study on the reproductive biology of the sprat (*Sprattus sprattus phalericus* (Risso, 1826)) in Turkish waters of the Black Sea. *Tr. J. of Zoology*, 18, 77–85.

Azzali, M., Cosini, G., Luna, M., 2001. Fluctuations in space and time of pelagic populations in the Adriatic Sea from 1976 to 1996. *Arch. Oceanogr. Limnol*. 22, 127–132.

Bakken, E., 1983. Recent history of Atlanto-Scandinavian herring stocks. *FAO Fish. Rep*. 291, 521-536.

Bat, L., Ivanova, P., Dobrovolov, Shulman, G. E., Nikolsky, V. N., Yuneva, T. V. Shchepkina, A. M., Kideys, A. E., 2007. The population structure of the Black Sea anchovy. *Aquaculture & Fisheries,* 2(8), 51-56.

Bell, M. V., Henderson, R. J., Sargent, J. R., 1986. The role of polyunsaturated fatty acids in fish. *Comp. Biochem. Physiol*. 83B(4), 711-719.

Bell, M. V., Sargent, J. R., 1996. Lipid nutrition and fish recruitment. *Mar. Ecol. Prog. Ser*. 134, 315-316

Bergeron, J., 2000. Effect of strong winds on the nutritional condition of anchovy (Engraulis encrasicolus) larvae in the Bay of Biscay, Northeast Atlantic, as inferred from an early field application of the DNA/RNA index. *ICES J. Mar. Science* 57(2), 249-255.

Boichenko, L., 2005. Major shifts in the phytoplankton community of the Romanian Black Sea waters. In: Velikova V., Chipev N. (eds.) Large-scale disturbances (regime shift) and recovery in aquatic ecosystems: challenges for management towards sustainability. UNESCO-ROSTE/BAS Workshop on Regime Shifts, 14–16 June. – Varna, (Power Point Presentation on CD).

Bryantseva, Yu. V., Bryantsev, V. A., Kovalchuk, L. A., Samyshev, E. Z., 1996. For the question of long-term changes of biomass in diatom and peridinien algae of the Black sea in relation with atmospheric activity. *Ekologiya Morya*, 45, 13-18 (in Russian).

Bulow, F. J., Zeman, M. E., Winningham, J. R., Kuddson, W. F., 1981. Seasonal variations in RNA-DNA ratios and in indicators of feeding, reproduction, energy storage and condition in a population of bluegill. *J. Fish Biology* 18, 237-244.

Caddy, J. F., Griffiths, R. C., 1990. A perspective on recent fishery-related events in the Black Sea. In: Recent trends in the fisheries and environment in the general fisheries for the Mediterranean (GFCM) area. *GFCM Stud. Rev*., 63, 43-71.

Caddy, J. F., Oliver, P. 1994. Some future perspectives for assessment and management of Mediterranean fisheries for demersal and shellfish resources, and small pelagic fish. In: Resource and environmental issues relevant to Mediterranean fisheries management. J. F. Caddy (ed.). *GFCM Stud. Rev*. 66, 19-60.

Chashchin, A. K., 1998. The anchovy and other pelagic fish stocks transformations in the Azov-Black Sea basin under environmental and fisheries impact. In: The Proceedings of the First International Symposium on Fisheries and Ecology. M. S. Celiikkale, E. Duzgunez, I. Okumus, C. Mutlu (eds.). 2 – 4 Sept. 1988, Trabzon, Turkey, 1-10.

Chashchin, A. K., Axelev O. I., 1990. Migrations of the stocks and availability of Black Sea anchovy to the fishery to the autumn and winter. In: Biological Resources of the Black Sea. V. A. Shlyakhov (ed.). VNIRO, Moscow, p. 80-93. (in Russian).

Chayanova, L. A., 1958. Nutrition of the Black Sea sprat. *Tr. VNIRO*, 36, 106-127. (in Russian).

Clemmensen, C., Sanchez, R., Wongtschowski, C., 1997. A regional comparison of the nutritional condition of SW Atlantic anchovy larvae, *Engraulis anchoita*, based on RNA / DNA ratios. *Arch. Fish. Meeresforsch*. 45 (1), 17-43.

Daskalov, G. M., 2002. Overfishing drives a trophic cascade in the Black Sea. *Mar. Ecol. Prog. Ser*. 225, 53-63.

Daskalov, G. M., 2003. Long-term changes in fish abundance and environmental indices in the Black Sea. *Mar. Ecol. Prog. Ser.*, 255, 259-270.

Dekhik, T. V., 1973 The ichthyoplankton of the Black Sea. Kiev, Naukova dumka, 242 pp. (in Russian).

Drazen, J. C. 2002. A seasonal analysis of the nutritional condition of deep-sea Macrourid fishes in North-East Pacific. *J. Fish Biol.* 60 (5), 1280-1295.

Dutil, J., Lambert, Y., Chabot, D., 2003. Winter and spring changes in condition factor and energy reserves of wild cod compared with changes observed during food deprivation in the laboratory. *ICES J. Mar. Sci*. 60 (4), 780-786.

Eremeev, V.N., Efimov, V.V., 2003. Regional aspects of global climate change. Visnik NAN Ukrainy. N2, 14-19 (in Ukrainian).

FAO, 2007. Fishery statistics. Capture Production 1950 – 2005. Downloadable at: http:// www.fao.org.

Farkas, T., Fodor, E., Kitajka, K., Halver, J.E., 2001. Response of fish membranes to environmental temperature. *Aquac. Res*. 32, 645-655.

Finenko, G. A., Romanova, Z. A., Abolmasova, G. I., Anninsky, B. E., 2006. Trophic relations in Black sea plankton community at present. *Ekologiya Morya*. 71, 50 – 54 (in Russian).

Fiorentini, L., Caddy, J. F., de Leiva, J. I., 1997. Long- and short-term trends of Mediterranean fishery resources. *GFCM Stud. Rev*. 69, 1–14.

Folch, J., Lees, M., Sloane-Stanley, G. H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. 226, 497–509.

Fraser, A. J., Gamble, J. C., Sargent, J. R., 1988. Changes in lipid content, lipid class composition of developing eggs and unfed larvae of cod (*Gadus morhua*). *Mar. Biol*. 89, 307-313.

Garcia-Franco, W., Vidal-Talamantes, R., Cota-Villavicencio, A., Ramon-Corrales, J., Prieto-Montalvo, J., 1999. Variation in fat content of northern anchovy (*Engraulis mordex*) in relation to the Baja California environment. *Reports of California Cooperative Oceanic Fisheries Investigations* [CalCOFJ Rep]. 40, 165 – 169.

Gershanovich, A. D., Markevich, N. B., Dergaleva, Z. T., 1984. On the employment of condition factor in ichthyological studies *Voprosy ichthyologii* 24, 740-752 (in Russian).

Glushchenko, T. I., Sorokolit, L. K., Negoda, S. A., 2005. Condition for feeding of the Black Sea sprat in the main regions of summer fishing in modern period. *Ribnoje khozjajstvo*. 3, 6-8 (in Russian).

Golenchenko, A. P., 1940. Sprat in the Black Sea. Fish industry (Rybnoe khozaystvo), Moscow, 6, 14–16 (in Russian).

Gorbunova, N. N., 1958. Reproduction and development (maturity) of Black Sea sprat *Sprattus sprattus phalericus* (Risso). *Tr. Seavastop. biol. stn*., 10, 108-126 (in Russian).

Grishin, A., Daskalov, G., Shlyakhov, V., Mihneva, V., 2007. Influence of gelatinous zooplankton on fish stocks in the Black Sea: analysis of biological time series. *Morsk. Ecol. Zhurn*. 6 (2), 5-24.

Grygiel, W., Wyszynski, M., 2003. Temporal (1980–2001) and geographic variation in the sexual maturity at age and length of herring and sprat inhabiting the southern Baltic. *Bull. Sea Fish. Inst*. 2 (159), 3-33.

Guisande, C., Riveiro, J., Sola, A., Valdes, L., 1998. Effect of biotic and abiotic factors on the biochemical composition of wild eggs and larvae of several fish species. *Mar. Ecol. Progr. Ser.* 163, 53 – 61.

Gwak, W. S., Tsusaki, T., Tanaka, M., 2003. Nutritional condition, as evaluated by RNA/DNA ratios, of hatchery-reared Japanese flounder from hatch to release. *Aquaculture*. 219 (1-4), 503-514.

Hakanson, J. L., 1989a. Analysis of lipid components for determining the condition of anchovy larvae *Engraulis mordax*. *Mar. Biol*. 102, 143-151.

Hakanson, J. L., 1989b. Condition of larval anchovy (*Engraulis mordax*) in the Southern California Bight, as measured through lipid analysis. *Mar. Biol*. 102, 153-160.

Hakanson, J. L., 1993. Nutritional condition and growth rate of anchovy larvae (*Engraulis mordax*) in the California Current: two contrasting years. *Mar. Biol*. 115 (2), 309-316.

Hakanson, J. L., Coombs, S. H., 1994. Lipid and elemental composition of sprat (*Sprattus sprattus*) larvae at mixed and stratified sites in the German Bight of the North Sea. *ICES J. Mar. Sci*. 51, 147-154.

Hazel, J. R., Williams, E. E., 1990. The role of alterations membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog. Lipid Res.* 29, 167-227.

Hubareva, E., Svetlichny, L., Kideys, A., Isinibilir, M., 2008. Fate of the Black Sea *Acartia clausi* and Acartia tonsa (Copepoda) penetrating into the Marmara Sea through the Bosphorus. *Estuarine Coastal and Shelf Science*. 76, 131-140.

Hunter, J. R., Leong, R., 1981. The spawning energetic of female northern anchovy *Engraulis mordax*. *Fish. Bull. U. S.*, 79, 215-230.

Iles, T. D., Wood, R. J., 1965. The fat/water relationship in North Sea herring (*Clupea harengus*) and its possible significance. J. *Mar. Biol. Ass. UK.*, 45 (2), 353-366.

Ivanov, L. S., Beverton, R. J. H., 1985. The fisheries resources of the Mediterranean. Part 2: Black Sea. *GFCM Stud. Rev.*, 60, 1-135.

Ivanova P. P., Dobrovolov I. S., 2006. Population – genetic structure on European anchovy (*Engraulis encrasicolus*, Linnaeus, 1758) (Osteichthyes: Engraulide) from Mediterranean Basin and Atlantic Ocean. *Acta Adriatica*, 47 (1), 13-22.

Ivlev, V. S., 1955. Experimental ecology of fish nutrition (In Russian). Pishchepromizdat, Moscow, 252 pp. [English translation 1961 "Experimental ecology of the feeding of fishes". Yale University Press, Newhaven, Connecticut].

Jones, P. D., Jonsson, T., Wheeler, D., 1997. Extension to the North Atlantic Oscillation using early instrumental pressure observations from Gibraltar and South-West Iceland. *Int. J. Climatol*. 17, 1433-1450.

Jonsson, J., 1996. Tagging of cod (*Gadus morhua*) in Icelandic waters 1948-1986. Rit Fiskideildar / *Journal of the Marine Research Institute, Reykjavik*, 14 (1), 82-84.

Kalnin, V. V., Kalnina, O. V., 1985. Genetic differentiation and reproductive relationship between the Black Sea race and Azov Sea race of European anchovy. 2. Genetic differences between the Black Sea race and the Azov Sea race and their intrarace heterogeny. *Genetics*, 20, 309-313 (in Russian).

Karzinkin, G. S., 1952. The principles of biological productivity of water bodies. Pishchepromizdat, Moscow, 372 pp. (In Russian).

Kates, M., 1975. *Techniques of lipidology*. Mir Publishing, Moscow (translated into Russian), 312 pp.

Kideys, A. E., 2002. Fall and rise of the Black Sea ecosystem. *Science*, 297, 1482 – 1484.

Kideys, A. E., Roohi, A., Bagneri, S., Finenko, G., Kamburska, L., 2005. Impacts of Invasive ctenophores on the fisheries of the Black Sea and Caspian Sea. *Oceanography*. 18 (2), 76-85.

Klyashtorin, L. B., 2003. Climate change and long-term fluctuations of commercial catches. The possibility of forecasting. *FAO Fish Tech. Pap*. 410, 1-86

Konovalov, S. M., 1995. Anthropogenic impact and ecosystems of the Black Sea. *Bull. Inst. Oceanogr*. 15, 53–82.

Kovalev A.V., Gubanova A.D., Kideys A.E., Melnikov V.V., Nierman U., Ostrovskaya N.A., Prusova I.Yu., Skryabin V.A., Uysal Z., Zagorodnyaya J., 1998. Long-term changes in the biomass and composition of fodder zooplankton in coastal regions of the Black Sea during the period 1957–1996. *In*: Ivanov L.I., Oguz T. (eds). Ecosystem Modelling as a Management Tool for the Black Sea. Klüwer Academic Publishers. Dordrecht/Boston/London. 1, 209–221.

Kreps, E. M., 1981. Lipids of cell membranes. Nauka, Leningrad, 339 pp. (In Russian).

Laevastu, T., 1993. Marine climate, weather and fisheries. Halsted Press, New York.

Lalli, C. M., Parsons, T. R., 1997. Biological oceanography. An introduction. 2nd edn. Oxford: Butterworth & Heinemann.

Lambert, Y., Dutil, J.-D., Onelet, P., 2000. Nutritional condition and reproductive success in wild fish populations. *Reproductive Physiology of Fish*, 77-84.

Lasker, R., 1970. Utilization of zooplankton energy by a Pacific sardine population in the California Current, p. 265-284. In: Marine Food Chains. J. H. Steele (ed.). Edinburgh.

Lasker, R., Theilacker, G. H., 1962. The fatty acid composition of the lipids of some Pacific sardine tissues in relation to ovarian maturation and diet. *J. Lipid Res.* 3, 60-64.

Last J. M., 1987. The food of immature sprat (*Sprattus sprattus* L.) and herring (*Clupea harengus* L.) in coastal waters of the North Sea. *ICES Journal of Marine Science*. 44 (1), 73-79.

Lee, R. F., Hagen, W., Kattner, G., 2006. Lipid storage in marine zooplankton. *Mar. Ecol. Prog. Ser*. 307, 273-306.

Levi, F., Bontonte, M., Mayzaud, P., 2005. Lipid composition of Symphodus ocellatus (Perciformes: Labridae) in the North-Western Mediterranean: Influence of two different biotopes. *Mar. Biol*, 146 (4), 805-814.

Lipskaya, N. Ya., 1960. On the diurnal and seasonal cycles of the Black Sea sprat (*Sprattus sprattus phalericus* (Risso)) nutrition. *Tr. Seavastop. Biol. Stn*., 13, 190-203 (in Russian).

Lleonart, J., Recasens, L., 1996. Fisheries and environment in the Mediterranean Sea. Caddy, J. F. (ed.) Resource and environmental issues relevant to Mediterranean fisheries management. *GFCM Stud. Rev*. 66, 5 – 18.

Lloret, J., Galzin, R., Gild, de Sola, A., Souplet, A., Demestre, M., 2005. Habitat related differences in lipid reserves of some exploited fish species in the north-western Mediterranean continental shelf. *J. Fish Biol*., 67, 51-65.

Lluch-Belda, D., Schwartzlose, R. A., Serra, R. *et al*., 1992. Sardine and anchovy regime fluctuations of abundance in four regions of the world oceans: a workshop report. *Fish Oceanogr.* 1, 339 – 347.

Lochman, S. E., Maillet, G. L., Frank, K. T., Taggart, C. T., 1995. Lipid class composition as a measure of nutritional condition in individual larvae Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquatic. Sci*. 52 (6), 1294 – 1306.

Lopez-Cazorla, A., Sanchez, P. E., 1986. Evaluacion del stock de la anchoa, *Engraulis* (L), de la pesqeria de Cataluna, a partir de datos de frecuencias de tallas. *Bol. Inst. Esp. Oceanogr*. 3, 67 – 72.

Love, R. M., 1970. The chemical biology of fishes. Vol. 1. Academic Press, London, New York, 547 pp.

Love, R. M., 1980. The chemical biology of fishes. Vol. 2. Academic Press, London, New York, 943 pp.

Lovern, J.A., 1964. The lipids of marine organisms. *Oceanography and Marine Biology, Annual Review*, 2, 169 – 191.

Marasovich, I., Pucher-Petkovic, T., Hernansez, A., 1998. Phytoplankton productivity of the Adriatic Sea in relation to pelagic fisheries. *Biljeske-Notes*. 72, 1 – 8.

Marshall, C. T., Yaragina, N. A., Lambert, Y., Kjesbu, O., 1999. Total lipid energy as a proxy for total egg production by fish stocks. *Nature*, 402, 288 – 290.

Masuda, R., 2003.The critical role of docosahexaenoic acid in marine and terrestrial ecosystems from bacteria to human behavior.. In: H. J. Browman and A. D. Skittesvik (eds). The big fish bang. Proceeding of the 26th annual larval fish conference. Norway: Publ. by Institute of Marine Research, p. 249-256

Mee, L. D., Friedrich, J., Gomoiu, M-T., 2005. Restoring the Black Sea in times of uncertainty. *Oceanography*, 18 (2), 100-111.

Mikaelyan, A. S., 1997. Long-term variability of phytoplankton communities in the open Black Sea in relation to environmental changes. pp 105–116 In: Ozsoy, E., Mikaelyan, A. (eds) Sensitivity to change: Black Sea, Baltic Sea and North Sea. Kluwer Academic Publishers, Dordrecht,.

Minuyk, G. S., Shulman, G. E., Shchepkin, V. Ya., Yuneva, T. V., 1997. Black Sea sprat (relation of lipid dynamics with biology and fisheries). *Sevastopol, Ekosi-Hydrophysika*, 139 pp. (in Russian).

Morris, R. J., Culkin, F. 2000. Fish. In: Marine biogenic lipids, fats and oils. R. G. Ackman (ed.). CRC Press, Boca Raton, p. 850-900

Nakano, H.Y., Ando, Y., Shirata, S. 1985. Changes of acid phosphatase, total protein, DNA and RNA during the early development of chum salmon (*Oncorhynchus keta*). *Bulletin of the Hokkaido Regional Fisheries Research Laboratory* 50, 71-77.

Navarro, J. C., Bell, H. V., Amat, F., Sargent, J. R., 1992. The fatty acid composition of phospholipids from brine shrimp *Artemia* sp. eyes. *Comp. Biochem. Physiol*. 103B, 89-81.

Nikolsky, G. V., 1963. The ecology of fishes (L. Birkett, transl.), Academic Press, London, New York, 352 pp.

Nikolsky, G. V., 1965. Theory of fish stock dynamics (in Russian). *Nauka*, Moscow, 382 pp.

Nikolsky, V. N. 2005. Statistical analysis of long-term variability of the Black sea sprat fatness. *Science letters (Naukovi zapiski) ser. Biology*. 4 (27), 164-166. (in Russian).

Nikolsky, V. N., Shulman, G. E., 2005. The sprat fat content variability in connection with long-term environmental changes in the Black Sea. In: Velikova, V., Chipev, N. (Eds.) Large-scale disturbances (regime shift) and recovery in aquatic ecosystems: challenges for management towards sustainability. UNESCO-ROSTE/BAS Workshop on Regime Shifts, 14–16 June, Varna, 159-168.

Nikolsky, V. N., Shulman, G. E., Yuneva, T. V., Shchepkina, A. M., Ivleva, E. V., Bat, L., Kideys, A., 2007. On the present condition of food supply of the Black Sea sprat. *Doklady Akademii Nauk Ukrainy*, 5, 194-198 (in Russian).

Oguz, T., Dippner, W., Kaymaz, Z., 2006. Climatic regulation of the Black Sea hydro-meteorological and ecological properties at interannual-to-decadal time scales. *J. Mar. Systems* 60, 235–254.

Orlova, E. L., Dolgov, A. V., Nesterova, V. N., Antsiferov, M. Yu., Konstantinova, L. L., 2000. Peculiarities of feeding behavior in Arcto – Norwegian cod in the Southern Barents when major objects are in deficiency. Counc. Meet. Bruges (Belgium), 27 – 30 Sep. 2000. ICES, Copenhagen (Denmark), 18 pp.

Orlova, E. L., Seliverstova, E. J., Dolgov, A. V., Nesterova, V. N., 1999. Herring abundance, food supply, and distribution in the Barents Sea and their availability for cod. Lowell Wakefield Fish. Symp. Series, N18. Herring Expectations for a New Millennium, 91 – 99.

Orlova, E. L., Ushakov, N. G., Nesterova, V. N., Boitsov V. D., 2002. Food supply and feeding of capelin (*Mallotus villosus*) of different size in the central latitudinal zone of the Barents Sea during intermediate and warm years. *ICES J. Mar. Sci*., 59 (5), 968 - 980.

Osborn, T. J., 2006. Recent variations in the winter North Atlantic Oscillation. *Weather* 61, 353-355.

Parrish, C. C., Abrajano, T. A., Budge, S. M., Helleur, R. J., Hudson, E.D., *et al*., 2000. Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In: P. Wangersky (ed). The Handbook of Environment Chemistry. 5, part D. *Marine Chemistry*, p. 193-223

Pavlovskaya, R. M., 1952. On reproduction of Black Sea sprat *Sprattus sprattus phalericus* (Risso*). Reports (Dokladi) Acad. Sci. USSR*, 82 (1), 165–166.

Pertierra, J. P., Lleonart, J., 1992. A study of the population dynamics of the Northwestern Mediterranean anchovy using LCA (length cohort analysis). *Rapp. PV Reun*. 33, 252 pp.

Polonsky, A. B., Voskresenskaya, E. N., Basharin, D. V., 2000. On the ocean influence on temperature variability of European and Mediterranean regions. *Marine hydrophysical journal*. 5, 30–45 (in Russian).

Pravdin, M. F., 1966. The Handbook of Ichthyology. Pischevaja promishlennost Publishing, Moscow (in Russian).

Prodanov, K., Mikhailov, K., Maxim, K., Chashchin., A., Arkhipov, A., Shlyakhov, V., Ozdamar E., 1997. Environmental management in the Black Sea and their rational exploitation. *GFCM Stud. Rev*., 68 FAO, Rome, 178 pp.

Rabinovich, A. L. A. Ripatti, P. O., 1991. On the conformational properties and functions of polyunsaturated acyl chains. *Biochem. et Biophys. Acta*. 1085, 53-62.

Rainuzzo, J. R., Reitan, K. I., Olsen, Yu. 1997. The significance of lipids at early stages of marine fish: a review. *Aquaculture*. 155, 103-115.

Sargent, J. R., 1976. The structure, metabolism and function of lipids in marine organisms. In: Malins, D. C., Sargent, J. R. (eds).Biochemical and biophysical perspectives in marine biology. Vol. 3. Academic Press, London, p. 149-212.

Sargent, J.R., 1995. Origins and functions of egg lipids: Nutritional implications. In: Bromage, N.R., Roberts, R.J. (eds.), Broodstock Management and Egg and Larval Quality. Blackwell, Oxford, p. 353-372.

Sargent, J. R., Henderson, R. J., 1986. Lipids. In: Coener, E., O'hara C. M. (eds.). The biological chemistry of marine copepods. Clarendon, Oxford, p. 59-108.

Sargent, J. R., Henderson, R. J., Tocher, D. R. 1989. The lipids. Chap. 4,. In J.E. Halver (ed.). Fish nutrition. Academic Press, Washington, D.C., pp. 153-218

Sargent, J. R., Parkes R. J., Mueller-Harvey, J. and Henderson, R. J., 1988. Lipid biomarkers in marine ecology. In: Sleigh, M. A. (ed.). Microbes in the sea. Ellis Horwood Ltd., Chichester, U.K., p. 119-138.

Sargent, J. R., Bell, J. G., Bell, M. V., Henderson, J., Tocher, D. R., 1993. The metabolism of phospholipids and polyunsaturated fatty acids in fish.. In: Lahlou, B., Vittiello, P. (eds.). Aquaculture: Fundamental and Applied Research, Coastal and Estuarine Studies 43. American Geophysical Union, Washington, D.C., pp.103-124

Sargent, J. R., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*. 177, 191–199.

Sargent, J. R., Tocher, D. R., Bell, J. G., 2002. The lipids.. In Halver, J.E., Hardy, R. W. (eds.), Fish Nutrition. Academic Press, New York., pp. 181-257

Sazhina, L. I. 1987. Reproduction, growth, productivity of marine copepods. Naukova Dumka, Kiev 156 p. (in Russian)

Shatunovsky, M. I., 1980. Ecological peculiarity of marine fish metabolism. Science, Moscow, 288 p. (in Russian)

Shelton, P. A., Armstrong, M. J., Rool, B. A. 1993. An overview of the application of the daily egg production method in the assessment and management of anchovy in the Southern Atlantic. *Bull. Mar. Sci*. 53, 778 – 794.

Shulman, G. E. 1960. Dynamics of fat content of the fish body *Uspechi sovremennoy Biologii*, 49, 225-239 (in Russian).

Shulman, G. E., 1963. Determination of fish food supply by intensity of fat accumulation and fat stores level in their body. *Zoologicheskiy zhurnal*, 42 (4), 581-588 (in Russian).

Shulman, G. E., 1974. Life cycles of fish. Physiology and biochemistry. Hulsted Press, John Wiley and Sons, New York, 253 pp.

Shulman, G. E., 2002. Anchovies of the Sea of Azov and the Black Sea: regularities of wintering migrations (brief review). *Morskoy Ekologicheskiy Zhurnal*, 1 (1), 67-77.

Shulman, G. E., Chashchin, A. K., Minyuk, G. S., Shchepkin, V. Ya., Nikolsky, V. N., Dobrovolov, I. S., Dobrovolova, S. G., Zhigunenko, A. S., 1994. Longterm monitoring of Black Sea sprat condition *Doklady Akademii Nauk* 335 (1), 124 – 126 (in Russian).

Shulman, G. E., Dobrovolov, I. S., 1979. The state of affairs in eco-physiological studies of Black Sea fishes pp. 321 – 340. In: The foundation of biological productivity of the Black Sea. V. N. Greze (ed.). Naukova dumka, Kiev (in Russian).

Shulman, G. E., Love, R. M., 1999. The biochemical ecology of marine fishes. *Adv. Mar. Biol.,*.36, Academic Press, London, 352 pp.

Shulman, G. E., Nikolsky, V. N., Yuneva, T. V., Minyuk, G. S., Shchepkin, V. Ya., Shepkina , A. M., Ivleva, E. V., Yunev, O. A., Dobrovolov, I. S., Bingel, F., Kideys, A. E., 2005. Fat content in Black Sea sprat as an indicator of fish food supply and ecosystem condition. *Mar. Ecol. Prog. Ser*., 293, 201-212.

Shulman, G. E., Shchepkina, A. M., 1983. Comparative evaluation of fish condition by morphological and physiological–biochemical parameters. *Rybnoe Khozyaistvo* 4, 25-27 (in Russian).

Shulman, G. E., Urdenko, S. Yu. 1989. Productivity of fishes of the Black Sea. Naukova Dumka, Kiev (in Russian).

Shulman, G. E., Yuneva, T. V., 1990. Docosahexaenoic acid and fish adaptation (review). *Hydrobiology*. 26, 43-51 (in Russian).

Sidorov, V.S., 1983. Ecological biochemistry of fish lipids. Nauka, Leningrad, 240 pp. (in Russian).

Silverstein, J.T.,Shearer, K.D., Dickhoff, W.W., Plisetskaya, E. M. 1998. Effects of growth and fatness on sexual development of Chinook salmon (*O. tshawytscha*) parr. *Can. J. Fisheries and Aquatic Sciences* 55, 2376-2382.

Sinovĉiĉ, G., 2000. Anchovy, *E. encrasicolus*: biology, population dynamics and fisheries case study. *Acta Adriatica*. 41, 3-53.

Sirotenko, M. D., Sorokalit, L. K., 1979. Seasonal changes of the Black sea sprat feeding. *Voprosi ichtiologii*. 19, 813-818 (in Russian).

Smith, T. R., Buckley, L. J., 2003. RNA-DNA ratio in scales from juvenile cod provides a nonlethal measure of feeding condition. *Trans. Am. Fish. Soc*., 132 (1), 9-17.

Sogard, S.M., Olla, B.L., 2000. Endurance of simulated winter conditions by age – 0 walleye pollock: effects of body size, water temperature and energy stores. *J. Fish. Biol*., 56, 1-21.

Sorokin, Yu. I., 2002. The Black Sea. Ecology and oceanography. Backhuys Publisher, Leiden, 875 pp.

Stergiou, K. I., 1988. Multivariate analysis of the commercial fishery of the Northwestern Coast of Greece, 1984–1991. *Acta Adriatica*. 29, 161-170.

Stoyanov, S. A. 1953. The Black Sea sprat *Sprattus sprattus sulina* (Antipa). *Tr. Inst. Zool*., 3. Varna, 90 pp. (in Bulgarian).

Svetovidov, A. N., 1964. The fishes of the Black Sea. Nauka, Moscow, Leningrad, 552 pp. (in Russian),

Thorpe, J. E., Talbot, C, Villarual, C., 1982. Bimodality of growth and smolting in Atlantic salmon. *Aquaculture*, 28, 123-132.

Trigari, G., Pirini, M., Ventrella, V., Pagliarani, A., TROMBETTY, F., BORGATTI, A.R., 1992. Lipid composition and mitochondrial respiration in warm- and cold-acclimated sea bass (Dicentrarchus labrax L.). Lipids. 27, 371-377.

Tudela, S., 1992. Assessment of the ecological footprint of fishing in the Catalon central coast (NE Spain). CIESM Workshop ser. Bull. Comm. *Int. Explor. Sci. Mer. Mediter*. 12, 79-82.

Varnavsky, V. S., Varnavskaya, N. V., Kalinin, S. V., Kinas, N. M., 1991. RNA/DNA index as a characteristic of growth rate during the early marine period of life in coho salmon (In Russian). *Voprosy Ichthiologii* 31, 783-789.

Velikova, V., Mihneva, V., 2005. High and low energy ecosystem structure in terms of regime shifts: examples from the western Black Sea. pp 43 – 57. In: Velikova V., Chipev N. (Eds.) Large-scale disturbances (regime shift) and recovery in aquatic ecosystems: challenges for management towards sustainability. UNESCO-ROSTE/BAS Workshop on Regime Shifts, 14–16 June, Varna,

Ventrella, V., Pagliarani, A., Pirini, M., Trigari, G., Trombetty, F., Borgatti, A. R., 1993. Lipid composition and mitochondrial ATPase activities in gills and kidneys of warm- and cold-acclimated sea bass (*Dicentrarchus labrax* L.). *Fish Physiol. Biochem*. 12, 293-304.

Vinberg, G. G., 1956. The Intensity of metabolism and food requirements of fish. Belorussian University Press, Minsk, 253 pp. (in Russian).

Vinogradov, M. E., Sapozhnikov, V. V., Shushkina, E. A., 1992. Ecosystem of the Black Sea Nauka, Moscow, 112 pp. (in Russian).

Vucetic, T.,1988. Long-term (1960–1983) fluctuations of zooplankton biomass in the Palagruze-Gargano area. FAO Fish. Rep. 394. FAO, Rome.

Watanabe, T., 1993. Importance of docosahexaenoic acid in marine larval fish. *J. World Aquacul*. 24, 152-161.

Watanabe, T., Takeuchi, T., Shimma, Y., 1978. Influence of SCP feed on lipid contents and fatty acid composition of rainbow trout. *Bull. Freshw. Fish Res. Lab*. 28, 37-46.

Yakobsson, Y., 1991. Recent variability in fisheries of the North Atlantic. ICES, 21 Variability Symp.: 1 – 12.

Yaragina, N. A., Marshall, C. T., 2000. Trophic influences on interannual and seasonal variation in the liver condition index of Northeast Arctic cod (*Gadus morhua*). *ICES J. Mar. Sci.,* 57 (1), 42-52.

Yunev, O. A., Vedernikov, V. I., Basturk, O. *et al*., 2002 Long-term variations of surface chlorophyll-a and primary production in the open Black Sea. *Mar. Ecol. Progress Ser*. 230, 11–28.

Yuneva T. V., 1990. Seasonal dynamic of the fatty acid content in the Black Sea anchovy and sprat. pp 196-206. In: Shulman, G. E., and Finenko, G. A. (eds.). Bioenergetics of hydrobionts. Naukova Dumka, Kiev.

Yuneva, T. V., Shchepkina, A. M. 2005. Fatty acid as trophic indicator of the Black Sea copepod *Acartia clausi* and *Calanus euxinus*. *Scientific notes (Naukovi zapiski). Ser. Biology*. 4 (27), 280-282 (in Russian).

Yuneva, T. V., Shulman, G. E., Chebanov, N. A., Shchepkina, A. M., Vilenslaya, N. I., Markevich, N. B., 1990. Relation between content of docosahexaenoic acid in parents with survival eggs and larvae of hunchback salmon. *Biol. Sci*., 10, 85-89 (in Russian).

Yuneva, T. V., Svetlichny, L. S., Shchepkina, A. M., 1998. Lipid content and moving activity of *Calanus euxinus* (Copepods) diapausing ecogroup. *Hydrobiol. Journal*. 34, 74-85 (in Russian).

Yuneva, T. V., Svetlichny, L. S., Yunev, O. A., Romanova, Z. A., Kideys, A. E., Bingel, F., Yilmaz, A., Uysal, Z., Shulman, G. E., 1999. Nutritional condition of female Calanus euxinus from cyclonic and anticyclonic regions of the Black Sea. *Mar. Ecol. Prog. Ser*. 189, 195-204.

Yur'ev, G. S. 1979 The Black Sea sprat. In: K. S. Tkachenko, Yu. K. Benko (eds). Fishery resources of the Black Sea. Food Industry Publ. Moscow, 73–79 (in Russian).

Zaitsev, Yu. P., 1993. Impacts of eutrophication on the Black Sea fauna. GFCM Stud. Rev., 64, 63-85.