EGG PRODUCTION RATE, FEEDING RATE, GONAD MATURITY AND GROWTH RATE OF *CALANUS EUXINUS* (COPEPODA) IN THE BLACK SEA

A THESIS SUBMITTED TO
THE INSTITUTE OF MARINE SCIENCES
OF
THE MIDDLE EAST TECHNICAL UNIVERSITY

BY

MURAT TELLİ

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE
IN
THE GRADUATE SCHOOL OF MARINE SCIENCES

SEPTEMBER 2002

Approval of the Graduate School of Marine Sciences

İlkay Salihoğlu

Prof. Dr. İlkay SALİHOĞLU
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Prof. Dr. Ferit BİNGEL
Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Assistant Prof. Şengül BEŞİKTEPE
Supervisor

Examining Committee Members

Prof. Dr. Ferit BİNGEL

Prof. Dr. Temel OĞUZ

Prof. Dr. Özden BAŞTÜRK

Assoc. Prof. Zahit UYSAL

Assistant Prof. Dr. Şengül BEŞİKTEPE

ABSTRACT

EGG PRODUCTION RATE, FEEDING RATE, GONAD MATURITY AND GROWTH RATE OF *CALANUS EUXINUS* (COPEPODA) IN THE BLACK SEA

Telli, Murat

M.Sc., Institute of Marine Sciences

Supervisor: Assistant Prof. Şengül BEŞİKTEPE

September 2002, 81 pages

The effects of gonad maturity and chlorophyll-a concentration on egg production rates of *Calanus euxinus* were examined in the Black Sea during October, 2000 and May, 2001. Egg production rates varied between 0 and 7.34 eggs female^{-1} d^{-1} in October, and between 1.82 and 7.02 eggs female^{-1} day^{-1} in May. Egg production rate of *C. euxinus* was found to be closely related to the proportion of mature females. However, egg production rate appears to be independent of the chl-a concentration in both cruises.

Ingestion rates of female *C. euxinus* ranged in between 2.08 - 40.54 ng chl-a female^{-1} day^{-1} in October and in between 6.68 - 64.93 ng chl-a female^{-1} day^{-1} in May. There was a positive correlation observed between ingestion rate and chl-a concentration. However, there was no statistically significant relationship between egg production rate and ingestion rates.

Reproductive growth rates of female *C. euxinus* were found to be negatively correlated to its dry weight. The mean growth rate was 0.01 d^{-1} in October and 0.03 d^{-1} in May. These results are much lower than previously recorded growth rates of broadcast-spawner copepods estimated under food saturation. This comparison may suggest that the present study was conducted under conditions of limiting resources.

Keywords: Black Sea, Copepod, *Calanus euxinus,* Egg Production Rate, Growth Rate, Feeding Rate.

ÖZ

KARADENİZ' DE *CALANUS EUXINUS*' UN (COPEPODA) YUMURTA ÜRETİM ORANI, BESLENME ORANI, GONAD GELİŞİMİ VE BÜYÜME ORANI

Telli, Murat

Yüksek Lisans, Deniz Bilimleri Enstitüsü

Tez Yöneticisi: Yar. Doç. Şengül BEŞİKTEPE

Eylül 2002, 81 sayfa

Calanus euxinus' un gonad gelişiminin ve besin bolluğunun yumurta verimine etkisi Ekim 2000 ve Mayıs 2001 müddetince Karadeniz' de incelendi. Yumurta üretim oranı Ekim ayinda 0 ila 7.34 yumurta dişi^{-1} gün^{-1}, Mayıs ayında 1.82 ila 7.02 yumurta dişi^{-1} gün^{-1} aralıklarında bulunmaktadır. Yumurta üretim oranının, gonad gelişimini tamamlamış dişilerin bulunma yüzdeleri ile yakın ilişkili olduğu gözlenmiştir. Ancak yumurta üretim oranının her iki örnekleme periyodunda da klorofil-a konsantrasyonundan bağımsız olduğu görülmektedir.

Dişi *C. euxinus*' un pigment tüketme oranları Ekim ayında 2.08 ila 40.54 ng chl-a dişi^{-1} gün^{-1}, Mayıs ayında 6.68 ila 64.93 ng chl-a dişi^{-1} gün^{-1} aralığında bulunmaktadır. *C. euxinus*' un klorofil tüketme oranı ile ortamın klorofil konsantrasyonu arasında positif bir korelasyon bulunmuştur. Fakat, klorofil tüketme oranlarının yumurta verimi ile istatistiksel kaydadeğer bir ilişkisi bulunamamıştır.

C. euxinus' un üretkenlik büyüme oranlarının kendi kuru ağırlığı ile ters orantılı olduğu bulunmuştur ve ortalama büyüme oranları Ekim ayı için 0.01 gün^{-1}, Mayıs ayı için 0.03 yumurta gün^{-1} dür. Bu sonuçlar daha önceden yumurtalarını yayarak yumurtlayan (broadcast-spawner) kopepodlar için bildirilen büyüme oranlarından oldukça düşüktür. Bu kıyaslama bize çalışmanın yapıldığı dönemlerde besin kıtlığı olabileceğini gösteriyor.

Anahtar kelimeler: Karadeniz, Kopepod, *Calanus euxinus*, Yumurta Üretim Oranı, Büyüme Oranı, Beslenme Oranı.

To My Mother and Father

ACKNOWLEDGEMENTS

I would like express my deep gratitude to my supervisor, Assistant Prof. Şengül Beşiktepe for her guidance and close collaboration at every stage of my research.

I am deeply thankful to Prof. Dr. Ilkay Salihoglu, director of the Institute of Marine Science for providing easy access to all the facilities throughout this research.

I would like to express sincere thanks to Assoc. Prof. Şükrü Beşiktepe, Assistant Prof. Erhan Mutlu for their support and valuable critisms throughout the cruises as chief-scientists.

My sincere appreciations are due to Assistant Prof. Erhan Mutlu for his help in statistics. I also like to thank my colleague, Elif Eker for her helpful discussions. I am grateful to R/V *Bilim* crews for their effort. I am also most grateful to Ethem Dipli for his help in sampling during the cruises.

I cordially thank to my examining committee members Prof. Dr. Ferit Bingel, Prof. Dr. Temel Oğuz, Prof. Dr. Özden Baştürk, Assoc. Prof. Zahıt Uysal and Assistant Prof. Şengül Beşiktepe for their valuable comments.

I cordially thank to my mother and my father for their moral and financial support.

In this study, sampling and experiments were carried out by R/V *Bilim* within the framework of TUBITAK (YDABÇAG – 100Y014, 101Y080 and 199Y122) and NATO SFP projects.

TABLE OF CONTENT

CHAPTERS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

1.1 GENERAL INFORMATION ON COPEPODA

The term plankton commonly includes both plant and animals that embrace all those organisms drifting in the water whose abilities of locomotion are insufficient to withstand currents (Omori and Ikeda, 1992).

Plankton are divided into two broad categories: *zooplankton* and *phytoplankton*. Phytoplankton are the free-floating plants of the sea that are capable of photosynthesis. They are important in the marine food chains because of their role as producers. Zooplankton are very small animals that feed on the phytoplankton and other microzooplankton (Nybakken, 1982).

The most important zooplankton group is the Crustacea, which acts as the main food item for a variety of fish populations and other predators. Among the crustacea, copepods are the most abundant and the best-known marine zooplankton. Most copepod species are in the orders Calanoida, Cyclopoida and Harpactocoida. Free-living copepods have fifteen somites (Figure 1.1). The first five comprise a head or cephalon, have the two pairs of antenna, the mandibules, and the two pairs of maxillae. The thorax is consisted of six somits, including a maxilliped and five pairs of swimming leg (McConnaughey, 1974).

Free-living planktonic copepods are generally small, between one and several millimeters in length. They swim weakly, using their five pairs of thoracic swimming legs, and have a characteristic jerky movement. They employ their very large antennae to slow their rate of sinking. Most free-living planktonic copepods have characteristic body shapes that are readily recognizable (Figure 1.1) (Omori and Ikeda, 1992).

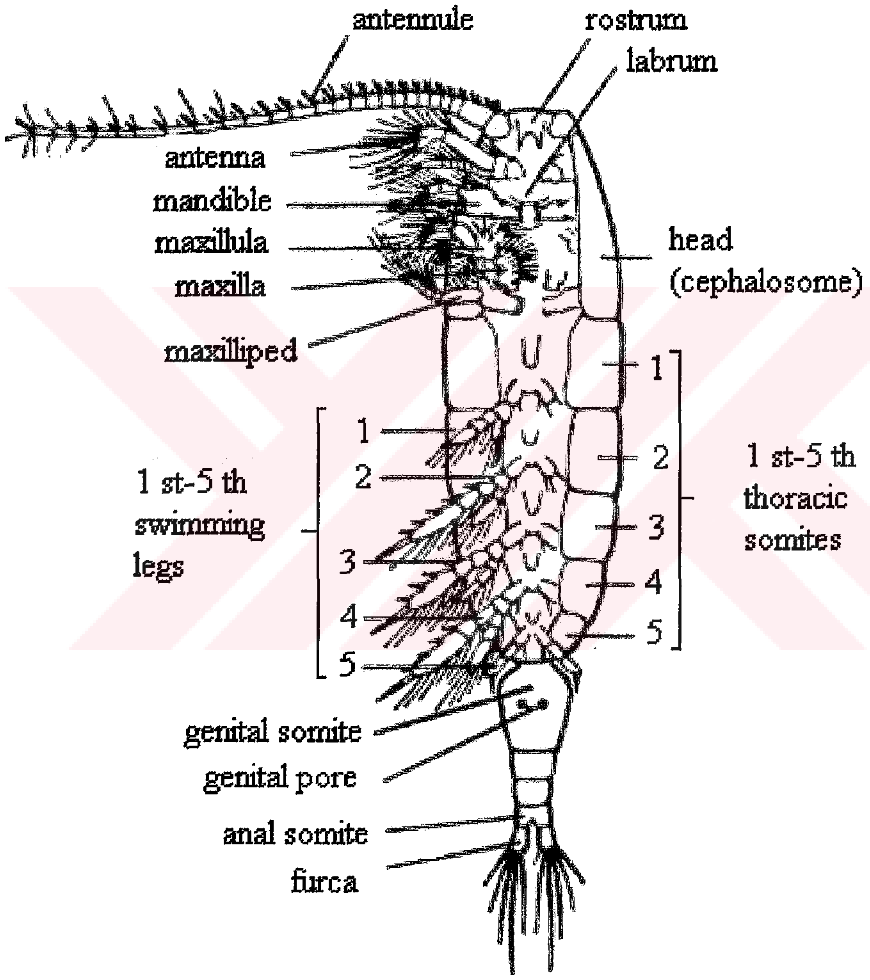

Figure 1.1: Ventral diagram of a calanoid female copepod (after Omori and Ikeda, 1992).

1.1.1 Feeding Rates of Copepods

Feeding is the main route for the transfer of energy and material from lower to higher trophic levels within an ecosystem, therefore its quantification will be a key factor when trophic interactions are studied. Food is also the source of energy and material for production and activity of zooplankton, so quantification of feeding behavior is fundamental in understanding the constraints of these processes (Bamstedt *et al.*, 2000). Copepods feeding rates is usually expressed as clearance rate or ingestion rate (Omori and Ikeda, 1992).

Clearance rate (formerly known as filtration rate) is described as the volume of water swept clear per animal per day. Filtering rate refers to the total volume of water filtered by animals over a particular time, whereas clearance rate refers to the volume of water from which food particles were completely removed over that time. Filtering rates is equal to clearance rate only when the filtering apparatus traps all food particles in the water, which passes through it. From a methodological viewpoint, most of the previously measured filtering rates of copepods do not present the real filtering rate but an apparent filtering rate, better termed clearance rate (Omori and Ikeda, 1992).

Ingestion rate is the amount (number of mass unit) of ingested food per unit time and predator. If number of food items is used, information on mass per unit of food should also be given. It is recommended that carbon is used as a general expression of mass. Alternatively, dry weight (DW), ash-free dry weight (AFDW) and nitrogen content are commonly used (Omori and Ikeda, 1992).

Feeding rate is a function of feeder's physiological condition, environmental variables and its behavioral repertoire and ability to deal with changing conditions and variables. Copepods can change their feeding rates over time, depending on endogenous rhythms and environmental variables (Paffenhöfer, 1988). In nature, because of varying abundance and qualities of food over time and space, and predation pressure from the invertebrate and vertebrate predators, feeding rates should vary with time (Frost, 1988; Paffenhöfer, 1988; Harris, 1988). Harris, (1988) recorded that number of copepod species maintain a close relationship with the surface chlorophyll maximum at the some stage of diel cycle. Durbin *et al.*, (1995) found a seasonal change in diel migratory and feeding behavior of calanoid copepod (especially in *Calanus* sp.), associated with increased predation and food limitation during the transition from spring to summer. They showed strong diel feeding periodicity during the spring bloom and didn't migrate vertically during the bloom event, and then began to migrate as the spring bloom ended.

Laboratory studies on the marine copepod suggested that ingestion rates of copepods are determined by their reaction to ambient food particle concentration, sizes, and biochemical properties (Paffenhöfer, 1988). The relationship between food abundance and ingestion rate is shown by a simple model, the functional response. An individual zooplankter encounters its prey in direct proportion to prey concentration. It is shown that ingestion rate is directly depends on food concentration at low food concentration, but constant and apparently independent of concentration at high food concentrations (Figure 1.2) (Frost, 1972; Rothhaupt, 1990; Huskin *et al.*, 2000; Støttrup and Jensen, 1990; Bergreen *et al.*, 1988; Paffenhöfer, 1988).

Clearance rate is also dependent on algal size and relative retention abilities of copepods. Most of the copepods are unable to retain particles < ≈ 3µm (Støttrup and Jensen, 1990). However, algal size is not important in determining algal quality. The functional response of feeding rates also dependent on the nutritional content of the algal species (Houde and Roman, 1987; Bergreen *et al.*, 1988). The sequence of clearance rates by the calanoid copepods on foods of different quality but similar volume and shapes was: living phytoplankton > dead phytoplankton > fecal pellets (Paffenhöfer, 1988; Houde and Roman, 1987).

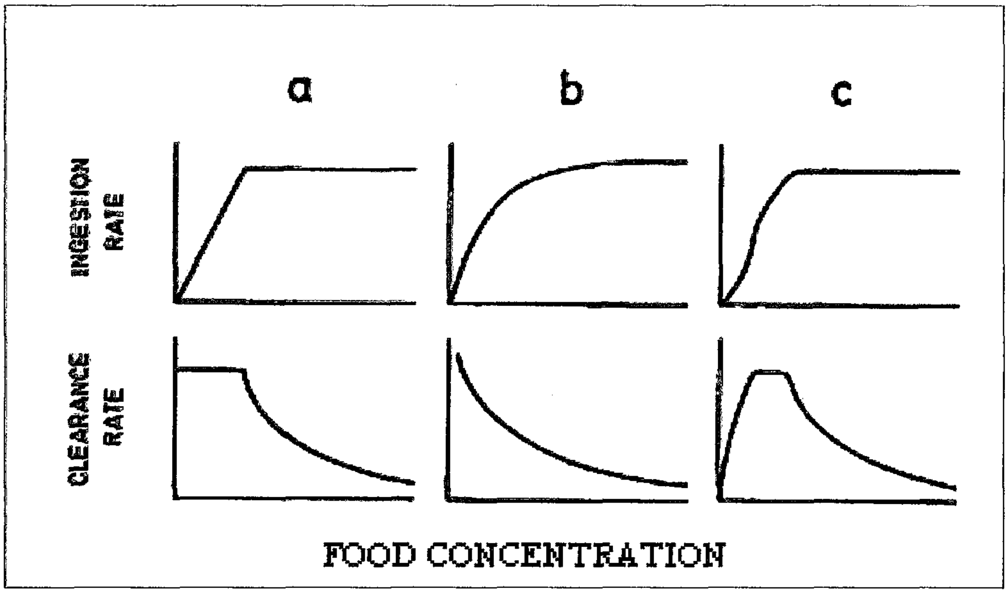

Figure 1.2: Schematic representation of three different feeding models for planktonic filter feeders. a. Rectilinear model. b. Curvilinear model. c. Decreasing clearance rates at low food concentrations (sigmoidal model) (after Rothhaupt, 1990).

1.2 GROWTH RATES OF COPEPODS

One of the ultimate goals of oceanography since it's beginning has been to determine and understand the different physical and biological characteristic of yield of fisheries. Within this scope, the study of copepods has become an important issue. Copepods are the main food item for many fish larvae and juveniles, and play an important role in the control of fish recruitment. Consequently, in the marine zooplankton ecology one of the first aims has been the determination of growth and fecundity of copepods, and biological, chemical and physical factors, which have an influence on it (Poulet *et al.*, 1995). In the last decades many researches have dealt with developing methods to determine the production, growth and fecundity in planktonic copepods in the sea (Bergreen *et al.*, 1988).

Basically there have been two main approaches: one is based on methods regarding the population dynamics of copepods; a second group of methods takes an approach that potentially makes time- and site-specific production estimates, thus allowing analysis of temporal and horizontal variations in productivity.

One group of methods considers the development of distinct cohorts and estimates the production from the changes in abundance and weight of individual age (stage) classes over time. This approach is very often not applicable to planktonic copepods, since populations are often continuously reproducing and distinct cohorts, therefore, cannot be separated. Also patchiness and advection processes make sampling of the same population cohort over the time difficult. One additional drawback of this approach is that it yields production estimates integrated over the fairly large areas and time periods (see the discussion in Bergreen *et al.*, 1988; Poulet *et al.*, 1995; Omori and Ikeda, 1992).

The second group of methods pursues the estimation of growth rates by different means. Some of the groups of methods are based on physiological bugged described by Huntley and Lopez, (1992). The impetus of the physiological bugged is the assumption that all physiological rates that influence individual growth respond (such as ingestion rate, filtration rates and respiration rate) in a quantitative and predictable manner to variety of environmental variables (such as food concentration). However, Huntlay and Lopez, (1992) pointed out that there are some inconveniences of an accumulated error due to the sometimes-huge variability obtained in the determination of the different component of the bugged.

The other approaches in the second groups based on measurements of *in situ* egg production rate and biomass of copepods. This method has the assumption that adult females express all their production not in somatic growth, but in egg mass (Berggreen *et al.*, 1988; Hopcroft and Roff, 1998; Poulet *et al.*, 1995). *In situ* growth rate is measured directly in the field. Individual stages are sorted ot of plankton tows and incubated in water from the collection site for 24 hour for the determination of *in situ* growth rates. The major disadvantage of this approach is that, it is extremely laborious since it needs time-specific and site-specific weight of copepods of each developmental stage. Also, the number of replicate incubations should be performed to eliminate errors coming from experiments and researchers. Consequently, the number of sites, times and species covered by this method is limited in practice (Poulet *et al.*, 1995).

A principle simplification of this approach has been based on two assumptions (Berggreen *et al.*, 1988). First, all stage-specific, instantaneous growth rates are equal. Second, they can be estimated from the specific egg production rates, which is equal to growth rates of female. However, these assumptions can be valid only in food saturation conditions. Previous studies performed in the field showed that growth rates of juvenile do not equal to growth rate of adult females and growth rates decline with the stages due to the food limitation in the field. In addition, planktonic copepods are often exposed to food limitation in nature (Runge, 1985; Champhell *et al.*, 2000; Kiørboe and Sabatini, 1995; Vidal, 1980; Hopcroft and Roff, 1998). Furthermore, Hirst and Lampitt, (1998) emphasized that adult female growth is more food limited than juvenile growth in nature. Therefore, egg production rate approach represents only adult female growth rate. Nevertheless, it gives several advantages such as short incubation time, explicability and accuracy of biomass and fecundity measurements (Poulet *et al.*, 1995).

1.3 GENERAL CHARACTERISTICS OF THE BLACK SEA

1.3.1 Physical and Chemical Properties

The Black Sea has been represented as the largest land-lock anoxic basin in the world with a maximum depth of ≈ 2200 m, a surface area of 4.2×10^5 km^2 and a volume of 5.3×10^5 km^3. The Black Sea waters deeper than 150-200 meters are anoxic, due to the permanent H_2S zone. The upper layer of 150 meters (containing 13 % of the sea water volume) is oxic. Depth of the anoxic zone is shallower at the central and deeper at the coastal parts of the Black Sea. A permanent halocline separates the oxic and anoxic layers (Özsoy and Ünlüata, 1997).

The surface layer has relatively lower salinity (ranged from 17.5 to 18.5 ‰) as compared to world seas, which is resulted from excess fresh water input and precipitation. Salinity of the northwestern part is relatively lower (between 13-15 ‰) than the mean Black Sea surface salinity due to the strong influence of three big rivers (Danube, Dniester and Dniepr). Below the surface layer, saltier Mediterranean waters (about 35 ‰) inflow to the mid-waters of the Black Sea and further sinks to the deeper layer of the Black Sea (Murray *et al.*, 1991; Oğuz *et al.*, 1992).

The Black Sea current system is characterized by a predominantly cyclonic basin wide circulation formed by Rim Current System. This system follows abruptly varying continental slope and margin topography all around the basin, a multi-centered cyclonic cell occupying the interior of the sea, and a series of inshore anticyclonic eddies confined by the Rim Current. Two separate cyclonic gyres are located in the western and eastern interiors of the basin and a few permanent anticyclonic eddy distributed over the basin (Oguz *et al.*, 1993) (Figure 1.3). Oğuz and Beşiktepe, (1999) recorded that Rim Current speeds as high as 50-100 cm s^{-1} within the upper 100 m of water column, and about 10-20 cm s^{-1} within the depth of 200 – 350 m recorded with the Acoustic Dropper Current Profiler (ADCP) measurements in the Black Sea.

Sea surface temperature varies seasonally because of the solar heating and decreases with the depth to a minimum located at depths between 50-100 meters where the temperature varies between 6.5-7°C and salinity between 18.50-18.53 ‰. This layer is called as the Cold Intermediate Layer (CIL). CIL is originated from the northwestern shelf and also at the center of cyclonic eddies located at the central part of the basin. Below the CIL, permanent halocline (between 50-200 meters) separates the surface water from the deep water. The basin-wide distribution of the oxygen-carrying CIL waters has important implications on the health and ecology of the Black Sea (Murray *et al.*, 1991; Oğuz *et al.,* 1993; Oğuz *et al.*, 1994; Ivanov *et al.*, 1997).

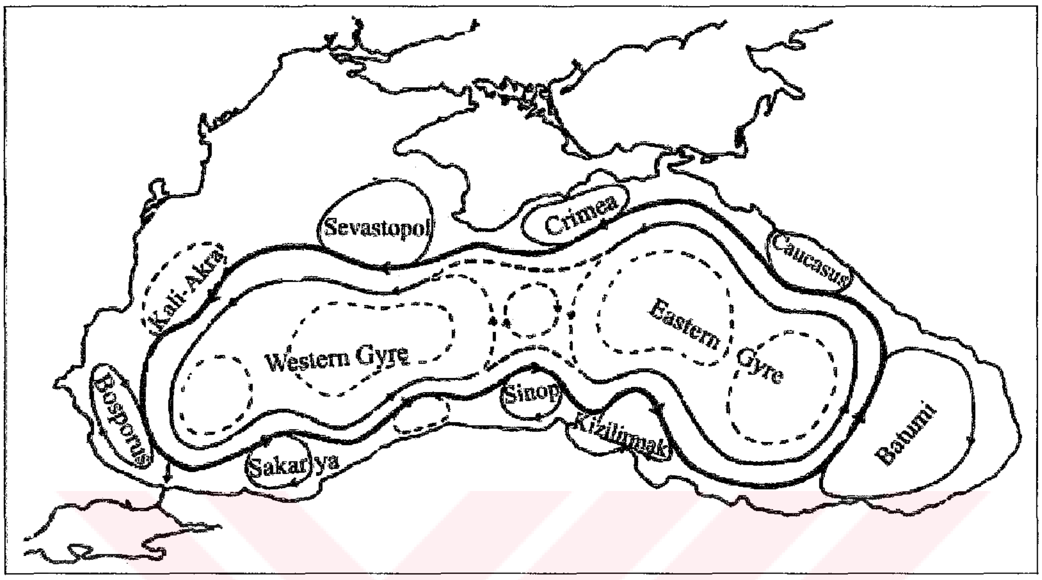

Figure 1.3: Circulation in the upper layer of Black Sea; solid line indicate quasi permanent flows, while dashed lines correspond to recurrent features of general circulations; (after Oğuz *et al.*, 1993).

The distributions of nutrient concentrations such as NO_2, NO_3 and PO_4, which are closely related to phytoplankton distribution (Vinogradov and Nalbandov, 1990), are high for the whole water column in the Black Sea. However their concentrations in the productive surface layer are low.

Nitrate concentrations start to increase at the depths of 14.0-14.2 density surface, and reach to a maximum of 8-9 µM at the depths of $\sigma t \approx 15.40 \pm 0.10$. This level of nitrate maximum coincides with the sharp drop in the oxygen concentrations below 20 µM as a result of the denitrification processes in the oxygen deficient water (Saydam *et al.*, 1993; Tuğrul *et al.*, 1992).

Phosphate profiles show two maxima in the water column. The upper peak coincides with the nitrate maximum and it is established at the lower boundary of the oxygenated layer due to the anaerobic oxidation of the biodegradable organic matter of algal origin. The deeper peak has been formed at the upper boundary of the anoxic sulphidic layer. It increases sharply from the minimum levels to the large values at $\sigma_t = 16.2 \pm 0.05$, significantly correlated with the first appearance of the anoxic sulphidic water, independent of geographical location (Saydam *et al.*, 1993; Tuğrul *et al.*, 1992).

Dissolved oxygen (DO) in the upper mixed layer is nearly at saturation level ranging from 250 to 350 μM. It's level then decreases steeply to the level of 20-30 μM through the main oxycline and become undetectable at the lower depths of suboxic layer (at about 16.15-16.2 density surfaces). At this layer H_2S starts to appear and its concentration incereases gradually with depth (Baştürk *et al.*, 1997). In addition, Tuğrul *et al.*, (1992), Saydam *et al.*, (1993), Baştürk *et al.*, (1997) have suggested that the upper and lower boundaries of the suboxic layer are independent of the circulation characteristics of the basin. However, Oğuz, (2002) say that the vertical physical processes are crucially important factors governing the basinwide distribution of the oxycline and suboxic layer properties. In the study of Oğuz, (2002), it is concluded that in the previous studies of Tuğrul *et al.,* (1992), Saydam *et al.,* (1993), Baştürk *et al.*, (1997), the oxygen data had been collected from the interior part of the western and central basins characterized by the same type of circulation system. Then, Oğuz, (2002) say that their assumption may not be uniformly valid when the composite is formed by pooling data from different parts of the basin with different circulation regimes. He found that while vertical diffusivity controls the position of the upper boundary of the suboxic layer, the upwelling velocity primarily controls its lower boundary. Consequently, Oğuz, (2002) suggested that composite plots of oxygen versus density for cyclonic and anticyclonic sites should be constructed separately.

1.3.2 Biological Properties

The aerobic layer of the Black Sea is biologically productive due to high run-off. The major rivers discharging to northwest shelf of the Black Sea are Danube, Dnepr and Dnester. Among these rivers, the Danube River is the greatest contributor of river run-off into the Black Sea accounting for about one half of the total riverine influx (Sur *et al.*, 1996).

Sorokin, (2002) indicated that peaks in the primary productivity of the Black Sea were known to occur twice a year, with a major bloom principally composed of diatom in early spring, followed by a second bloom mainly comprising coccolithophorids in autumn. Additional summer blooms with a predominance of dinoflagellates and coccolithophorids have been increasingly observed in the region in recent years (Uysal and Sur, 1995).

A sharp increase in the eutrophication of the Black Sea has been observed in the 1960s and early 1970s after a rise in nutrients discharge by Danube. This rise resulted from mainly man made impacts through industrial runoff, and domestic sewage discharges and nutrient-loads from these sources (Sorokin, 2002; Zaitsev and Alexandrov, 1997; Caddy, 1993; Bologa, 1986; Saydam, 1993). The consequence of eutrophication of the Black Sea is very important for its ecosystem (Aubrey *et al.*, 1996). This eutrophication processes started with phytoplankton blooms, followed with a decrease in transparency, large-scale anoxia especially on the Northwest Shelf and benthic mortality (references cited in Sorokin, 2002).

Ecological changes in open sea regions of the Black Sea have received less attention than coastal regions. These open areas constitute a partially isolated ecosystem in which water masses are separated from polluted coastal areas by a meandering Rim Current frontal zone (Oğuz *et al.*, 1993). Yunev *et al.*, (2002) analyzed the seasonal and long-term variability in the surface chl-a and depth integrated primary production in the open Black Sea over the period of 1964 to 1996. They found that surface chl-a concentration showed significant increase in winter-spring months. In contrast to chl-a variations, the seasonal dynamics of depth integrated primary production exhibited an increase in summer months in addition to winter-spring maximum. The chl-a concentrations for the entire open Black Sea reveal a distinct stable long-term trend (Chl-a values were with the mean value of 0.15 mg m^{-3}) except for the sharp increase in 1993 (mean value was 0.99 mg m^{-3}).

However, as a result of much higher nutrient input, phytoplankton cell densities in the coastal waters of northwestern Black Sea increased dramatically within the last two decades (Cociasu *et al.*, 1996). Cociasu *et al.*, (1996) pointed out that from 1974 onwards a distinct increase in the bloom frequency, the number of bloom-forming species and cell density were observed in Romanian coastal areas. From 1980 to 1990 intense blooms, particularly of *Skeletonema costatum* and *Prorocentrum costatum* were observed. Both species developed blooms nearly every year.

In the last few decades the number of dinoflagellates species was usually lower than that of diatoms (Bologa, 1986). However, recently, the situation has been reversed due to the proceeding eutrohication. The dominant species at the surface were dinoflagellates in March-April and diatom coccolithophore (*Emiliana huxleyi*) in October (Eker *et al.*, 1999; Sorokin, 2002).

Accompanying the rise in primary production, biomass and production of zooplankton increased by at least 1 or 2 orders of magnitude. The population numbers of gelatinous planktons such as moon jelly *Aurelia aurita*, *Noctiluca scintillans*, jellyfish *Rhizostoma pulmo* and outburst of the rainbow comb jelly *Mnemiopsis leidyi* greatly increased (Caddy, 1993; Sorokin, 2002; Zaitsev and Alexandrov, 1997).

On the other hand, the populations of some zooplanktons species including copepods *Centropages, Pontella, Pseudocalanus* have become sparse. Large species of crustacean plankton have been replaced by smaller detritivore and predatory opportunists species like *Acartia* (Zaitsev and Alexandrov, 1997; Sorokin, 2002).

Due to the increased densities, detritus, *Noctiluca*, jellyfish and other suspended organism and particles, the transparency of Black Sea water has significantly decreased (Vladimirov *et al.*, 1997).

1.3.2.1 *Calanus euxinus* in the Black Sea

In the pelagic food chains of the Black Sea the most important species of zooplankton are the copepods (Sorokin, 2002). Among them *Calanus euxinus* has major importance in transferring the organic matter from primary producer to the higher taxa including pelagic fish (Beşiktepe *et al.*, 1998).

Calanus euxinus is the most dominant species among the mesozooplankton of the Black Sea and occurs in the aerobic zone of the Black Sea for all seasons. It accounts for over one-third of the total zooplankton biomass and its maximum concentration always exceeds 250-500 ind. m^{-3} (Vinagradov *et al.,* 1992). Ünal, (2002) has studied the seasonality of zooplankton at the inshore and offshore stations in southern Black Sea, and identified *C. euxinus* as the most dominant copepod species in terms of biomass, constituting 43% at inshore and 55% at offshore of the total copepod biomass. Both at the inshore and offshore waters of the Black Sea, *C. euxinus* shows the major peak in February and relatively smaller peaks in April, July and September in terms of biomass. In addition, Ünal, (2002) found that the biomass and abundance values of *Calanus euxinus* were always much higher in offshore than inshore waters throughout the year.

Calanus euxinus is present in the aerobic zone of the Black Sea during all seasons and reproduces whole year, since nauplii and eggs were present throughout the year in the Black Sea (Ünal, 2002; Sazhina, 1996). Sazhina, (1996) also reported that *Calanus euxinus* produces about 7 generations in a year, with the maximum production occurring in early spring. Beşiktepe, (2001) reported the same results that metanauplii of *C. euxinus* was presented in all seasons. Since the data obtained by Besiktepe (2001) comprised more than half of the *C. euxinus* population in April 1995, it led to reach a conclusion that April is the main reproductive season of *C. euxinus* in the southern Black Sea. The maximum abundance of early copepodite stages generally occurs in the late spring. In late summer and autumn, the age composition of the population is characterized by the C-5 and adults (Vinogradov *et al.*, 1995; Vinogradov and Shushkina, 1992; Ünal, 2002).

In the Black Sea, younger copepodites and nauplii inhabited upper 100 m throughout the day. The C-5 and adults of *Calanus euxinus* undergo daily vertical migration from the oxygen minimum zone to the surface water. The fifth copepodite stage of *Calanus euxinus* is divided into two different ecological groups during the summer months. The first group is concentrated in the deeper layer of the oxygen minimum zone (diapausing group); the second group shows diel vertical migration and feeding activity (Beşiktepe, 2001; Vinogradov *et al.*, 1995; Vinogradov and Shushkina, 1992). Vinogradov *et al.*, (1992) pointed out that in February-March, almost all stage of C-5 show diel vertical migration and feeding activity. In April, only ~ 10 % and in august > 85 % of stage C-5 remains constantly at the depth of oxygen minimum zone for diapausing.

1.4 IMPORTANCE AND AIMS OF THE PRESENT STUDY

In spite of long history of the investigation of the Black Sea zooplankton, the reproductive patterns of the zooplankton have been poorly investigated. Only a few number of egg production studies have been recorded in the Black Sea (Sazhina, 1996; Arashkevich *et al.*, 1998). The main goal of this study was to contribute to knowledge on ecology and reproduction of the common calanoid copepod *Calanus euxinus* in the southern Black Sea.

The specific aims of this study were as follows:

1) To determine the feeding rate of *Calanus euxinus*, and examine the effect of food availability on feeding rate of the copepod.

2) To quantify egg production rates of *Calanus euxinus* in order to estimate growth rate of the copepod in the Black Sea.

3) To examine the effects of variations in environmental conditions (i.e. food availability) and internal factors (i.e. maturity) on egg production of *Calanus euxinus*. An additional goal was to describe the diel egg-laying pattern of the copepod.

CHAPTER II

MATERIALS AND METHODS

2.1 AREA SURVEYED

Sampling was carried out during two cruises aboard the R/V 'BİLİM' in the southern Black Sea in October (6-17) 2000 and May (23-27) 2001. In October 2000, 13 stations were visited in the central and eastern part of the Black Sea (Figure 2.1). In May 2001, 9 stations were visited in west part of the Black Sea (Figure 2.2).

Sampling stations names, their locations and parameters measured during the study were summarised in Table 2.1.

2.2 HYDROGRAPHIC AND BIOLOGICAL SAMPLING

2.2.1 CTD Measurements

Temperature, salinity and *in situ* fluorescence profiles were taken at each station using a Seabird CTD and Chelsea fluorometers attached to it.

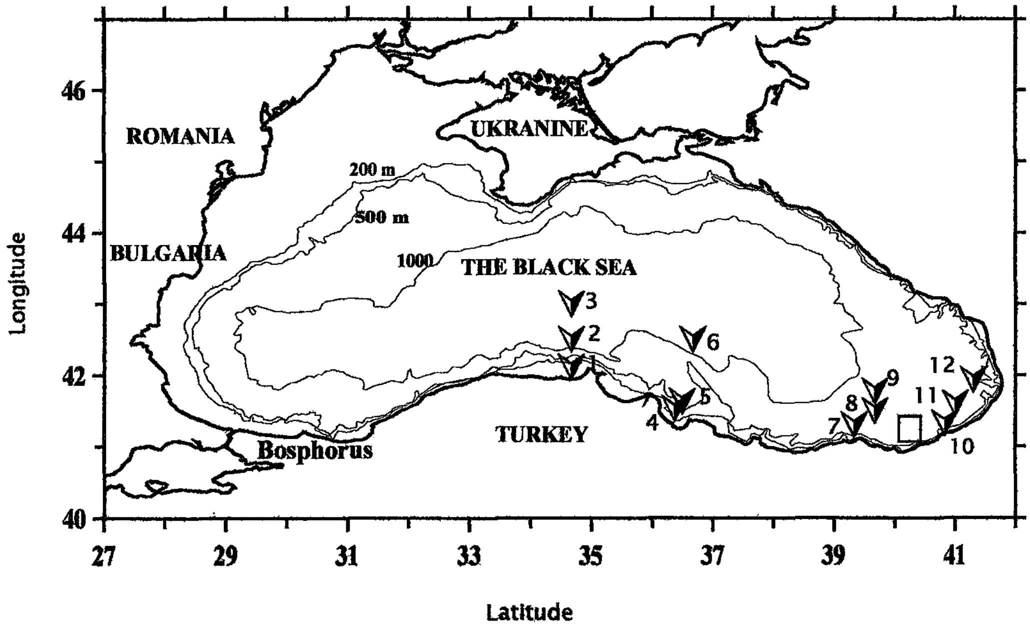

Figure 2.1: Location of sampling stations visited in October 2000. (V) Egg Production stations, (☐) Egg Laying Time station.

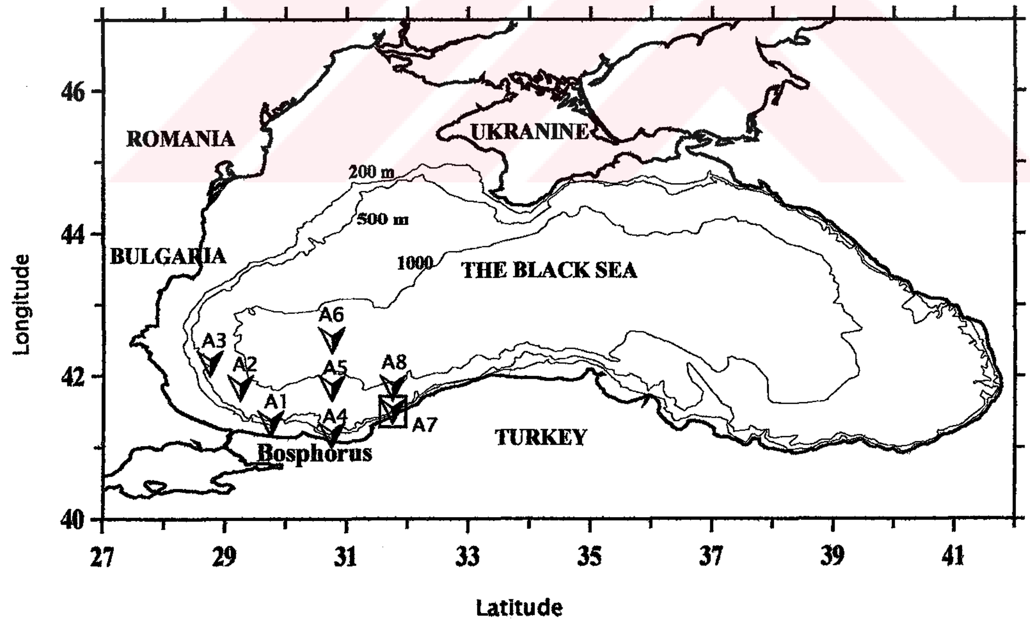

Figure 2.2: Location of sampling stations visited in May 2001. (V) Egg Production stations, (☐) Egg Laying Time station.

Table 2.1: Sampling stations, characteristics of the stations and parameters measured during the study. {COS = Coastal Regions, ACR = Anticyclonic Region, CR = Cyclonic Region, RCR= Rim Current Region. CTD = Conductivity, Temperature, Depth; Abd = Abundance; Chl-a = Total Chlorophyll-a; Fl = Fluorescence; EPR = Egg Production Rate; Gr = Growth Estimation; ELT = Egg Laying Time Experiments}.

St. Name & Numbers	*Date*	*Regions*	*Longitude*	*Latitude*	*Measured Parameters*	*Total depth (m)*	*σ_θ Density Gradient*	*Fluorescence max, depth (m)*
M0650R40 (1)	06.10.00	COS	42.11	34.67	CTD, Abd, Chl-a, Fl, EPR, Gr	100	-	17
N00R40 (2)	07.10.00	CR	43.00	34.67	CTD, Abd, Chl-a, Fl, EPR, Gr	2200	140	23
M30R40 (3)	06.10.00	RCR	42.50	34.67	CTD, Abd, Chl-a, Fl, EPR, Gr	2200	100	35
L40W40 (4)	16.10.00	RCR	41.77	39.67	CTD, Abd, Chl-a, Fl, EPR, Gr	450	165	23
L30Y00 (5)	14.10.00	RCR	41.60	41.00	CTD, Abd, Chl-a, Fl, EPR, Gr	920	170	25
L50Y30 (6)	14.10.00	RCR	41.93	41.30	CTD, Abd, Chl-a, Fl, EPR, Gr	2100	170	25
L32T22 (7)	17.10.00	ACR	41.53	36.37	CTD, Abd, Chl-a, Fl, EPR, Gr	680	168	25
L3750T30 (8)	09.10.00	ACR	41.63	36.50	CTD, Abd, Chl-a, Fl, EPR, Gr	1800	165	30
M30T40 (9)	09.10.00	ACR	42.50	36.67	CTD, Abd, Chl-a, F, EPR, Gr	2200	135	25
L00W20 (10)	16.10.00	ACR	41.30	39.33	CTD, Abd, Chl-a, Fl, EPR, Gr	950	200	20
L15W40 (11)	16.10.00	ACR	41.50	39.67	CTD, Abd, Chl-a, Fl, EPR, Gr	1950	138	25
L0750X50 (12)	13.10.00	ACR	41.33	40.83	CTD, Abd, Chl-a, Fl, EPR, Gr	1700	140	20
L15X15	15.10.00	ACR	41.30	40.30	CTD, Chl-a, Fl, ELT	1155	170	25
M30M45 (A1)	24.05.01	CR	42.50	30.75	CTD, Abd, Chl-a, Fl, EPR, Gr	2200	115	20
L50L15 (A2)	23.05.01	RCR	41.83	29.25	CTD, Abd, Chl-a, Fl, EPR, Gr	1800	150	10
M10K45 (A3)	23.05.01	RCR	42.17	28.75	CTD, Abd, Chl-a, Fl, EPR, Gr	796	156	12
L50M45 (A4)	25.05.01	RCR	41.83	30.75	CTD, Abd, Chl-a, Fl, EPR, Gr	1900	125	20
L30N45 (A5)	26.05.01	RCR	41.50	31.75	CTD, Abd, Chl-a, Fl, EPR, ELT, Gr	376	153	2
L20L45 (A6)	27.05.01	ACR	41.33	29.75	CTD, Abd, Chl-a, Fl, EPR, Gr	270	170	4
L10M45 (A7)	27.05.01	ACR	41.17	30.75	CTD, Abd, Chl-a, Fl, EPR, Gr	667	178	15
L50N45 (A8)	26.05.01	ACR	41.83	31.75	CTD, Abd, Chl-a, Fl, EPR, Gr	1600	160	10

2.2.2. Total Chlorophyll-a Measurements

Water samples for determination of chlorophyll-a (chl-a) concentration at chosen depths (according to fluorescence profiles) were collected with CTD rossette sampler then filltered throught Whatman GF/F filters (0,7 µm pore size 47 mm in diameter) and kept frozen at –20 °C untill analysis.

After the cruise, samples were extracted in 5 ml 90% acetone solution by using a grinder. Following the extraction samples were kept in refrigerator for 12 hour and then centrifuged (3000 rpm, 10 minutes).

A Hitachi F-3000 Model Fluorescence Spectrofluorometer was used to measure chlorophyll-a concentration (JGOFS, 1994). Emission wavelength of 669 nm and excitation wavelength of 420 nm were used for the spectrofluorometric analysis. Before the samples analyses the fluorometer was calibrated using a commercially available chlorophyll-a standard from Sigma and concentration of the standard stock solutions was determined spectrophotometrically. Fluorometer readings were recorded before and after acidification with two drops of 1N of 10 % HCL to calculate chlorophyll-a and phaeopigments concentration. Total chlorophyll-a concentration was determined by adding chlorophyll-a and phaeopigments (JGOFS, 1994).

2.2.3. Sample collection, preservation and caunting

Copepod samples for determination of abundance were collected using a Hansen Plankton Net (70 cm mouth opening and 300µm mesh size) towed vertically at 0.5 m sec^{-1} from the depth of the beginning of anoxic water layer to the surface. At shallow stations where there was no anoxic water layer, net was towed from the bottom to the surface. The sampling depths were estimated from the angle and length of the hauling wire. The samples were preserved in 4% borax buffered formaldehyde for later analyses.

After the cruise, samples were sub-sampled (from 1/1 to 1/8, depends on abundance) with a folsom splitter. Copepodite stages and adults of *Calanus euxinus* were identified and counted under an Olympus SZX-12 model stereomicroscope. Their prosome lengths were measured under the stereomicroscope with an ocular micrometer (Figure 2.3).

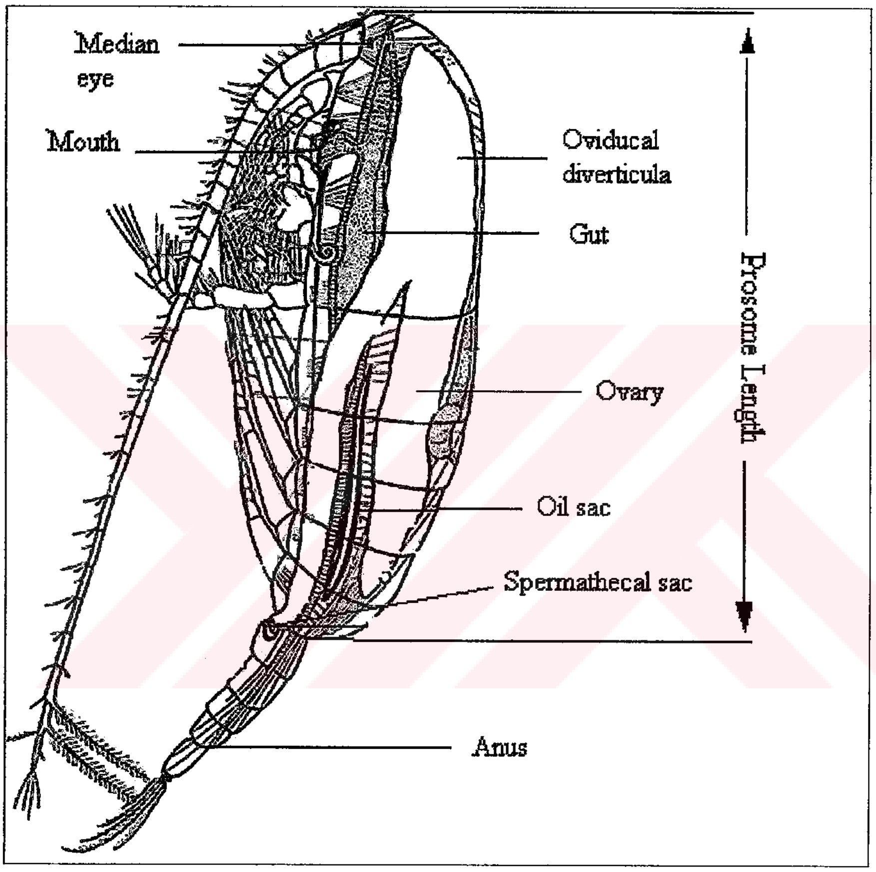

Figure 2.3: Diagram of a female *Calanus* sp., from the side (after Marshal and Orr, 1972).

2.2.4 Egg Production Experiments, Ingestion and Filtration Rate Measurements

Egg production and ingestion rates of female *Calanus euxinus* were determined using the bottle incubation method at twelve stations in October 2000 and eight stations in May 2001. Copepod samples for the egg production and ingestion rate experiments were collected with a Hansen net (mouth opening 70 cm, mesh size 300 µm) via vertical hauls from the depth of 16.2 density layer to the surface.

The contents of cod-ends of the net were kept in 15-liter plastic container filled with *in situ* surface seawater until microscopic sorting. Females were sorted under a stereomicroscope with wide-mouth plastic pipettes within a few hours. The groups of 10 (occasionally 5 or 15) females were transferred to 2000 ml translucent bottles previously filled with water from the fluorescence maximum. This water had been screened through 56 µm sieves in order to remove eggs and other zooplankters. Egg production rate and ingestion rate measurements were carried out within the same experimental bottles. That`s why 1-2 liters of the water were filtered on GF/F to measure initial chlorophyll-a concentration to calculate ingestion rate.

Usually 3 replicate bottles with copepods and two control bottles without copepods were set to determine egg production and ingestion rates. The experimental bottles were kept on deck in an incubator with surface water running through. Incubations were performed under the natural light cycle. After approximately 24 h, the contents of the bottles were gently back-siphoned by using a hose with 56 µm mesh mounted on the end of hose and then filtered on GF/F filters to measure chlorophyll-a concentration for estimating of natural phytoplankton growth and ingestion rates of *C. euxinus*.

The rest of the samples in the bottles were filtered onto 56 µm sieves and the copepods were checked for activity, and eggs and copepods were preserved in 4 % buffered formaldehyde for counting and measuring later in the laboratory.

After the cruise, all eggs and empty shells were counted. Egg diameter and copepod prosome length were determined under an Olympus SZX-12 model stereomicroscope.

Ingestion rates of adult female of *Calanus euxinus* (I, µg Chl-a individual^{-1} day^{-1}) were calculated using the equations of by Frost, (1972) given below.

Phytoplankton growth rate, *k*, was calculated from:

$$C_2 = C_1 e^{k(t_2 - t_1)} \qquad (2.1)$$

Where C_1 and C_2 are chl-a concentration (µg Chl-a l^{-1}) in the control bottles at time t_1 and t_2. Grazing coefficient, *g*, was calculated from:

$$C_2^* = C_1^* e^{(k-g)(t_2 - t_1)} \qquad (2.2)$$

Where C_1^* and C_2^* are chl-a concentration (µg Chl-a l^{-1}) in the bottle with copepods at time t_1 and t_2. Using value of *k* and *g*, the average natural phytoplankton concentration, C_{ave}, for each bottle with copepod during the experimental time interval t_2 and t_1 was calculated from:

$$C_{ave} = C^*_1 [e^{(k-g)(t_2 - t_1)}]/(t_1 - t_2)(k - g) \qquad (2.3)$$

The volume swept clear by the copepods (volume of water passing throught the maxillary filter per unit time) was calculated by the equation:

$$F = V g / N \text{ (liter copepod}^{-1}\text{ day}^{-1}) \qquad (2.4)$$

Where V is the volume (liter) of the experimental bottle and N is the number of copepods in the bottle. The ingestion rate, I, is then calculated from:

$$I = C_{ave} F \text{ (µg chl-a eaten copepod}^{-1}\text{ day}^{-1}) \qquad (2.5)$$

2.2.5. Diel Changes in Egg Production of *Calanus euxinus*

To describe the diel egg-laying pattern of *Calanus euxinus*, two experiments were performed seperately in October 2000 and May 2001 cruises. In each cruise, two sets of experimental bottles were prepared for the experiments. Each set consisted of 4 replicates of 1000 ml translucent bottles. The groups of 5 females were transferred to these bottles previously filled with water from the fluorescence maximum. This water had been screened through 56 µm sieves in order to remove eggs and other zooplankters.

One set of the bottles was incubated at daytime and the other was incubated at nighttime period during 12 h. The experimental bottles were kept on deck in an incubator with surface water running through.

After incubation the contents of the bottles were filtered onto 56 µm sieves, the animals checked for activity, and eggs and copepods preserved in 4 % buffered formaldehyde for counting and measuring later in the laboratory. After the cruise, all eggs were counted under an stereomicroscope.

2.2.6. Gonad Maturity Determination

Gonad maturity was measured in *Calanus euxinus* females from the preserved tows for each of the stations and females from each of the egg production experimental bottles. Between 20 and 90 females from preserved samples and all females from experimental bottles were stained in borax carmin solution according to Niehoff and Hirche (1996). Individuals were kept in borax carmine solution during 12 h until all tissues were stained. To remove excess color from tissue, the samples were submerged in ethanol (50%) with 0.5-1% concentration of HCL for 10 h. In order to dehydrate tissues, female were rinsed in an ethanol series (50, 70, 90 and 95 %; 2 h at each conc.) and kept in glycerin to clear tissues and to store animals.

A simple classification for assessment of gonad maturity based on the presence of eggs in diverticula, ovary and oviducts were performed under stereomicroscope as described in Kosobokova, (1999). The genital system in mature stage of an adult female *Calanus glacialis* is shown in Figure 2.4. Figure 2.5 exhibits morphology of each gonad maturity stages in adult female *Calanus glacialis* according to Kosobokova, 1999. Table 2.2 provides the brief description of gonad maturity stages based on Kosobokova's, (1999) morphological definition.

The internal organization of the gonads of *Calanus* species has a dorsally laying ovary, the early development of oocytes take placae. From the ovary, two anterior diverticula extent into the head region and two posterior diverticulaes (oviducts) extent in to the abdomen. The oocytes stay at ovary during the first maturation processes and then pass into the anterior diverticulas and oviducts. Here they continue their maturation processes untill they are released from the spermathecal sacs into sea (Figure 2.4) (Niehoff, 1998).

Young immature females have a compact ovary containing few small oocytes and there were no oocytes in divetuculae and oviduct. The maturation is associated with the appearance of small oocytes in diverticulae and oviduct in the stages of semimature -a and -b. The first female in spawning conditions is in the mature stages. The semi-spent female indicates termination of the reproductive periods. The stage of spent includes empty gonads, oviducts and diverticulas. Remature females indicates the beginning of the next reproductive activity in a female (Table 2.2).

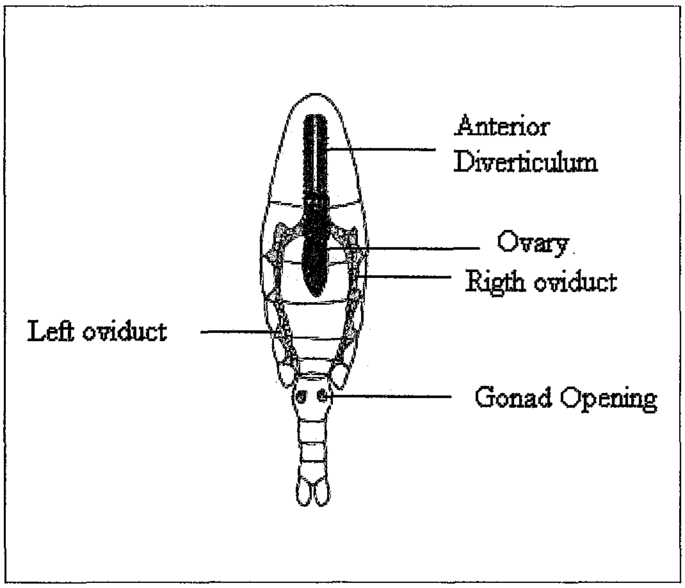

Figure 2.4: The genital system in mature stage of an adult female *Calanus glacialis* (after Kosobokova, 1999).

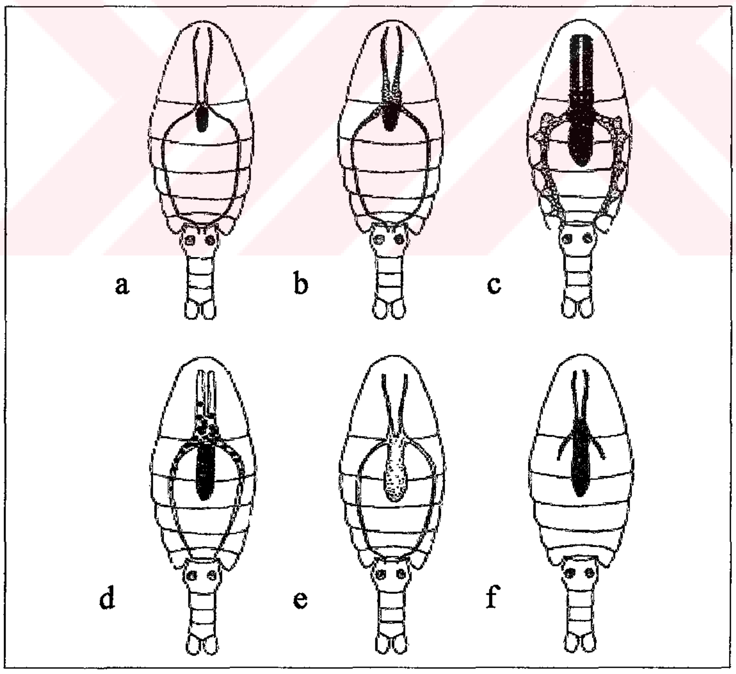

Figure 2.5: Morphology of gonad maturity stages in adult females *Calanus glacialis*: *a*- Immature, *b*- Semimature-b, *c*- Mature, *d*- Semispent, *e*- Spent, f- Remature (after Kosobokova, 1999).

Table 2.2: Gonad maturity stages of *Calanus euxinus* adult female (modified from the Kosobokova, 1999).

Maturity Stages - Morphological description

1. *Immature*; ovary compact, a few small oocytes in ovary, no oocytes in diverticulae and oviduct.

2. *Semi-mature a*; anterior ends of diverticulae widely separated, diverticulae and oviduct with single row of small oocytes.

3. *Semi-mature b*; diverticulae is widely separated and filled with several rows of small oocytes. There is more than one row of oocytes in oviducts.

4. *Mature*; diverticulae close together, filled with several rows of oocytes; pouches of large oocytes in oviducts.

5. *Semi-spent*; few large oocytes in ovary, diverticulae is filled with several rows of large oocytes and not very close. Oviducts are filled with single irregularly spread oocytes and pouches of oocytes can be seen on it.

6. *Spent*; diverticula and oviducts thin bands, no oocytes in ovary, posterior of ovary extends to the third thoracic segment.

7. *Rematuring;* large ovary filled with small oocytes extends to third thoracic segment; anterior ends of diverticula widely separated, diverticulae and oviducts with one or two rows of small oocytes.

2.3 LENGTH-WEIGHT RELATIONSHIP OF *CALANUS EUXINUS*

The same samples of abundance collections were used to determine length-weight relationship of *Calanus euxinus*. Prosome lengths of copepod (from C-1 to adults) were measured using an Olympus SZX-12 model stereomicroscope with an ocular micrometer.

After length measurements individuals were washed in distilled water, placed in pre-weighted aluminum pans, and dried at 55 °C for 24 h; samples were then cooled in a desicator during 6 h, then weighted. Blank pans without copepod washed with distilled water were prepared to ensure if there were any salts additions coming from distilled water. According to Chisholm and Roff, (1990) there were no differences between dry weights of preserved samples and freshly collected copepodites. Thus no correction for shrinkage or loss of weight on preservation is necessary.

The observations of both the independent (length) and dependent (weight) variables were commonly transformed to natural logarithms in order to conform more closely to the linear regression analyses regarding homogeneity of error variance over the range of equation. Then, length-weight regression was expressed using the equation described by Bird and Prairie (1985).

$$\ln \mathrm{W} = (b \times \ln \mathrm{L}) + \ln a + RMS/2 \qquad (2.6)$$

Where ln means natural logarithms, W is the weight (µg), *b* is the slope, L is the length (µm), *a* is the constant and *RMS* is the Residual Mean Square used as a correction factor (equivalent to the standard error of the estimation).

Students` *t* test was applied for the comparisons of the slopes of linear regressions of length-weight relationships derived from two cruises (Zar, 1984).

2.4 GROWTH RATE ESTIMATION OF *CALANUS EUXINUS*

Female instantaneous growth rates (g_r, as egg production) were derived using the equation described by Hopcroft and Roff (1998).

$$g_r = \ln (W_{Female + Egg} / W_{Female}) / t \qquad (2.7)$$

Where $W_{Female + Egg}$ is the carbon weight of female and eggs observed during the experiments, W_{Female} is the carbon weight of female, t is the incubation time (day^{-1}).

The dry-weights of female were predicted from the length-weight relationships. Dry weights were converted to carbon content assuming C (µg carbon) = 40 % of dry weight (Kiorboe and Sabatini, 1994). Egg weights, as carbon, were predicted from the direct measurements of egg diameters assuming carbon content of 0.14×10^{-6} µg C μm^{-3} (Huntlay and Lopez, 1992).

CHAPTER III

RESULTS

3.1 ENVIRONMENTAL CONDITIONS

The vertical profiles of temperature, salinity, relative fluorescence and chlorophyll-a from the stations are shown in Figure 3.1.

Sea surface temperature was ~ 20 °C and a well-developed seasonal thermocline was present during the October 2000 cruise (Figure 3.1 A). The depth of the thermocline was formed between 20 and 55 m. Fluorescence values decreased markedly below the thermocline. Sea surface salinity ranged from 17.56 to 18.22 ‰. The depth of the halocline was observed between 120 and 200 m (Figure 3.1 A).

In May 2001; the depth of the thermocline was observed between 10 and 30 m (Figure 3.1 B). Sea surface temperature was ~ 16 °C and a well-developed seasonal thermocline was observed. Remarkable decrease was observed in fluorescence values below the thermocline. Sea surface salinity generally varied between 16.90 and 18.09 ‰. The depth of halocline was observed between 130 and 180 m (Figure 3.1 B).

The total chlorophyll-a concentrations were coincident with the relative fluorescence values in October and May (Figure 3.1 A and B).

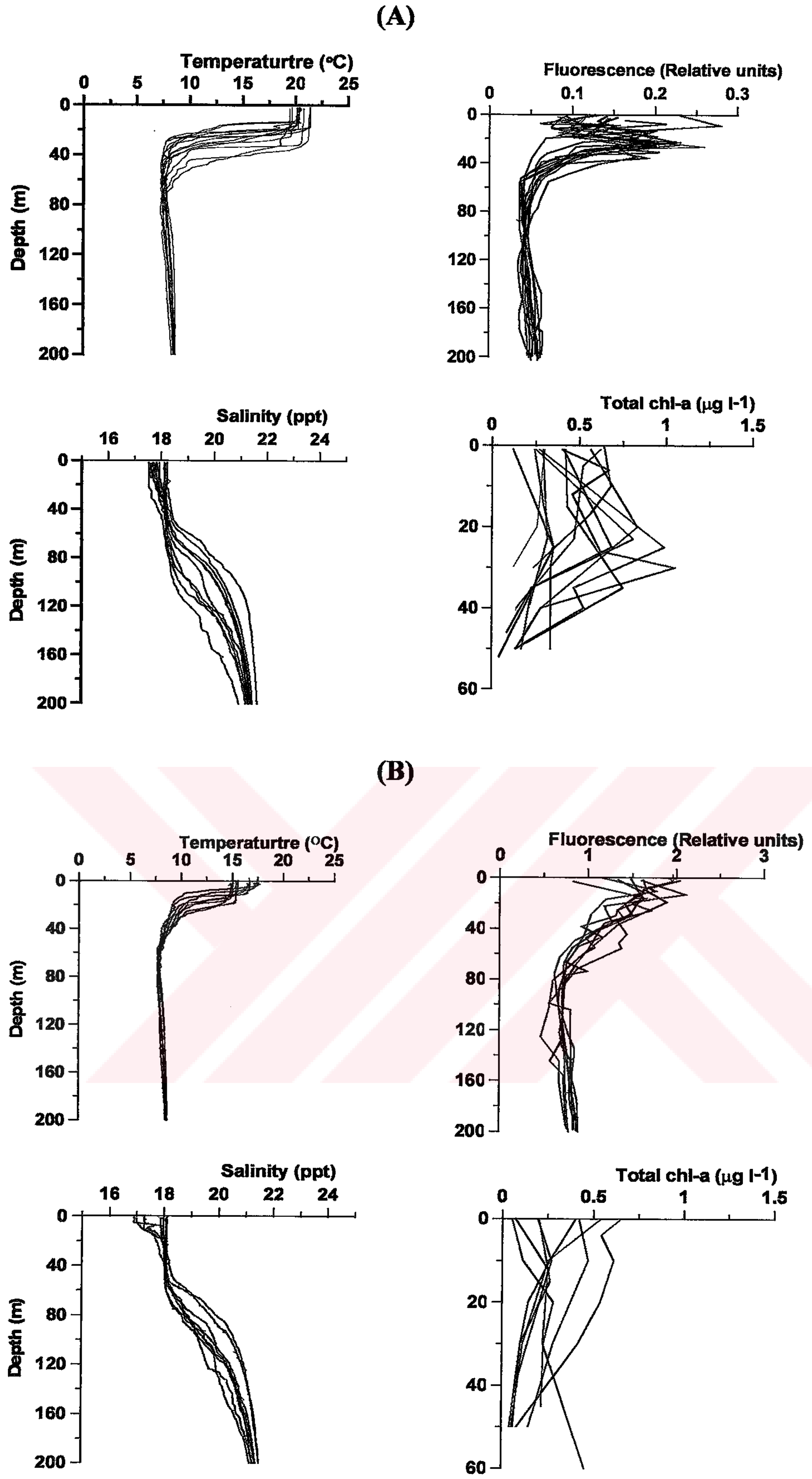

Figure 3.1: Vertical distribution of temperature, fluorescence, salinity and total chlorophyll-a concentrations in October 2000 (A) and May 2001 (B) cruises.

3.2 EGG PRODUCTION RATE OF *CALANUS EUXINUS*

In October 2000, daily egg production rates based on the 24 h experiments ranged from 0 to 7.34 ± 2.16 eggs female^{-1} day^{-1} (Figure 3.2-a). Average egg production rate for the Southern Black Sea was 1.69 ± 2.31 egg female^{-1} day^{-1}.

In May 2001, daily egg production rates ranged from 1.82 ± 1.14 to 7.02 ± 2.21 egg female^{-1} day^{-1} (Figure 3.2-b). Average egg production rate was 3.86 ± 1.93 egg female^{-1} day^{-1}. Egg production rates of *C. euxinus* in May were significantly higher than those in October (Mann-Whitney Rank Sum Test, $P \leq 0.001$).

3.2.1 Egg Production Rate and Gonad Maturity

Because the gonad stage is a measure of the reproductive status of the population, the proportion of mature female should be most closely related to egg production rates. The proportion of mature female in the experimental bottles ranged from 7.9 to 65.8 % in October and 59 to 81 % in May 2001 (Table 3.1). Figure 3.3 exhibits the relationship between proportion of mature female in the experimental bottles and egg production rates. There was a statistically significant relationship between egg production rates and the proportion of mature female in both cruise (Linear regression analysis, $P < 0.05$, $r^2 = 0.73$ in October, $P < 0.005$, $r^2 = 0.73$ in May, $P < 0.005$, $r^2 = 0.71$ for data combined from both cruises).

3.2.2 Egg Production Rate and Chlorophyll-a Concentration

Figure 3.4 shows the relationship between chlorophyll-a concentration and egg production rates. Chl-a concentration (estimated by depth integrated chlorophyll-a concentration, 0-50 m) was used as an index of available food source in the study. There are no significant relationships between egg production rates and depth integrated chlorophyll-a concentrations in October 2000 and May 2001 (Spearman Rank Correlation, $P > 0.05$).

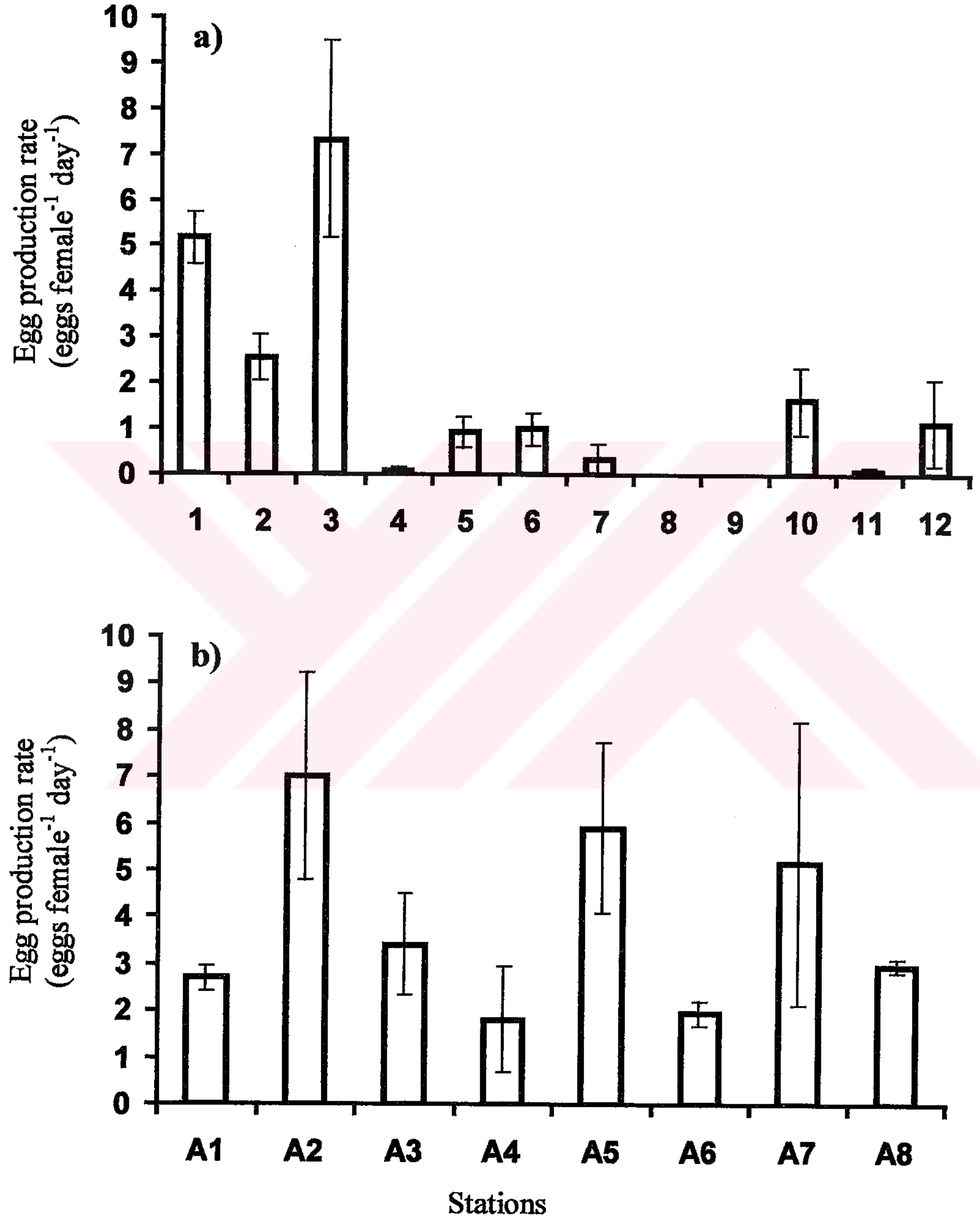

Figure 3.2: Egg production rates of *Calanus euxinus* in October 2000 (a) and May 2001 (b).

Table 3.1: Egg production rates (EPR) with standard deviations, depth integrated chlorophyll-a concentrations (<Chl-a>), chlorophyll-a concentrations < 56 µm (Chl-a). Ingestion rates and filtration rates with standard deviations, the percentages of mature female (% mature) in experimental bottles for both cruises.

St. Name	EPR (egg female^{-1} day^{-1})	<Chl-a> (mg m^{-2})	Chl-a (µg chl-a l^{-1})	Ingestion Rate (ng Chl female^{-1} day^{-1})	Filtration Rate ml ind^{-1} day^{-1}	% Mature
M0650R40 (1)	5.18 ± 0.56	22.59	0.72	-13.02 ± 9.32	-0.71 ± 0.49	65.83
M30R40 (2)	2.54 ± 0.50	30.78	0.44	40.54 ± 00.00	4.43 ± 3.13	55.00
N00R40 (3)	7.34 ± 2.16	26.46	0.22	27.61 ± 15.50	5.27 ± 3.39	65.30
L32T22 (4)	0.07 ± 0.11	19.44	0.46	21.55 ± 09.46	3.83 ± 1.93	07.87
L3750T30 (5)	0.93 ± 0.33	27.42	0.42	-51.96 ± 20.19	-4.57 ± 1.47	39.81
M30T40 (6)	0.99 ± 0.35	36.80	0.75	-22.90 ± 30.85	-1.70 ± 2.28	40.18
L00W20 (7)	0.36 ± 0.30	13.21	0.45	17.91 ± 00.44	1.86 ± 0.05	25.00
L15W40 (8)	0.00 ± 0.00	14.03	0.23	03.78 ± 20.68	0.93 ± 3.70	11.11
L40W40 (9)	0.00 ± 0.00	17.66	0.31	02.08 ± 11.79	0.81 ± 0.51	07.87
L0750X50 (10)	1.63 ± 0.73	35.73	0.40	-51.96 ± 20.19	-4.57 ± 1.47	33.75
L30Y00 (11)	0.08 ± 0.07	29.69	0.38	00.30 ± 00.00	-2.89 ± 4.84	08.33
L50Y30 (12)	1.14 ± 0.94	19.11	0.29	-12.60 ± 12.36	-2.18 ± 2.09	43.05
L20L45 (A1)	2.69 ± 0.27	23.97	0.92	36.90 ± 00.00	1.71 ± 0.00	59.23
L50L15 (A2)	7.02 ± 2.21	20.43	0.43	06.69 ± 11.99	0.69 ± 1.16	85.83
M10K45 (A3)	3.41 ± 1.09	17.28	0.61	06.16 ± 0.00	0.52 ± 0.00	77.83
L10M45 (A4)	1.82 ± 1.14	10.66	0.20	03.24 ± 0.00	0.48 ± 0.00	57.14
L50M45 (A5)	5.92 ± 1.83	9.44	0.28	00.00 ± 0.00	0.79 ± 0.00	75.22
M30M45 (A6)	1.95 ± 0.26	12.31	0.32	-33.24 ± 17.74	-3.37 ± 1.59	66.67
L30N45 (A7)	5.16 ± 3.04	14.75	0.58	64.93 ± 60.48	3.87 ± 2.63	80.81
L50N45 (A8)	2.96 ± 0.14	12.22	0.41	10.96 ± 20.03	0.95 ± 1.72	64.81

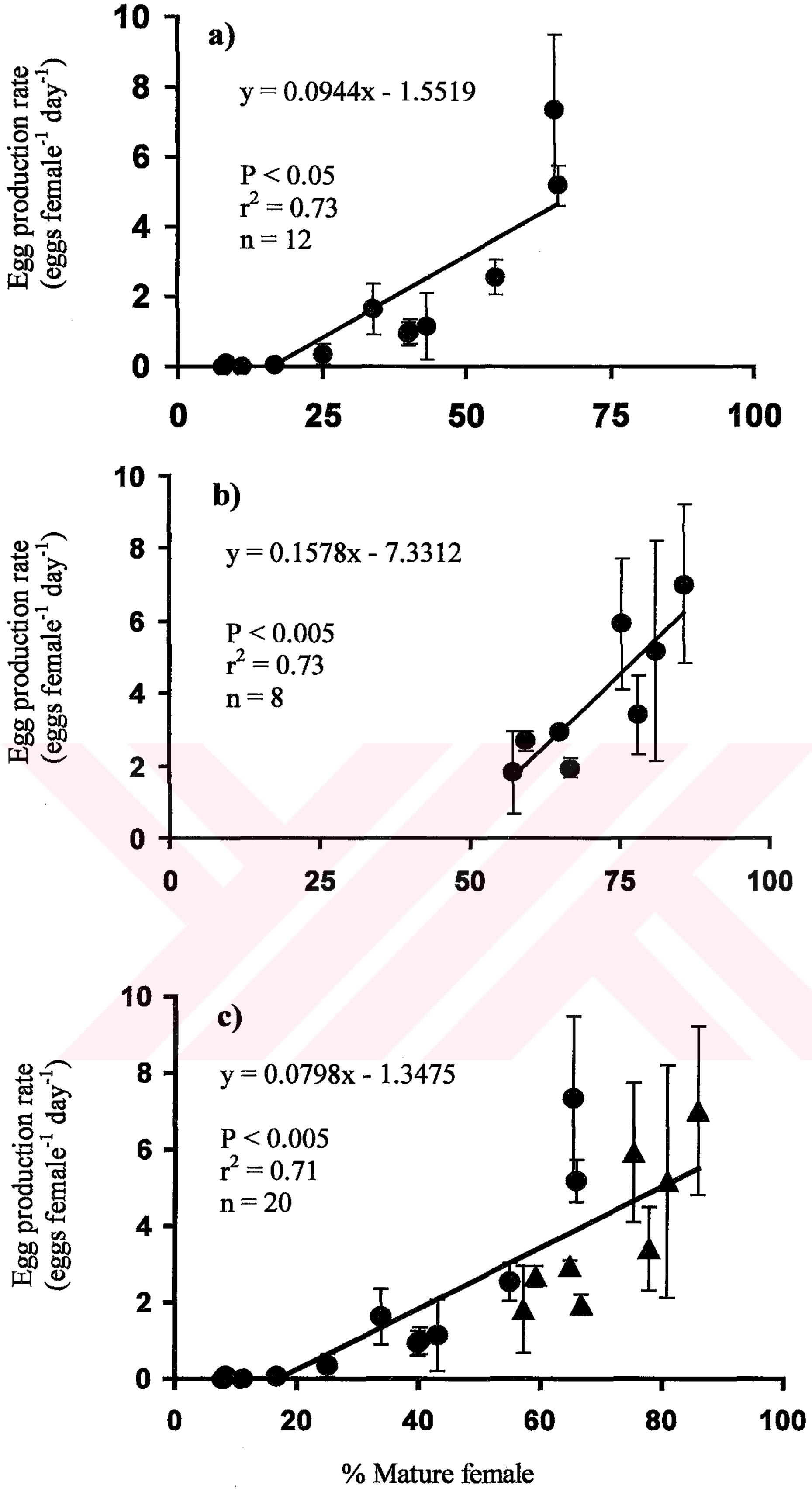

Figure 3.3: The relationship between the percentages of mature female and egg production rates (egg female^{-1} day^{-1}) in October (a), in May (b) and combined data from both cruises (c) (● October 2000, ▲ May 2001).

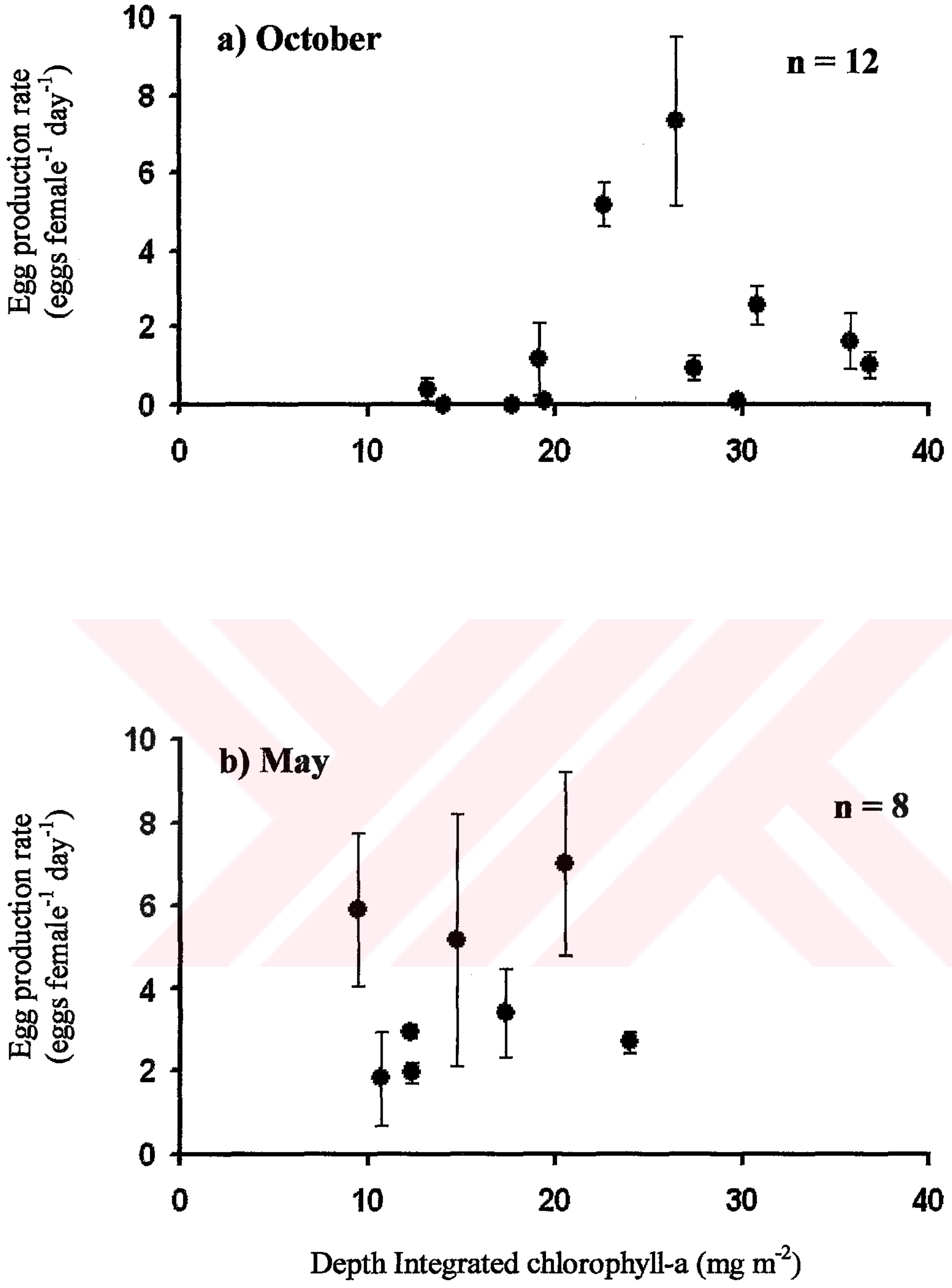

Figure 3.4: The relationship between egg production rates (egg female^{-1} day^{-1}) of *Calanus euxinus* and depth integrated chlorophyll-a concentrations (0-50 meters) in October 2000 (a) and May 2001(b).

3.3 FILTRATION AND FEEDING RATES OF *CALANUS EUXINUS* ON < 56 µm PARTICULATE CHL-A

Feeding rates of *Calanus euxinus* were measured in the same bottles used for egg production experiments. That's why; grazing rates of *Calanus euxinus* were estimated on chlorophyll –a containing particles in < 56-µm size. *Calanus* grazing on < 56 µm particulate chl-a ranged from 0.30 to 40.54 ng chl-a female^{-1} day^{-1} in October 2000 and from 0 to 64.93 ng chl-a female^{-1} day^{-1} in May 2001. Ingestion rates of *C. euxinus* in October were not significantly different than the ingestion rates in May (t-test, $P = 0.73$).

Some negative filtration rate values were estimated in this study; this is probably due to the enhanced growth of algae in experimental bottles caused by ammonium excretion by copepods. These problems have been recorded by previous studies (Roman and Rublee, 1980; Peters and Downing, 1984; Miller at *al.*, 1995). These negative values were excluded when the Figures 3.5 and 3.6 were fitted.

The relationships between chlorophyll-a concentrations and ingestion rates of *C. euxinus* are illustrated in Figure 3.5. There were no significant relationship between chl-a concentrations and ingestion rates of *C. euxinus* in October and May cruises (Figure 3.5-a and b), but when the data from both cruise were pooled, the positive correlation were observed between ingestion rate and chl-a concentration (Figure 3.5-c) (Spearman Rank Correlation $r=0.56$, $P<0.036$).

The relationship between filtration rates of *Calanus euxinus* and < 56 µm chlorophyll-a concentrations is exhibited in Figure 3.6. Filtration rates seem not to be correlated with chlorophyll-a concentration.

The relationship between egg production rates and ingestion rates of *Calanus euxinus* is presented in Figure 3.7. There was no statistically significant relationship between egg production rates and ingestion rates.

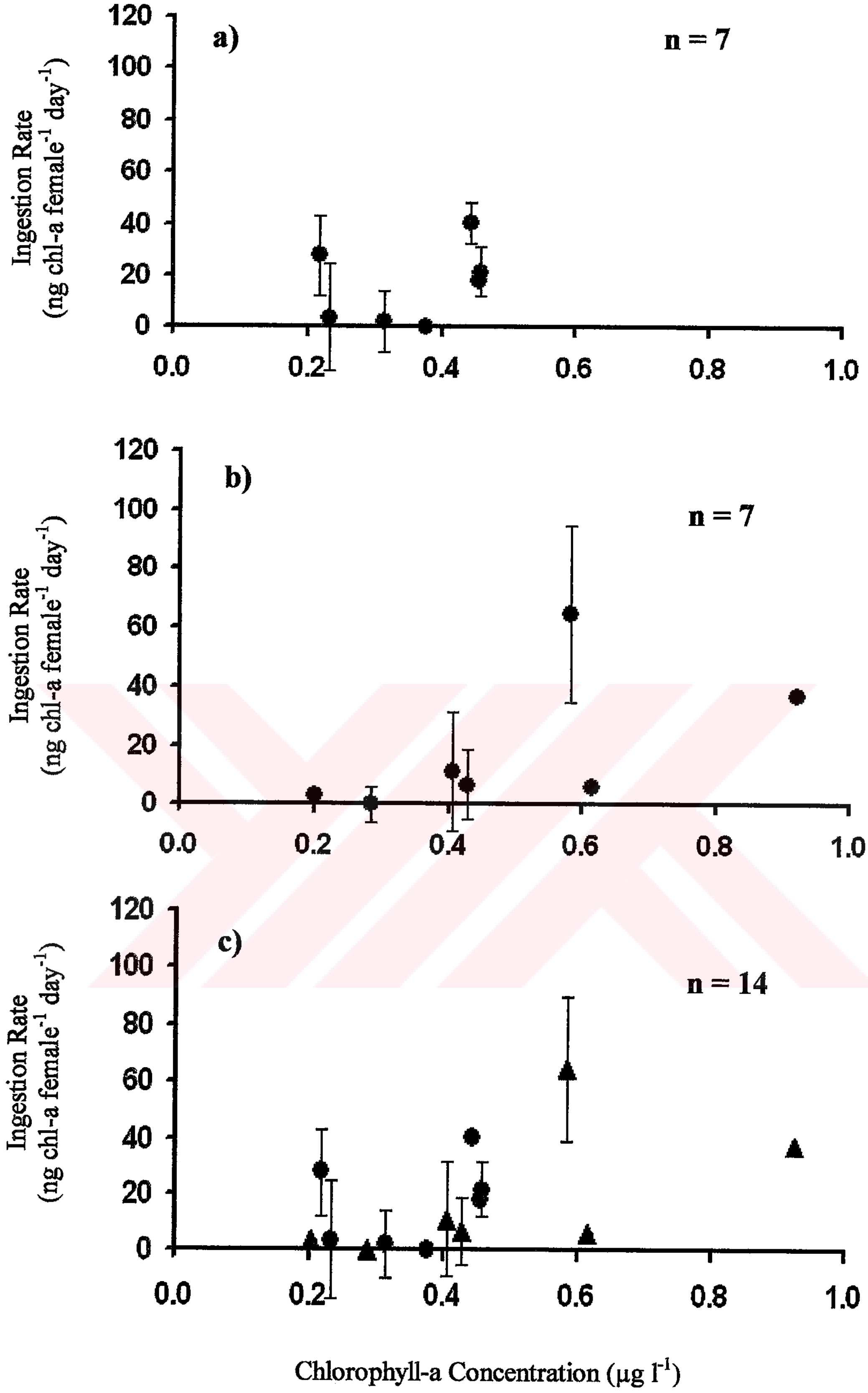

Figure 3.5: Relationship between ingestion rate of *Calanus euxinus* and chlorophyll-a concentration measured in the experimental bottles containing < 56 µm filtered seawater from the depth of fluorescence maximum in October (a), in May (b) and data combined from both cruises (c) (● October, ▲ May).

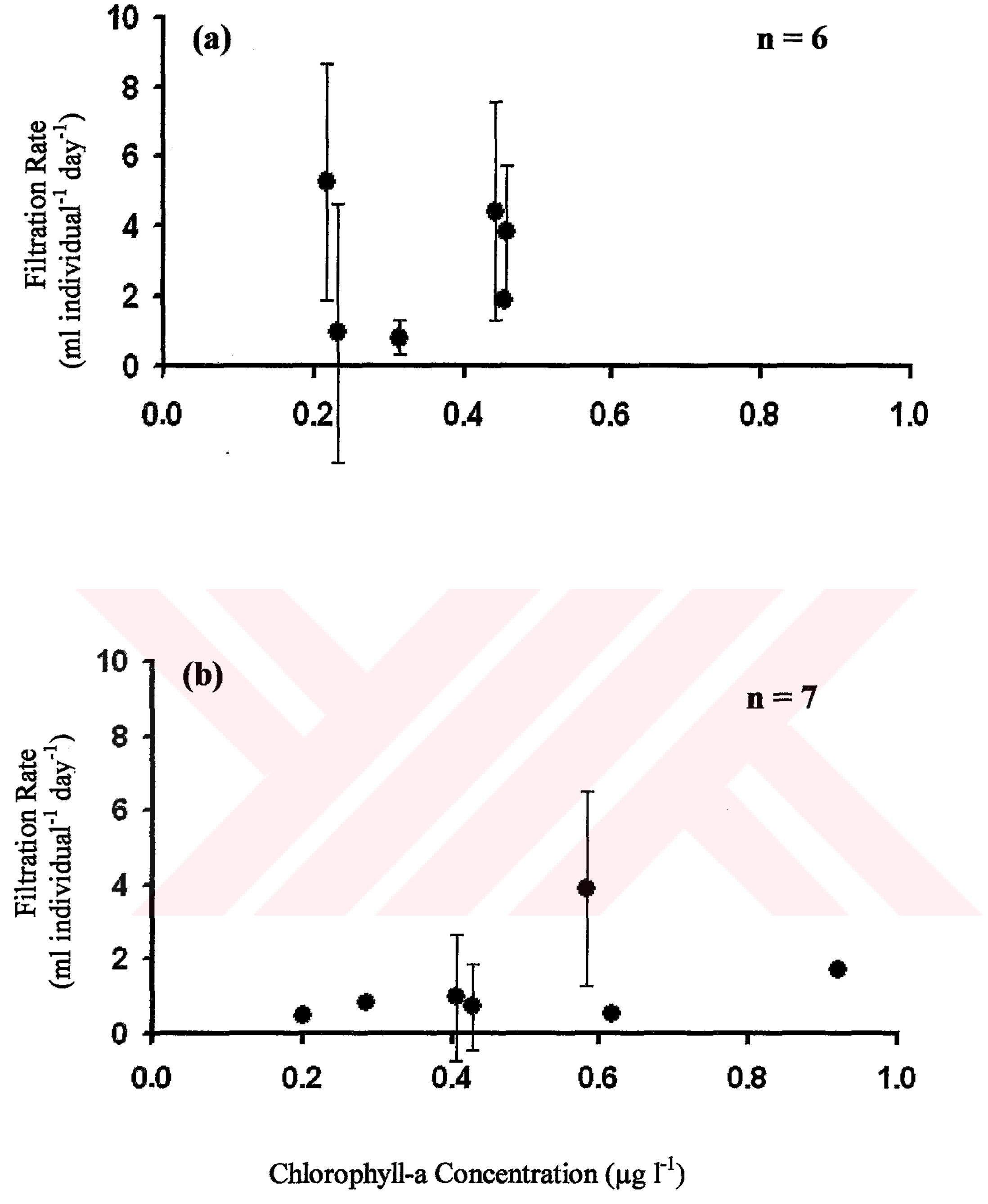

Figure 3.6: Relationship between filtration rates of *Calanus euxinus* and chlorophyll-a concentration measured in the experimental bottles containing < 56 μm filtered seawater from the depth of fluorescence maximum in October 2000 (a) and May 2001 (b).

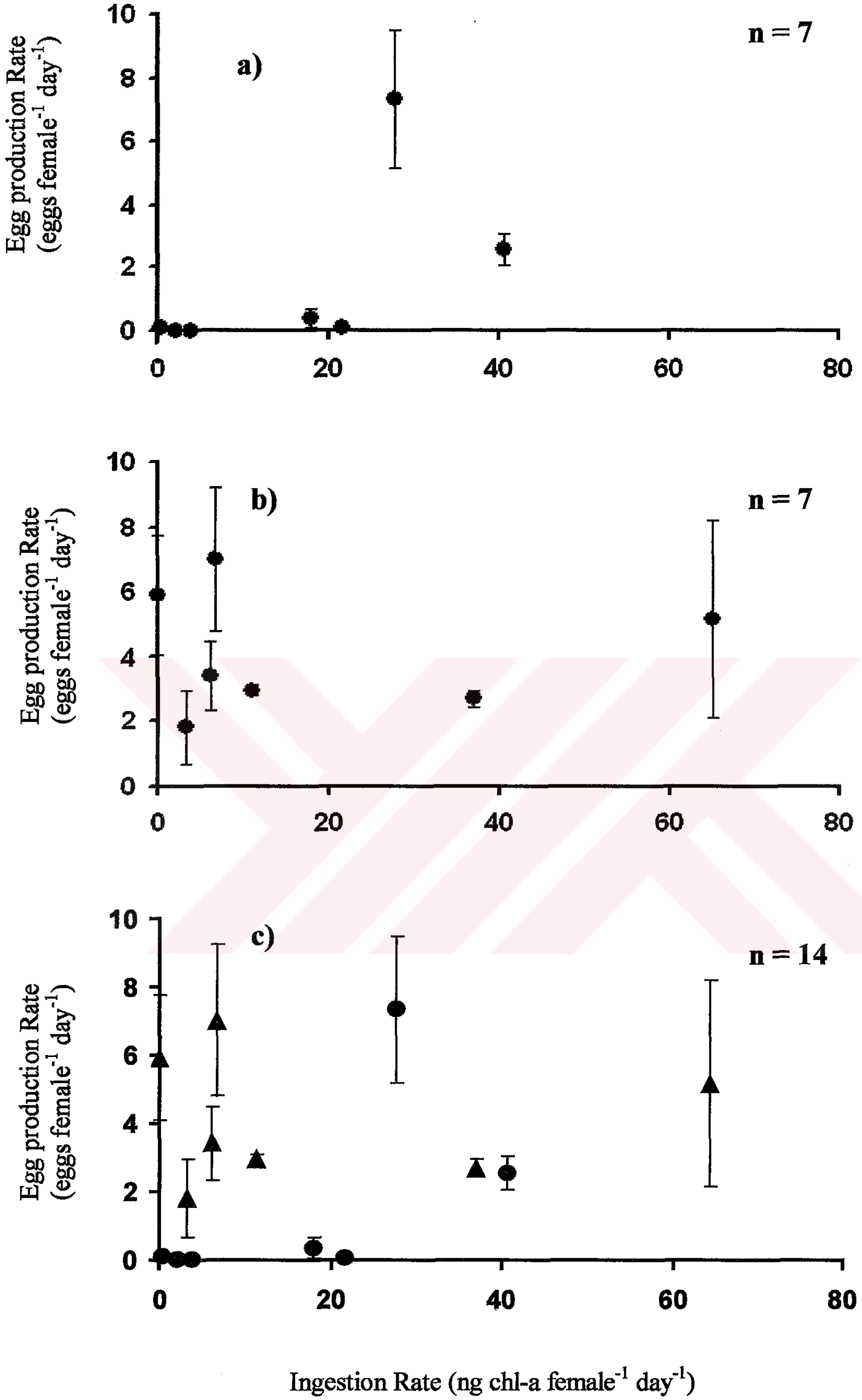

Figure 3.7: Egg production rates versus ingestion rates of *Calanus euxinus* in October 2000 (a), May 2001 (b) and data from both cruises (c) (● October 2000, ▲ May 2001).

3.4 DIEL CHANGES IN EGG PRODUCTION RATE OF *CALANUS EUXINUS*

Diurnal differences in egg production rates of *Calanus euxinus* are shown in Figure 3.8. Female *Calanus euxinus* showed strong nocturnal egg laying behavior. Mean egg production was 0.2 egg female^{-1} h^{-1} at nighttime and 0.01 egg female^{-1} h^{-1} at daytime in October 2000. In May 2001, the mean egg production rate was 0.6 egg female^{-1} day^{-1} at nighttime and 0.06 egg female^{-1} day^{-1} at daytime.

To understand the diel egg laying behavior of *C. euxinus* in the Black Sea, different groups of females were used for daytime and nighttime incubation periods. From the previous results it is shown that maturity stage of the individuals is most closely related parameter to egg production rates (see Fig. 3.3). To compare the day and nighttime egg production rate, the percentage of mature females used during the day and nighttime incubation should be almost same. To make sure that the day and night time egg production rate is comparable, the percentages of mature females used in these experiments were determined. In October 2000, the percentage of mature females was 69 % during the day time and 75 % during the night time; in May 2001, the percentage of mature females was 82 % during the day time, and 77 % during the night time. Thus the daytime and nighttime results are comparable.

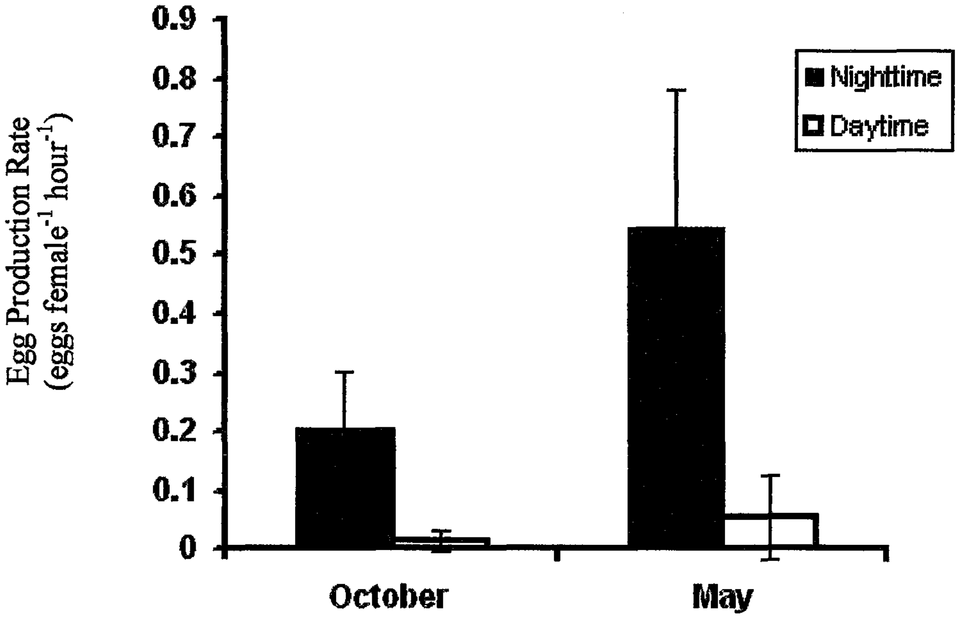

Figure 3.8: Diel changes in egg production rates of *Calanus euxinus* in October 2000 and May 2001.

3.5 ABUNDANCE AND STAGE COMPOSITION OF *CALANUS EUXINUS*

Table 3.2 and 3.3 show the abundances (individual m^{-3}) and prosome length (μm) of copepodite stages and adult of *Calanus euxinus* from each station in October and May, respectively. The abundance of *Calanus euxinus* did not differ much between October and May, with 33 – 35 individuals m^{-3}.

In October, older copepodite stage C-5 (36 %) and female (25 %) formed the half of the population. Copepodite stage C-1 and males made up only 3 – 4 % of the population. In May, early copepodite stages C-1, C-2, C-3, C-4 were made up 64 % of the population (Figure 3.9). Males were very rare and made up only 2 % of the population.

Females were always found in much higher numbers than males during the two sampling periods. Female : male ratios varied from 2:1 to 21:1 in May 2001 and 2:1 to 14:1 in October 2000.

Mean prosome length of all copepodites stages and adults is slightly higher in October than May. In both sampling periods, the prosome length of female is always slightly longer than the male prosome length (Table 3.2 and 3.3).

Figure 3.10 exhibits the relationship between depth integrated chlorophyll-a concentration and the dry weight of copepodite stages and adults (female + male). Dry weight of copepodite stages C-2, C-3, C-4 seems to be negatively correlated with the depth integrated chlorophyll-a concentration. However, dry weight of adult (female + male) seems to be correlated positively with the depth integrated chlorophyll-a concentration. On the other hand, the dry weight of C-1 and C-5 did not show any tendency with the depth integrated chlorophyll-a concentration.

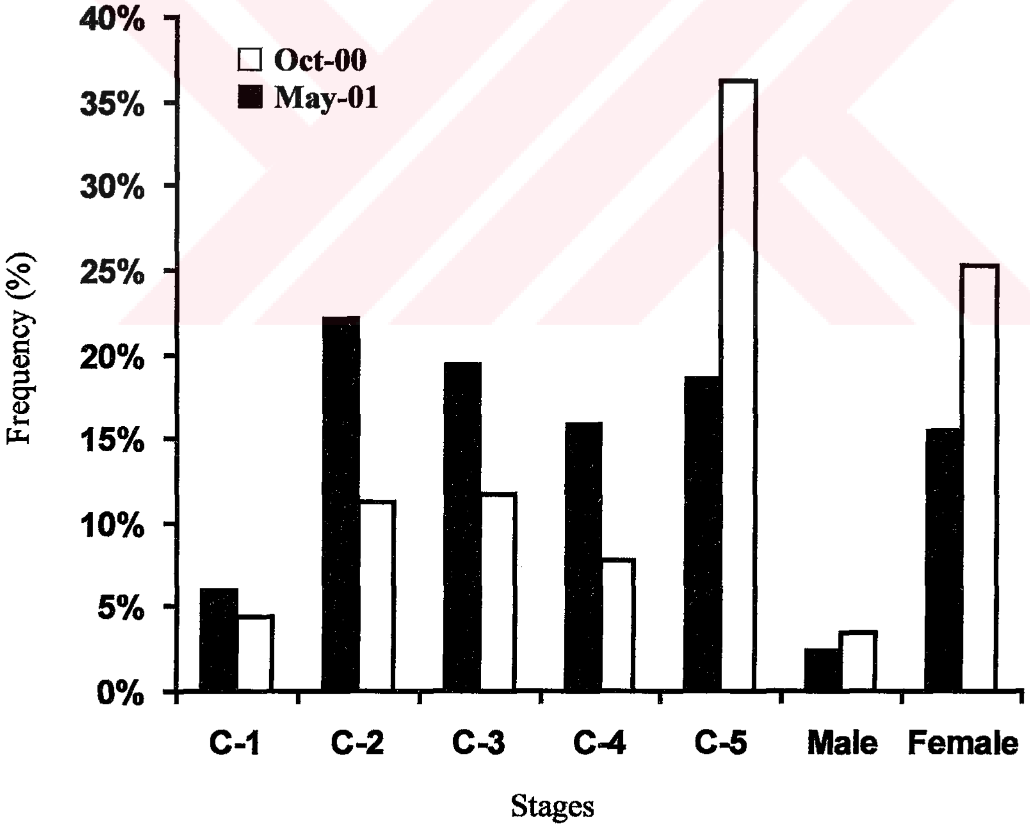

Figure 3.9: The average frequency of stage composition of *Calanus euxinus* in October 2000 and May 2001.

Table 3.2: Abundance (Abd., individual m^{-3}) and Prosome Length (P.L., µm) of copepodite stages and adults *C. euxinus* from each station in October 2000.

Stations	C-1		C-2		C-3		C-4		C-5		Male		Female	
	Abd.	P.L.	Abd.	P.L.	Abd.	P.L.	Abd.	P.L.	Abd.	P.L.	Abd.	P.L.	Abd.	P.L.
M0650R40 (1)	1.30	740	3.64	970	4.42	1320	8.06	1788	14.82	2340	0.78	2544	16.64	2706
N00R40 (2)	2.23	715	1.19	938	1.19	1263	1.04	1865	3.71	2289	1.78	2513	15.60	2642
M30R40 (3)	1.12	732	0.51	989	1.02	1283	0.51	1643	13.87	2399	1.12	2558	10.81	2679
L40W40 (4)	1.36	722	9.18	976	9.11	1322	4.96	1708	3.94	2265	1.09	2489	3.60	2701
L30Y00 (5)	3.01	749	3.01	936	1.92	1319	1.56	1729	13.34	2249	1.08	2569	4.81	2689
L50Y30 (6)	0.52	748	0.61	989	0.43	1296	0.26	1672	16.55	2326	0.95	2523	10.31	2664
L32T22 (7)	0.52	740	1.77	961	5.87	1355	3.70	1717	4.59	2290	0.49	2523	0.95	2698
L3750T30 (8)	0.96	680	2.55	955	6.38	1277	4.08	1771	6.38	2312	1.28	2519	5.10	2658
M30T40 (9)	2.80	730	3.12	988	1.44	1268	1.28	1725	17.12	2346	0.80	2565	7.44	2670
L00W20 (10)	2.50	685	14.56	944	13.62	1274	4.16	1713	20.69	2287	2.29	2505	13.31	2688
L15W40 (11)	0.90	737	3.09	977	2.49	1277	1.51	1690	12.51	2368	1.13	2527	7.99	2705
L0750X50 (12)	1.44	703	4.33	980	1.73	1285	1.44	1747	25.28	2319	1.90	2508	10.11	2673
MEAN	**1.55**	**723**	**3.96**	**967**	**4.14**	**1295**	**2.71**	**1731**	**12.73**	**2316**	**1.22**	**2529**	**8.89**	**2681**
STDV	**0.86**	**23**	**4.04**	**20**	**4.00**	**28**	**2.31**	**58**	**6.91**	**43**	**0.52**	**25**	**4.83**	**20**

Table 3.3: Abundance (Abd., individual m^{-3}) and Prosome Length (P.L., μm) of copepodite stages and adults *C. euxinus* from each station in May 2001.

Stations	**C-1**		**C-2**		**C-3**		**C-4**		**C-5**		**Male**		**Female**	
	Abd.	**P.L.**	**Abd.**	**P.L.**	**Abd.**	**P.L.**	**Abd.**	**P.L.**	**Abd.**	**P.L.**	**Abd.**	**P.L.**	**Abd.**	**P.L.**
M30M45 (A1)	1.23	694	1.05	943	0.68	1283	1.05	1687	0.83	2179	0.18	2402	0.37	2484
L50L15 (A2)	0.14	732	2.13	952	3.10	1249	2.62	1632	2.69	2190	0.21	2305	2.48	2511
M10K45 (A3)	0.26	757	3.95	931	9.21	1249	9.21	1607	7.37	2120	0.66	2411	2.24	2572
L50M45 (A4)	2.17	724	4.29	1034	4.82	1345	4.11	1793	7.99	2046	0.47	2336	4.05	2460
L30N45 (A5)	0.26	685	3.75	941	6.05	1262	4.18	1617	7.50	2109	0.34	2399	4.77	2626
L20L45 (A6)	2.95	708	8.15	920	7.37	1231	4.33	1612	3.03	2074	1.13	2409	7.11	2609
L10M45 (A7)	6.10	672	12.83	957	11.23	1300	7.63	1675	6.79	2282	0.90	2548	8.94	2687
L50N45 (A8)	3.14	689	23.02	969	9.42	1297	9.42	1589	13.60	2153	2.62	2454	11.51	2558
MEAN	**2.03**	**708**	**7.40**	**956**	**6.48**	**1277**	**5.32**	**1652**	**6.23**	**2144**	**0.81**	**2408**	**5.18**	**2563**
STDV	**2.04**	**28**	**7.34**	**35**	**3.54**	**37**	**3.08**	**66**	**4.01**	**74**	**0.80**	**73**	**3.75**	**77**

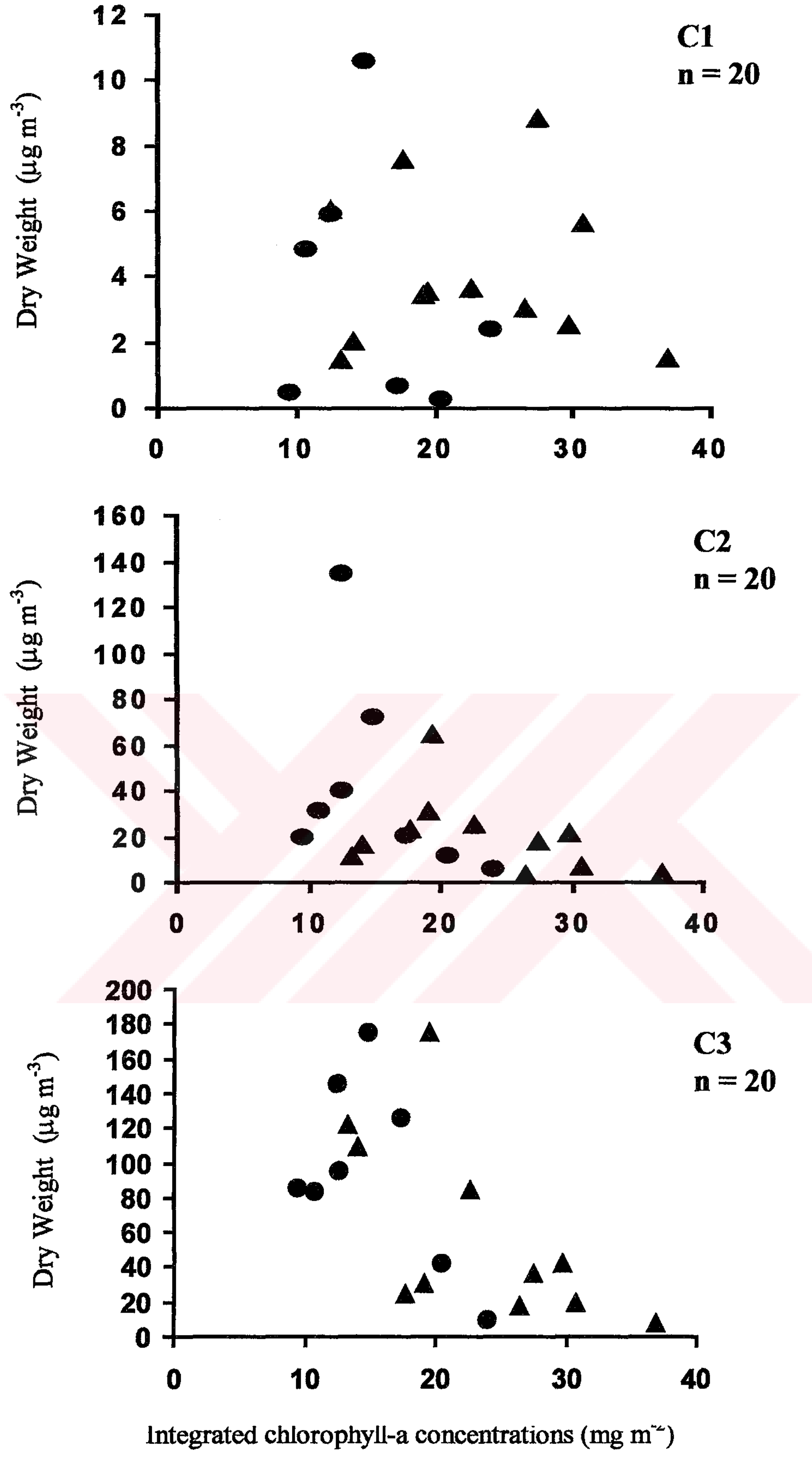

Figure 3.10: Dry weight versus depth integrated chlorophyll-a concentrations (0-50 m, mg m^{-2}) in each copepodite stages and adults. (Data from both cruises were combined, ▲ October 2000 and ● May 2001).

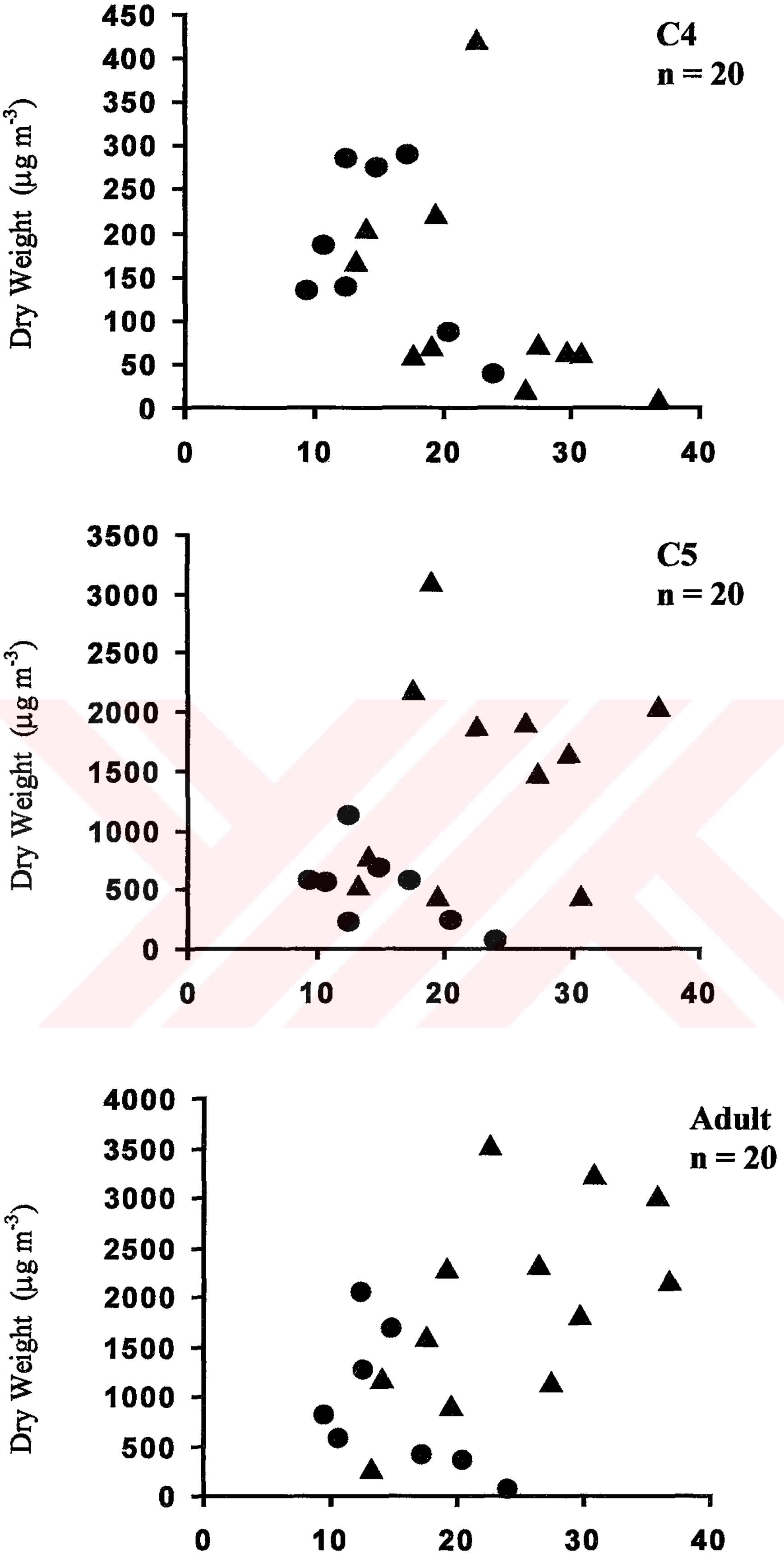

Figure 3.10: Continued

3.6 GONAD MATURITY

The percentages of gonad maturity stages in the female *Calanus euxinus* in October 2000 and May 2001 are shown in Table 3.4.

The mature female formed 65 and 60 % of the female population in October 2000 and May 2001, respectively. The proportion of young immature females did not exceed 1-6 % of the female population in the two sampling periods.

Semimature females made up only 17 and 26 % of the population in October 2000 and in May 2001 respectively.

The semispent female indicates termination of the reproductive period and it consists of 15 to 12 % of the population in October 2000 and in May 2001, respectively. Spent females, including empty gonads, and remature females, indicating the beginning of the next reproductive activity, were not observed from the preserved samples in both sampling period.

3.7 LENGTH-WEIGHT RELATIONSHIP OF *CALANUS EUXINUS*

Length-weight relationship for *C. euxinus* is shown in Figure 3.11. Regression coefficients (r^2) for October 2000 period (Figure 3.11-a) is calculated as 0.96 and for May 2001 (Figure 3.11-b) is calculated as 0.95.

There were no significant differences between the slopes and intercepts of the Length-Weight regressions for May 2001 and October 2000 periods (Students' t test, comparisons of slopes, $P < 0.0005$; comparisons of elevation, $P < 0.005$, Table 3.5).

Table 3.4: The percentage of gonad maturity stages in female *Calanus euxinus* in October 2000 and May 2001 (Imt = Immature, S.Mt-a = Semi Mature-a, S.Mt-b = Semi Mature-b, Mt = Mature, S. Spt = Semi Spent, Spt = Spent, Rmt = Remature).

Stations	**% Imt**	**% S. Mt - a**	**% S. Mt - b**	**% Mt**	**% S. Spt**	**% Spt**	**% Rmt**
October - 00							
M0650R40 (1)	0	0	13.30	85.00	1.66	0	0
N00R40 (2)	0	0	18.61	37.20	44.18	0	0
M30R40 (3)	0	1.81	5.45	80.00	12.72	0	0
L40W40 (4)	0	3.27	3.27	77.00	16.39	0	0
L30Y00 (5)	2.60	3.89	3.89	75.30	14.28	0	0
L50Y30 (6)	0	1.78	7.14	66.10	25.00	0	0
L32T22 (7)	0	6.89	25.86	48.30	18.96	0	0
L3750T30 (8)	0	3.26	9.78	77.20	9.78	0	0
M30T40 (9)	0	0	12.30	63.10	24.61	0	0
L00W20 (10)	1.40	8.21	10.95	68.50	10.95	0	0
L15W40 (11)	5.89	11.78	44.11	38.20	0	0	0
L0750X50 (12)	0	1.78	25.00	73.20	0	0	0
Mean	**0.82**	**3.56**	**14.98**	**65.76**	**14.88**	**0.00**	**0.00**
May - 01							
M30M45 (A1)	5.263	15.8	36.84	42.11	0	0	0
L50L15 (A2)	0	5.26	10.53	75.41	8.77	0	0
M10K45 (A3)	0	4.35	19.57	63.02	13.00	0	0
L50M45 (A4)	1.695	5.08	16.95	50.85	25.41	0	0
L30N45 (A5)	0	2.3	8.046	79.33	10.35	0	0
L20L45 (A6)	0	4	18.67	54.74	22.72	0	0
L10M45 (A7)	0	1.23	20.99	67.90	9.88	0	0
L50N45 (A8)	0	11.9	28.57	50.00	9.52	0	0
Mean	**0.87**	**6.24**	**20.02**	**60.4**	**12.5**	**0.00**	**0.00**

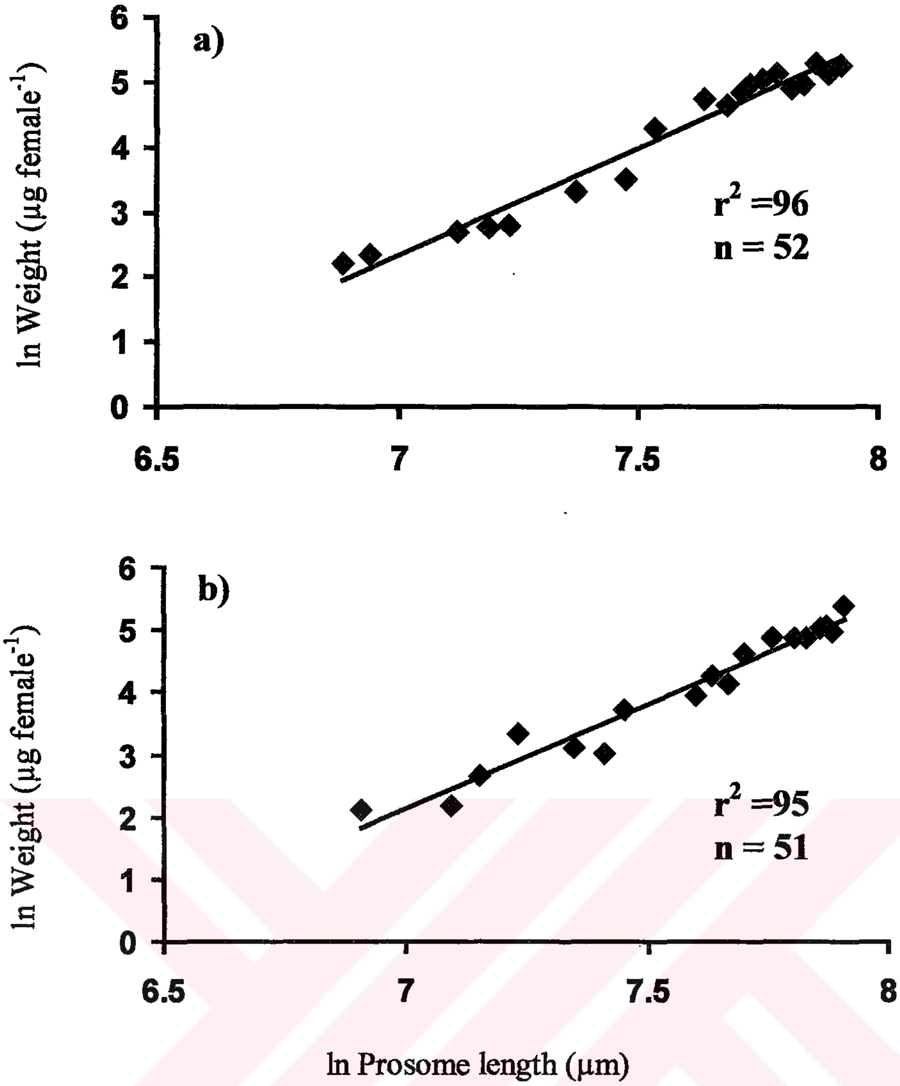

Figure 3.11: Length versus dry weight of *Calanus euxinus* in October 2000 (a) and May 2001 (b).

Table 3.5: Length- dry weight regressions of *Calanus euxinus*. W: dry weight (µg), L: Prosome length (µm), n: Number of data, RMS: Residual Mean Square of the regressions.

Season	**Regression**	**n**	**RMS**	**r^2**	**P - value**
Oct-00	ln W = 3.30 lnL-20.76	52	0.0465	0.96	<0.0005
May-01	ln W = 3.33 lnL-21.13	51	0.0657	0.95	<0.0005

3.8 GROWTH RATES OF *CALANUS EUXINUS*

Instantaneous growth rate derived from egg production experiments (i.e. the reproductive growth rate of females, g_r) ranged between min 0.00 to max 0.051 ± 0.01 d^{-1} (mean value was 0.011 ± 0.015 d^{-1}) in October 2000 and from 0.013 ± 0.002 to 0.053 ± 0.016 d^{-1} in May 2001 (mean value was 0.029 ± 0.015 d^{-1}).

Figure 3.12 shows the relationship between instantaneous reproductive growth rate (g_r) and depth integrated chlorophyll-a concentration. There was no any trend found between growth rates and chlorophyll-a concentration. On the other hand, instantaneous reproductive growth rate of *Calanus euxinus* decreases significantly with the increasing dry weight in October 2000 (Spearman Rank Correlation; r^2=-0.63, $P<0.05$), but there is no correlation between growth rate and dry weight in May 2001 (Figure 3.13-a and b). However, when the data from both cruise were pooled, negative significant correlation was observed (Spearman Rank Correlation; r^2=-0.45, $P<0.05$) (Figure 3.13-c).

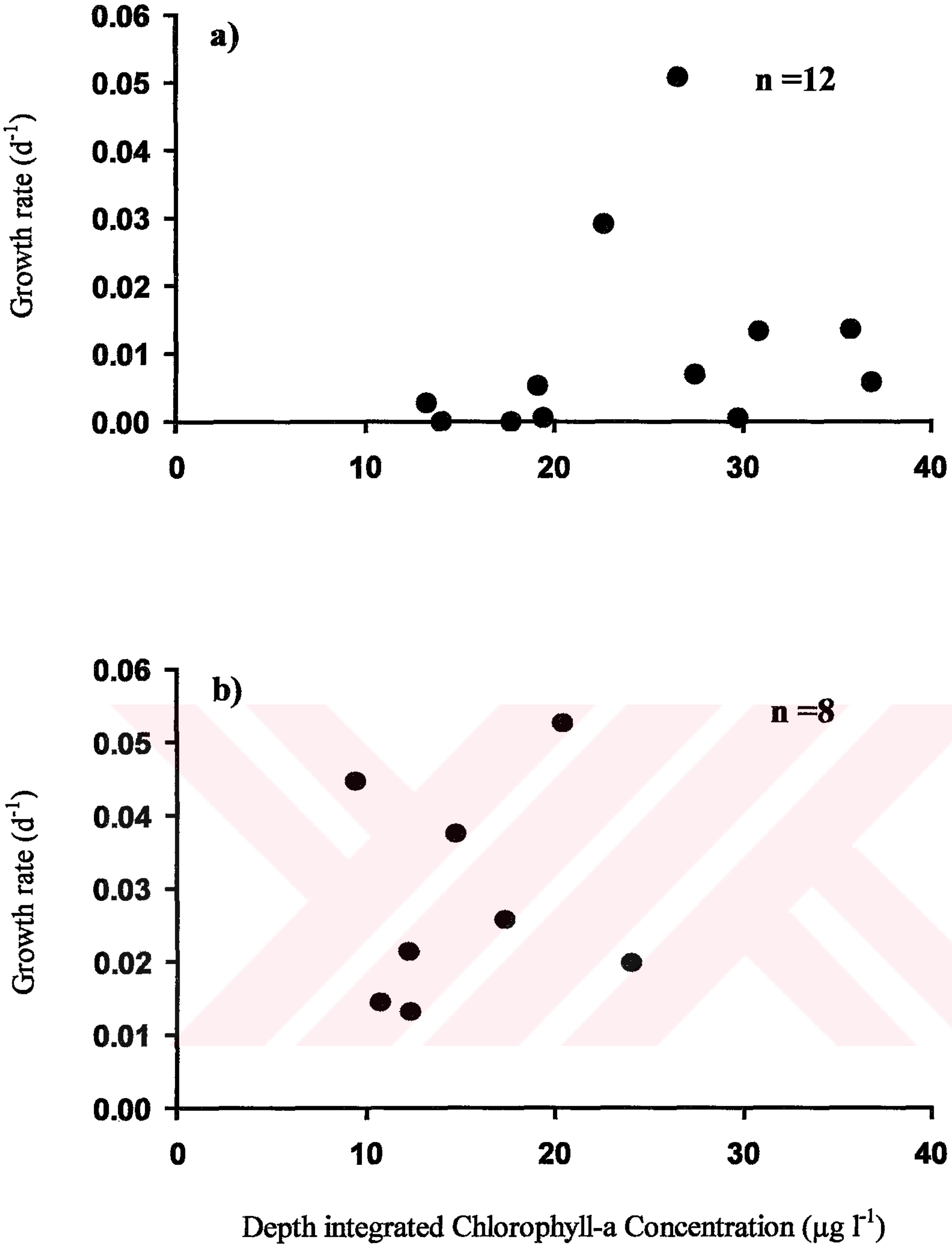

Figure 3.12: The relationship between instantaneous reproductive growth rates and depth integrated chlorophyll-a concentrations in October 2000 (a) and May 2001 (b).

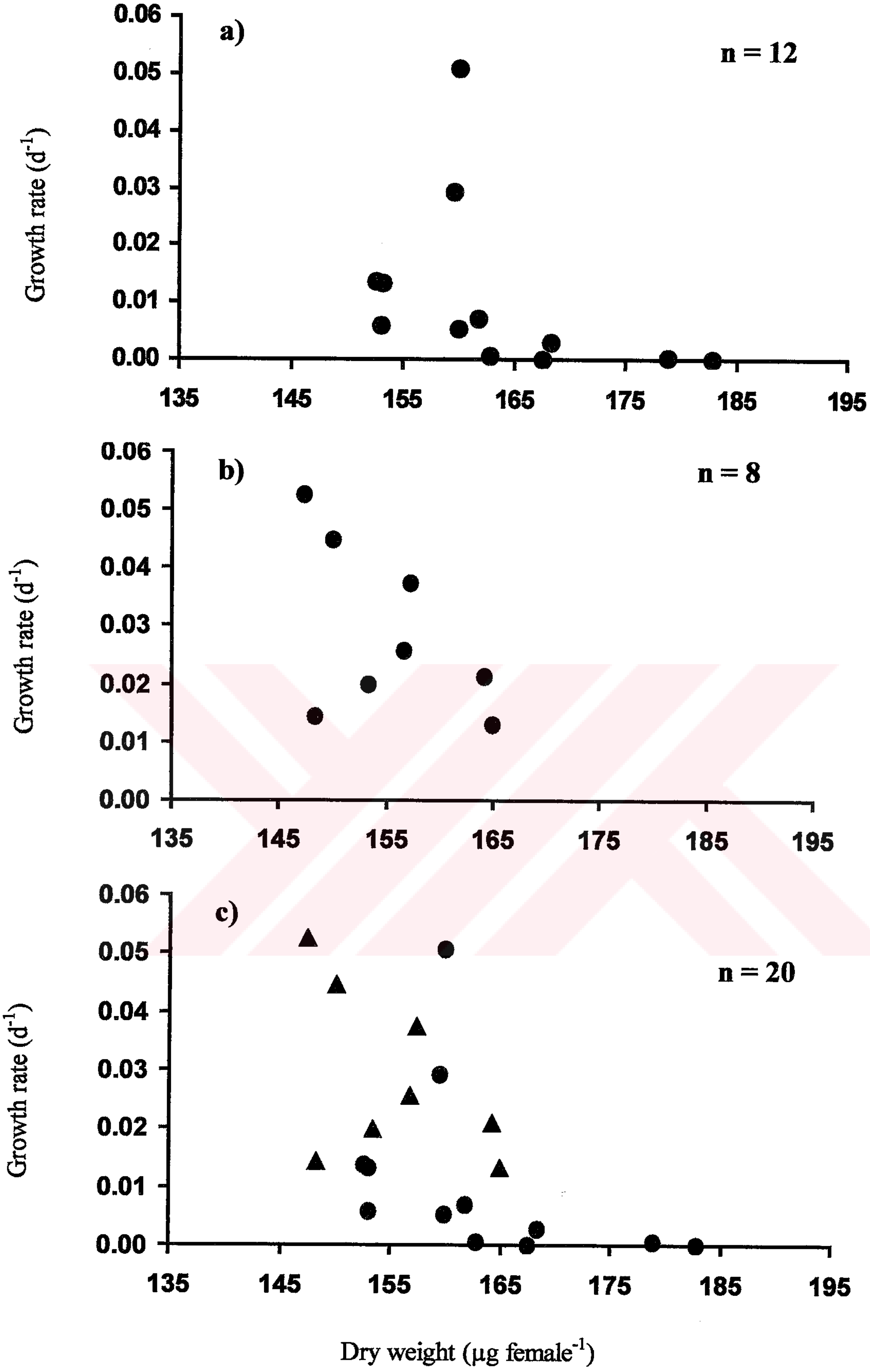

Figure 3.13: The relationship between instantaneous growth rates and adult female dry weight of *Calanus euxinus* in October 2000 (a), May 2001(b), and data from both cruises (c) (● October 2000, ▲ May 2001).

CHAPTER IV

DISCUSSION

4.1. EGG PRODUCTION RATES OF *CALANUS EUXINUS*

Average daily egg production rates based on the 24 h bottle experiments were identical to the egg production rates reported earlier for *Calanus euxinus* in the Black Sea (Arashkevich *et al.*, 1998; Sazhina *et al.* 1996). Egg production rates of *Calanus euxinus* were reported to be in the range of 2 to 11 egg female^{-1} day^{-1} in September – October 1996 in the southern Black Sea by Arashkevich *et al.*, (1998) and 3 and 7 egg female^{-1} day^{-1} in September in the western Black Sea by Sazhina *et al.*, (1996). However, the results found in this study are lower (0 to 7 egg female^{-1} day^{-1}) than those reported for the other *Calanus* spp. from the other regions of the world. Egg production rates of calanoid copepods are regulated by multiplicity of endogeneus and environmental factors, dominant factors being temperature (Huntley and Lopez, 1992; Runge and Plourde, 1996) and food availability (Runge ,1985; Kleppel, 1992; Kleppel *et al.*, 1998; Jónasdóttir, 1994; Durbin *et al.*1983). The reproductive behavior of big copepods like *Calanus* is linked strongly to phytoplankton production. *Calanus* can only produce eggs at maximum rates under conditions of dense phytoplankton blooms (Gutiérrez and Peterson, 1999). Generally phytoplankton concentration is determined by measuring chlorophyll-a concentration in the field. Previously recorded egg production rates of different *Calanus* spp. from different regions show that egg production rates of *Calanus* sp. are higher in the conditions of higher *in situ* chlorophyll-a concentration. Table 4.1 exhibits egg production rates of *Calanus* sp. measured in the field by using the bottle incubation techniques from different regions. It can be concluded that egg production rates tended to increase with the increasing chl-a concentration. In the present study, chlorophyll-a concentrations were found lower than these regions given in Table 4.1. Egg production rates of copepods can be species-specific. For example, in the study of Gutiérrez and Peterson, (1999), *C. pacificus* and *C. marshallae* shows different egg production rates in the same environmental conditions and in the same place and time (average daily egg production rates were about 43 and 27 egg female^{-1} day^{-1} respectively).

Table 4.1: The range of egg production rates of different *Calanus* sp. from different regions (Eggs = egg female^{-1} day^{-1}, Chl-a = Chlorophyll-a concentration).

Species	Eggs	Seasons	Regions	Chl-a (µg l^{-1})	References
C. pasificus	28 - 52	Summer	North Atlantic	13.5 - 16.2	Gutiérrez and Peterson, (1999)
C. marshallae	15 - 37	Summer	North Atlantic	13.1 - 15.3	Gutiérrez and Peterson, (1999)
C. simillimus	3 - 20	Autumn	South Atlantic	0.5 - 1.0	Miralto *et al.*, (1998)
C. finmarchicus	2 - 46	Summer	Southwest Iceland	2 - 10	Gislason and Astthorsson, (2000)
C. finmarchicus	0 - 7	Spring	Southwest Iceland	0.2 - 1	Gislason and Astthorsson, (2000)
C. finmarchicus	2 - 33	Spring	North Norway	3 - 8	Diel and Tande, (1992)
C. euxinus	2 - 7	Spring	The Black Sea	0.2 - 0.9	The present study
C. euxinus	0 - 7	Autumn	The Black Sea	0.2 - 0.8	The present study

4.1.1 Egg Production and Chlorophyll-a Concentration

Previous laboratory and field studies have shown that food availability is one of the major environmental factors controling the copepod reproductivity (Durbin *et al.*, 1983; Runge, 1985; Kleppel, 1992; Kleppel *et al.,* 1998; Kleppel and Hazzard, 2000). In this study, total Chl-a concentration was measured as an index of available food source in the field. Egg production rates of *Calanus euxinus* were only weakly related to in situ food concentration (as estimated by depth integrated chlorophyll concentration). Lack of relationship between egg production and chlorophyll concentration have been reported for some copepod species in a few studies (Saiz e*t al.,*1999; Gutiérrez and Peterson, 1999), but statistically significant relationship between egg production and *in situ* chlorophyll concentration was observed by many scientists (Richardson and Verheye, 1998; Camphell and Head, 2000; Runge, 1985; Durbin *et al.*, 1983). In the present study, the lack of significant relationship may due to the small range of chlorophyll concentration values found during the sampling periods; depth integrated chlorophyll concentration was between 13.21 and 35.73 mg m^{-2} in October 2000 and 9.44 and 23.97 mg m^{-2} in May 2001. In this study, total chlorophyll-a concentration did not appear to be a good estimator of egg production at particular study periods in the Black Sea.

It should be considered that egg production rate is affected by not only food availability but also food quality. Since production of eggs is energetically expensive, sufficient nutritional energy is essential for the female to meet energy expenditure of egg production. Previous studied have shown that size and the chemical composition of phytoplankton such as protein and specific fatty acids could greatly affect the egg production of copepods (Kleppel, 1992; Kleppel *et al.*, 1998; Jónasdóttir, 1994; Richardson and Verheye, 1998; Rung, 1985; Frost, 1972).

4.1.2 Egg Production and Gonad Maturity

Gonad maturity is one of the most important endogenous factor affecting egg production rates (Hircle, 1996; Niehoff and Hircle, 1996; Kosobokova, 1999; Hircle and Niehoff, 1996; Pasternak *et al.*, 2001). The percentage of mature female is an indicator of the proportion of females capable of spawning during the incubation. Due to this fact that egg production rate is closely related to the state of reproductive maturity of the female (Campbell and Head, 2000; Niehoff and Hircle, 1996; Hircle and Niehoff, 1996). One of the goals of this study was to evaluate the possible relationship between egg production and proportion of mature female in 24 h bottle experiments. Few studies (e.g., Campbell and Head, 2000; Hirche, 1996; Hirche and Kwasniewski, 1997) have examined the relationship between proportion of mature female and egg production in copepods. In the present study, these two parameters were linearly related (see Figure 3.3). In October 2000, egg production rates were found either zero or near zero (0.07 egg female^{-1} day^{-1}) in four stations located in the central and western parts of the Black Sea (Table 3.1). In these stations, the proportions of mature females were very low (between % 8 – 17).

4.2 FEEDING AND FILTRATION RATES OF *CALANUS EUXINUS*

In the present study, pigment ingestion rates of female *Calanus euxinus* on < 56 μm size fraction responded to changes in chlorophyll concentration in the Black Sea. Feeding rate of *C. euxinus* has been studied before in the Black Sea (Arashkevich *et al.*, 1998; Besiktepe *et al.*, 1998). The results of the present study are similar to those of Arashkevich *et al.*, (1998) who measured the ingestion rate of *C. euxinus* directly from the rate of removal of chl-a concentration in the incubation bottles during the 24 h egg production experiments in September – October 1996. An important difference between present study and the study of Arashkevich *et al.*, (1998) is that Arashkevich *et al.*, (1998) did not sieve seawater from any mesh, they used ambient seawater collected from the depth of the fluorescence maximum. Although they performed their experiments with ambient seawater, their chlorophyll concentrations are in the range of chlorophyll concentrations in the present study. Thus food availability of these two studies are comparable. However, the feeding rates of present study are lower than those of Beşiktepe *et al.,* (1998). They measured ingestion rates by using gut fluorescence method, and recorded very high ingestion rates, 140 ng pigment female^{-1} d^{-1} in April 1995 and 441 ng pigment female^{-1} d^{-1} in September 1995.

A variety of methods involving incubation and gut pigment analyses have been employed to estimate *in situ* feeding rates. Gut fluorescence method is described to quantify *in situ* algal grazing from the gut analyses of freshly caught copepods (Bamstedt *et al*., 2000). Kiørboe *et al*., (1985) and Dagg and Grill, (1980) compared *in situ* feeding rates obtained from the different methods in several planktonic copepod species. They found that *in situ* grazing rates estimated by different methods were in fair agreement, however they concluded that the gut-fluorescence method generally yields the highest and the egg production method the lowest estimates. In addition, the gut fluorescence method provides short-term measurements, but the incubation technique from the rate of removal of chl-a concentration by copepods in the incubation bottle provides estimates integrated over one diurnal cycle (Kiørboe *et al*., 1985).

In the present study, ingestion rates and filtration rates observed from egg production rates have negative values in most of the stations (Table 3.1). Potential bottle effects of incubation methods have been discussed by some other researchers (Roman and Rublee, 1980; Peters and Downing, 1984; Miller *at al*., 1995). Ammonium excretion by copepods may enhance the growth of algae in the experimental bottles compared with control bottles. This effect is more pronounced in the nitrate-limited waters. Therefore these negative ingestion rates are not surprising for the Black Sea, because under optimum light conditions the phytoplankton population was shown to be nitrate limited in open waters of the Black Sea (Yayla *et al*., 2001).

4.2.1 Ingestion Rates Versus Food Concentration

The relationship between food concentrations and copepod ingestion rates have been described by many scientists for both field and laboratory studies for different copepod species (Frost, 1972; Bergreen *et al.*, 1988; Støttrup and Jensen, 1990). A copepod individual in a food environment will encounter its prey in direct relation to the abundance of it. Thus, in the absence of other limiting factors, feeding rate should increase linearly with food concentration. However, there is a mechanical and physiological upper limit for feeding rate, due to the actual mechanical processing at captures and ingestion, the volumetric constraints of the digestive tract and the time needed for digestion. Then feeding rates become independent of food concentration under saturating food concentration (Frost, 1972; Bergreen *et al.*, 1988; Støttrup and Jensen, 1990; Huskin *et al.*, 2000; Bamstedt *et al.*, 2000). These circumstances produce a response that can be illustrated by one of three models (see section 1.1.1). In the present study, there was a positive correlation observed between ingestion rate and chl-a concentration (Figure 3.5-c). Unfortunately, it was not observed full picture of one of the functional response models in the section 1.1.1 (see Fig. 1.2). This may be explained by the low food concentration in both sampling period.

4.2.2 Ingestion Rates Versus Egg production

Few field studies (e.g. Kleppel, 1992; Dam *et al.*, 1994; Durbin *et al.*, 1995) have examined the relationship between feeding and egg production rates of copepods. In the present study, the egg production rate was not related to ingestion rate (Fig. 3.7). Tester and Turner, (1990) concluded that there is considerable interspecific variability in the lag period for conversion of ingested food to egg production in marine copepods. They showed that for *Anomalocera ornato* and *Centropages typicus,* the lag period was ≈ 67-91 h. Hirche and Bohrer, (1987) reported that *Calanus glacialis* continued to lay eggs for a further 3-6 d when starved. Kiorboe *et al.*, (1985) stated that egg production in the larger species (e.g. *Calanus*) reflects food intake during the post-starvation period for several days. Uncoupling of these two parameters may be related to quality of food, nitrogen content or the C:N ratio of ingested cells (Houde and Roman, 1987; Cowles *et al.*, 1988; Kiorboe, 1989) and feeding prehistory of copepods (Saiz e*t al.,* 1999). Because *C. euxinus* has stored food reserves as oil sac, its egg production may closely associated with stored energy reserves, not with feeding within the short time period.

4.3 DIEL CHANGES IN EGG PRODUCTION RATE OF *CALANUS EUXINUS*

Calanus euxinus showed strong nocturnal egg-laying behavior in the Black Sea. The same fecundity pattern has been recorded for *Calanus sp*. from other regions (Marshall and Orr, 1972; Runge and Plourde, 1996). A few studies have been directed to understand what triggers the nocturnal egg-laying behaviour. Durbin *et al.,* (1983) found a significant, positive correlation between the daily egg production rate of *Acartia tonsa* and food availability (chlorophyll a) during 24 to 48 h bottle experiments. However Stearns *et al.*, (1989) found no evidence suggesting that pigment concentrations may trigger the nocturnal egg laying in *A. tonsa* during 2 field experiments. While there are experimental results that propose an endogenous rhythm in egg-laying and feeding behavior (Spindler, 1971 and Marcus, 1985; references cited in Stearns *et al.*, 1989). *Calanus euxinus* showed nocturnal feeding (Beşiktepe *et al.*, 1998) and nocturnal egg-laying (present study) behavior in the Black Sea. It should be studied in detail whether a diel feeding rhythm in this species directly controls the diel timing of egg-laying or there is an independent endogenous rhythm in egg production rate other than an endogenous rhythm in nocturnal feeding behaviour.

4.4 ABUNDANCE AND STAGE COMPOSITION OF *CALANUS EUXINUS*

Calanus euxinus, a key species in the Black Sea, is observed throughout the year with higher abundance and biomass values in February (Ünal, 2002). The development stages of *Calanus euxinus* (metanauplii, copepodite and adult stages) were observed for whole year and showed a definite distribution pattern in the Black Sea. The early copepodite stages and nauplii are dominant in early spring and winter seasons while in late summer and autumn older stages (C-5 and adult) are dominant (Ünal, 2002; Beşiktepe, 2001; Vinogradov *et al.*, 1995; Vinogradov and Shushkina, 1992). Results found in the present study are coincident with the previous studies. In October 2000, C-5 and female shows dominant pattern among the stages of *Calanus euxinus*. Older stages of copepods (C-5 and adults) were comprised of more than 65 % of the population. In May 2001, early stages of *Calanus euxinus* (C-1, C-2, C-3 and C-4) generally showed dominant pattern among the stages and they were comprised of 64 % of the population (Figure 3.9).

The adult sex ratios of *Calanus euxinus* (female : male) often favor females in all seasons in the Black Sea (Beşiktepe, 2001; Vinogradov *et al.*, 1990). This skewed sex ratio is a common finding for *Calanus* sp. from the other regions in all seasons (Pasternak *at al.,* 2001; Irigoien *at al.,* 2000; Tande and Hopkins, 1981). The disproportionately large numbers of females versus males have been attributed to differential mortality acting against males. There has also been speculation that males not live as long as females (Pasternak e*t al.,* 2001; Irigoien *et al.,* 2000; Crain and Miller, 2000; Tande and Hopkins, 1981). Results found in the present study corroborate the previous findings that the number of males is usually outnumbered by females with the ratio of 2:1 to 14:1 in October 2000 and 2:1 to 21:1 in May 2001.

In marine ecology, it is a well known fact that the amount of phytoplankton in the sea is controlled mainly by the grazing of zooplankton (Marshall and Orr, 1972; Head *et al.*, 2000; Madsen *et al.*, 2001). Also phytoplankton concentration is one of the important factors effecting the zooplankton distribution and development in the field. Especially in temperate waters, zooplankton cannot reproductively active until primary productivity has occurred in spring (Marshall and Orr, 1972). In this study, the biomass of copepodite stages and adults (female + male) exhibited different pattern according to depth integrated chlorophyll concentration in both sampling periods. The biomass of early copepodite stages (C-2, 3, 4) tended to increase with increasing depth integrated chlorophyll-a concentration, whereas in the biomass of adult individuals (female + male) an increasing tendency with increasing chlorophyll-a concentration has been observed in the present study (Figure 3.10). On the other hand, C-5 and C-1 did not show any tendency. This lack of relationship can be attributed for C-5 that they show seasonal diel migration in the Black Sea. While some of them continue to their diel vertical migration, the other part of C-5 remained at the depth of Oxygen Minimum Zone (OMZ) during their summer diapausing periods when they do not show any feeding activity (Beşiktepe, 2001; Vinogradov *et al.*, 1990). This may be an explanation for lack of relationship between biomass of C-5 and integrated chlorophyll concentration (Figure 3.10).

4.5 GONAD MATURITY

The adult gonad morphology of the *Calanus* species has been described by Marshall and Orr, (1972), Niehoff and Hirche, (1996), Niehoff, (1998), Kosobokova, (1999) in order to distinguish gonad development stages. This knowledge is essential to understand the processes of reproduction and also to fill the gaps in our understanding of life cycle of copepods.

Gonad development in *Calanus* species starts at C-4. Sexual differentiation takes places during late C-5 development, when potential males and females can be easily distinguished by anatomy of their gonads (Tande and Hopkins 1981). Basically, young copepodite stages show actively feeding behavior and accumulate lipid reserves in the upper layer of water column, the older stages of C-5 stops their feeding activity and descends into the deep layer, where they reduce their metabolic rates and enter a diapausing period. During this period, gonad growth and development are sustained by internal energy deposits of oil sac (Hirche, 1996).

In the Black Sea, like most *Calanus* sp., life cycle of *Calanus euxinus* includes a diapause phase. After the winter-spring phytoplankton bloom, a part of the C-5 population enters the diapause. C-5 consists of two groups: one of group is concentrated in the deeper layer near the OMZ for whole day for diapausing; the other group shows diel vertical migration and feeding activity (Arashkevich *et al.*, 1998; Vinogradov *et al.*, 1992; Besiktepe, 2001). Lipid content of the diapausing C-5 stages is much higher than the migrating groups (Vinogradov *et al.*, 1992). However, whether *Calanus euxinus* in the Black Sea develops their gonads during the diapausing periods or not, is unclear.

The gonad development stages of females passes through several stages which can be distinquished according to the number of oocyctes increases both in the anterior diverticula and posterior diverticulaes (oviducts) (Kosobokova, 1999; Niehoff, 1998; Hircle, 1996). In the present study, the gonad morphology of *Calanus euxinus* concides with other *Calanus* species described by Niehoff, (1998), Hircle, (1996), Kosobokova, (1999) and Tande and Hopkins, (1981). In the present study, determination of gonad stages were based on the morphological descriptions of Kosobokova, (1999) (Table 2.2 and Figure 2.5). Although, Kosobokova, (1999) reported seven stages in the *Calanus glacialis* maturation cycle, we identified five stages for *Calanus euxinus* in the Black Sea. Spent and Remature stages were not found in our samples.

In the present study, the mature females formed more than half of the population in both sampling periods. The immature stages of females were observed only at few stations and had the smallest proportion, i.e. not more than 5 %. Semimature-a and semimature-b made up only ~ 5 % and ~ 17 % of the population in both sampling periods, respectively. The semi-spent female formed ~ 13 % the population.

These findings show that *Calanus euxinus* is actively reproductive in our sampling seasons. Sazhina (1996) reported *Calanus euxinus* reproduce during the whole year and the female population was represented mainly by mature females in the Black Sea.

4.6 LENGTH-WEIGHT RELATIONSHIP OF *CALANUS EUXINUS*

In the zooplankton studies especially with copepods, length weight relation is essential for the calculation of biomass, growth and secondary production (Chisholm and Roff 1990). In this study, length-weight regressions have been generated for *Calanus euxinus* in the Black Sea during the two different seasons (spring and autumn). The length-weight regressions can differ considerably between seasons due to the variabilities in temperature and food availability, which are reflected as variations in energy density of copepods mainly as a consequence of lipid storage. For instance, the regression developed from the copepod collected in summer may not coincident with that for winter season (Durbin and Durbin, 1978; Ergun, 1994). In the present study there was no any differences observed in length-weight regressions between two seasons probably due to similar environmental conditions in both sampling periods.

4.7 GROWTH RATES OF *CALANUS EUXINUS*

The common assumption used in the determination of growth in copepods is that, over short periods, net growth of an individual adult female is equal to the amount of material expelled as eggs. This assumption relies upon adult body mass being in steady state between the start and end of the same period. Berggreen *et al.*, (1988) and Poulet *et al.,* (1995) stated that instantaneous reproductive growth rate of female can be representative of the specific growth rate of all development stages of copepods when food conditions are not limiting. However, planktonic copepods are food limited in nature especially in open waters (Runge, 1985; Champhell *et al.*, 2000). Growth rate of broadcasting copepods (e.g. *Calanus* sp.) decreases with increasing body weight (Hirst and Lampitt, 1998; Hutchings *et al.*, 1995; Hopcroft *et al.*, 1998; Harris *at al.,* 2000; Kiørboe and Sabatini, 1995; Vidal, 1980). Furthermore, Hirst and Lampitt, (1998) emphasized that adult female growth is more food limited than juvenile growth in nature. In the present study, instantaneous reproductive growth rates of female *Calanus euxinus* were negatively related to female dry weight with a slope of –0.0006 in October 2000 and -0.001 in May 2001 (Figure 3.13). Hirst and Lampitt (1998) suggested that the slope of growth rate versus weight for broadcast-spawners was -0.28 under food-saturated conditions at 15 °C, but the relationship for *in situ* growth is not significant in their data. Additionally, they suggested that for an individual with 1 μg C weight, the predicted food-saturated growth rate is 0.336 d^{-1}, while *in situ* value is 0.107 d^{-1}. In the present study, the mean growth rate was 0.01 in October 2000 and 0.03 in May 2001. These results are much lower than those of Hirst and Lampitt, (1998) predictions for food saturated conditions. These comparisons may suggest that the present study was conducted under conditions of limiting resources. Furthermore, in order to understand whether *Calanus euxinus* is experiencing food limitated conditions in the Black Sea or not, potential food limitation experiments were performed during both sampling periods. Female *Calanus euxinus* acclimatized in the natural seawater from the fluorescence maximum depth, was enriched with the laboratory cultured alga *Thalassiosira weissfloggii* for 24 h and then incubated in the same conditions as was in egg production experiments. Control groups were set up containing seawater from fluorescence maximum depth without food addition. Table 4.2 shows the response of egg production to the enrichment with *Thalassiosira weissfloggii.* It is experimentally demonstrated that the egg production rates of *C. euxinus* was significantly (Student's *t* test, $P > 0.25$) higher in enriched water in both sampling periods. This response suggests that during the present study copepod egg production was limited by food availability.

Table 4.2: Comparison of average egg production rates of *Calanus euxinus* (eggs ind^{-1} d^{-1}) from incubations in natural (*in situ*) and enriched water.

Stations	Date	Long.	Lati.	Food Con. in Acclimatation (μg C l^{-1})	Food conc in Incubation (μg C l^{-1})	# of egg without food add.	# of egg with food add.
M00W00	Oct-00	42.00	39.00	186.21	258.2	0.00	4.84
L3750Y10	Oct-00	41.62	41.17	191.47	155.4	0.00	0.50
M20L45	May-01	42.33	29.75	269.69	150.7	0.70	3.73
M00O00	May-01	42.00	32.00	150.73	220.2	5.69	6.45

CHAPTER V

SUMMARY

This study allowed us to measure egg production rates of *Calanus euxinus* in the southern Black Sea and to examine the relationships between egg production rates and total chlorophyll-a concentrations (used as an index of food quality). In the present study, average daily egg production rates of *C. euxinus* ranged from 0 to 7 egg female^{-1} day^{-1} in the southern Black Sea. These results are identical to the previously recorded egg production rates of *C. euxinus* in the Black Sea. Total chlorophyll-a concentration did not appear to be a good estimator of food availability. Egg production rates were not affected by the total chlorophyll-a concentration.

Furthermore, the state of reproductive maturity of the female population and its influence on egg production rate were studied. The proportion of female capable to spawn (mature female) is a prerequisite parameter to interpret observation of the reproductive status of the population. Indeed, females do not possess mature oocytes will not spawn. In the present study, egg production rate seems to be most closely related to the state of reproductive maturity of the female. In both sampling period, *Calanus euxinus* is actively reproductive as the proportion of mature female formed more than half of the population. It formed 65 and 60 % of the female population in October and May respectively.

In the present study, pigment ingestion rates of female *Calanus euxinus* on < 56 μm size fraction was also investigated in the Black Sea. There was a positive correlation observed between ingestion rate and chl-a concentration. On the other hand, the egg production rate was not related to ingestion rate. This lack of relationship can be attributed that larger species like *C. euxinus* reflects food intake during the preceding several days (Kiorboe *et al.*, 1985). Because they have food reserves as oil sac, and their egg production may closely associated with stored energy reserves, they may not respond to feeding within the recent past day. In the present study, *Calanus euxinus* showed strong nocturnal egg-laying behaviour in the Black Sea.

Moreover, abundance of all copepodite stages of *Calanus euxinus* was also studied in each experimental station in order to examine copepod population structure and female per male ratios. In October 2000, older stages of copepods (C-5 and adults) showed dominant pattern among the stages of *C. euxinus*. They comprised of more than 65 % of the population. However, in May 2001, early stages of *C. euxinus* (C-1, C-2, C-3 and C-4) were found to be more abundant comprising of 64 % of the population. The adult sex ratios of *Calanus euxinus* (female : male) often favor females in both sampling study in the Black Sea. This ratio ranged from 2:1 to 14:1 in October 2000 and 2:1 to 21:1 in May 2001.

In addition, length-weight regressions formulas were derived for *Calanus euxinus* in both season, May 2000 and October 2001. These regression formulas were used for calculating reproductive instantaneous growth rates (g_r) of adult female copepods. The length-weight regression of *Calanus euxinus* did not vary significantly between October 2000 and May 2001.

In the present study, instantaneous reproductive growth rates of female *Calanus euxinus* ranged from 0.00 to 0.051 d^{-1} October 2000 and from 0.013 to 0.053 d^{-1} in May 2001. The growth rate negatively correlated with the dry weight. The growth rate of *C. euxinus* are much lower than previously recorded growth rates of broadcast-spawners copepods (Hirst and Lampitt, 1998). In this manner, it may be suggested that the present study was conducted under conditions of limiting resources. Evidently, potential food limitation experiments performed in both sampling period have shown us the egg production rate of *C. euxinus* was limited by food availability in the Black Sea.

REFERENCES

Arashkevich, E., Svetlichny, L., Gubareva, E., Besiktepe, Ş., Gucu, A. C. and Kideyş, A. E. (1998) Physiological and ecological study of *Calanus euxinus* (Hulseman) from the Black Sea with comments on its life cycle. In NATO Science Series. 2. Environmental series Vol: 47. Ivanov, L. I., Oguz, T., Ecosystem Modeling as a Management Tool for the Black Sea, 351-365. Kluwer Academic Publishers. Printed in Netherlands.

Aubrey, D., Moncheva, S., Demirov, E., Diaconu, V. and Dimitrov, A. (1996) Environmental changes in the western Black Sea related to anthropogenic and natural conditions. J. Mar. System, 7: 411- 425.

Bamstedt, U., Gifford, D. J., Irigoien, X., Atkinson, A., and Roman, M. (2000) ICES Zooplankton Methodology Manual. Edited by Harris R.P., Wiebe P.H., Lenz J., Skjoldal H.R. and Huntley M.. A Harcourt Science and Technology Company, Academic Press. 684 pp.

Baştürk, O., Tuğrul, S., Konovalov, S. and Salihoğlu, I. (1997) Variation in the vertical structure of water chemistry within the tree hydrodinamically different regions of the Black Sea. In NATO ASI Series. Özsoy, E. and Mikaelyan, A. (eds.), Sensitivity to change: Black Sea, Baltic Sea and North Sea, 183-196. Kluwer Academic Publishers. Printed in Netherlands.

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

Bird, D. F. and Prairie, Y. T. (1985) Practical guidelines for the use of zooplankton length-weight regression equations. J. Plankton Res., 7 (6): 955-960.

Beşiktepe, Ş., Kideyş, A. E., Ünsal, M. (1998) *In situ* grazing pressure and dial vertical migration of female *Calanus euxinus* in the Black Sea. Hydrabiologia, 363: 323-332.

Beşiktepe, Ş. (2001) Dial vertical distribution, and herbivory of copepods in the southwestern part of the Black Sea. J. Mar. System 28: 281-301.

Bologa, A. S. (1986) Planktonic primary production of the Black Sea: A revive. Thalassia Jugoslavica, 21/22 (1/2): 1-22.

Caddy, J. (1993) Toward a cooperative evaluation of human impacts on fishery ecosystem of enclosed and semi enclosed seas. Reviews in Fishery Science, 1 (1): 57- 95.

Campbell, R. W. and Head, J. H. E. (2000) Egg production rates of *Calanus finmarchicus* in the western North Atlantic: Effects of gonad maturity, female size, chlorophyll concentration, and temperature. Can. J. Aquat. Sci., 57: 518-529.

Camphell, R. G., Runge, J. A. and Durbin, E. G. (2000) Evidence for food limitation of *Calanus finmarchicus* production rates on the southern flank of Georges Bank during April 1997. Deep-Sea Res. II. 48: 531-549.

Chisholm, L. A and Roff, J. C. (1990) Size-weight relationships and biomass of tropical neritic copepods off Kingston, Jamaica. Mar. Biol., 106: 71-77.

Cociasu, A., Dorogan, L., Humborg, C. and Popa, L. (1996) Long-tem ecological changes in Romanian coastal waters of the Black Sea. Mar. Pollution Bul., 32 (1): 32-38.

Cowles, T. J., Olson, R. J., and Chisholm, S. W. (1988) Food selection by copepods: discrimination on the basis of food quality. Mar. Biol., 100: 41-49.

Crain, J. A. and Miller, C. B. (2000) Detection of sex and sex ratio in *Calanus finmarchicus* early stage fifth copepodites. ICES J. Mar. Sci., 57: 1773-1779.

Dagg, M. J. and Grill, D. W. (1980) Natural feeding rates of *Centropoges typicus* females in the New York Bight. Limnol. Oceanog., 25: 597- 609.

Dam, H. G., Peterson, W. T., Bellantoni, D. C. (1994) Seasonal feeding and fecundity of the calanoid copepod *Acarcia tonsa* in Long Island Sound: Is omnivory important to egg production? Hydrabiologia, 292/293: 191-199.

Diel, S. and Tande, K. (1992) Does the spawning of *Calanus finmarchicus* in high latitudes follow a reproducible pattern? Mar. Biol., 113: 21-31.

Durbin, E. G. and Durbin, A. G. (1978) Length and weight relationship of *Acarcia clausi* from the Narragangestt Bay. Rhode Island. Limnol. Oceanog., 23: 958-969.

Durbin, E. G., Durbin, A. G., Smayda, T. J., and Verity, P. G. (1983) Food limitation of production by adult *Acarcia tonsa* in Narragansett Bay, Rhode Island. Limnol. Oceanog., 28 (6): 1199-1213.

Durbin, E. G., Campbell, R. G., Gilman, S. L., and Durbin, A. G. (1995) Diel feeding behavior and ingestion rate in the copepod *Calanus finmarchicus* in the southern Gulf of Maine during late spring. Continental shelf Res. 15 (4/5): 539-570.

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

Ergun, G. (1994) Distribution of five calanoid copepod species in the southern Black Sea. M. S. Thesis. METU – Institute of Marine Sciences, Erdemli, 117 pp.

Frost, B. W. (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pasificus*. Limnol. Oceanog.,17 (6): 805-815.

Frost, B. W. (1988) Variability and possible adaptive significance of dial vertical migration in *Calanus pacificus*. A plankton marine copepod. Bulletin of Marine Science, 43 (3): 675-694.

Gislason, A. and Astthorsson, O. S. (2000) Winter distribution, ontogenic migration, and rates of egg production of *Calanus finmarchicus* southwest of Iceland. ICES J. Mar. Sci., 57: 1727-1739.

Gutiérrez, J. G. and Peterson, W. T. (1999) Egg production rates of eight calanoid copepod species during the summer 1997 off Newport, Oregon, USA. J Plankton Res., 21 (4): 637-657.

Harris, R. P. (1988) Interactions between dial vertical migratory behavior of marine zooplankton and the subsurface chlorophyll maximum. Bulletin of Marine Science, 43 (3): 663-674.

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

Head, E. J. H., Harris, L. R., and Campbell, R. W. (2000) Investigation on the ecology of *Calanus* spp. In the Labrador Sea. I. Relationship between the phytoplankton bloom and reproduction and development of *Calanus finmarchicus* in spring. Mar. Ecol. Prog. Ser., 193: 53-73.

Hircle, H. J. (1996) The reproductive biology of the marine copepod, *Calanus finmarchicus* – a review. Opelia 44: 111-128.

Hirche, H. J. and Bohrer, R. N. (1987) Reproduction of the Arctic copepod *Calanus glacialis* in Fram Strait. Mar. Biol., 94: 11-17.

Hirche, H. J. and Niehoff, B. (1996) Reproduction of the Arctic copepod *Calanus hyperboreus* in the Greenland Sea. Field and laboratory observations. Polar Biol., 16: 209-219.

Hirche, H. J. and Kwasniewski, S. (1997) Distribution, reproduction and development of *Calanus* species in the Northeast Water in relation to environmental conditions, J. Mar. System, 10: 299-317.

Hirst, A. G. and Lampitt, R. S. (1998) Towards a global model of *in situ* weight-specific growth in marine planktonic copepods. Mar. Biol., 132: 247-257.

Houde, S. E. L. and Roman, M. R. (1987) Effects of food quality on the functional ingestion response of the copepod *Acarcia tonsa*. Mar. Ecol. Prog. Ser., 40: 69-77.

Hopcroft, R. R., Roff, J. C., Webber, M. K. and Witt, J. D. S. (1998) Zooplankton growth rates: the influence of size and resources in tropical marine copepodites. Mar. Biol., 132: 67-77.

Hopcroft, R. R. and Roff, J. C (1998) Zooplankton growth rate: the influence of female size and resources on egg production of tropical marine copepods. Mar. Biol., 132: 79-86.

Huntlay, M. and Lopez, M. D. G. (1992) Temperature-dependent production of marine copepods: A global synthesis. Am. Nat., 140:201-242.

Huskin, I., Anadón, R., Álvarez-Marqués, F., and Harris, R. P. (2000) Ingestion, faecal pellet and egg production rates of *Calanus helgolandicus* feeding coccolithophorid diets versus non- coccolithophorid diets. J. Exp. Mar. Biol. Ecol., 248: 239-254.

Hutchingd, L., Verheve, H. M., Mitchell-Innes, B. A., Peterson, W. T., Hugget, J. A., and Painting, S. J. (1995) Copepod production in the south Benguela system. ICES J. Mar. Sci., 52: 439-455.

Irigoien, X., Obermuller, B., Head, R. N., Harris, R. P., Rey, C., Hansen, B. W., Hygum, B. H., Heath, M. R., and Durbin, E. G. (2000) The effect of food on determination of sex ratio in *Calanus* spp.: Evidence from experimental studies and field data. ICES J. Mar. Sci., 57: 1752-1763.

Ivanov, L. I., Beşiktepe, Ş. and Özsoy, E. (1997) The Black Sea cold intermediate layer. In NATO ASI Series. Özsoy, E. and Mikaelyan (eds.), Sensitivity to change: Black Sea, Baltic Sea and North Sea, 253-264. Kluwer Academic Publisher. Printed in Netherlands.

JGOFS. (1994) Protocols for the join Global Ocean Flux Study (JGOFS) Core Measurements, Manual and Guides 29, UNESCO, 170 pp.

Jónasdóttir, S. H (1994) Effects of food quality on the reproductive success of *Acarcia tonsa* and *Acarcia hudsonica*: laboratory observations. Mar. Biol., 121: 67-81.

Kiørboe, T. (1989) Phytoplankton growth rate and nitrogen content: Implications for feeding and fecundity in a herbivorous copepod. Mar. Ecol. Prog. Ser., 55: 229-234.

Kiørboe, T., Møhlenberg, F., and Rusgard, H. U. (1985) In situ feeding rates of planktonic copepods: a comparison of four methods. J. Exp. Mar. Biol. Ecol., 88: 67-81.

Kiørboe, T. and Sabatini, M. (1994) Reproductive and life cycle strategies in egg-carrying cyclopoid and free-spawning calanoid copepods. J Plankton Res., I: Vol 16 No: 10: 1353-1366.

Kiørboe, T., and Sabatini, M. (1995) Scaling of fecundity, growth and development in marine planctonic copepods. Mar. Ecol. Prog. Ser., 120: 285-298.

Kleppel, G. S. (1992) Environmental regulation of feeding and egg production by *Acarcia tonsa* off southern California. Mar. Biol., 112: 57-65.

Kleppel, G. S., Burkart, C. A. and Houchin, L. (1998) Nutrution and regulation of egg production in the calanoid copepod *Acarcia tonsa*. Limnol. Oceanog., 43 (5): 1000-1007.

Kleppel, G. S., and Hazzard, S. E. (2000) Diet and egg production of the copepod *Acarcia tonsa* in Florida Bay. II. Role of the nutritional environment. Mar. Biol., 137: 111-121.

Kosobokova, K. N. (1999) The reproductive cycle and life history of the Arctic copepod Calanus *glacialis* in the White Sea. Polar Biol., 22: 254-263.

Madsen, S. D., Nielsen, T. G., and Hansen, B. W. (2001) Annual population development and production by *Calanus finmarchicus, C. glacialis* and *C. hyperboreus* in Disco Bay, western Greenland. Mar. Biol., 139: 75-93.

Marcus, N. H. (1985) Endogenous control of spawning in a marine copepod. J. Exp. Mar. Biol. Ecol., 91: 263-269.

Marshall, S. M. and Orr, A. P. (1972) The biology of a marine copepod. Springer-Verlag, Heidelberg / New York 195 pp.

McConnaughey, B. H. (1974) Introduction to marine biology. The C. V. Mosby Company Saint Louis. 125 pp.

Miller, C. A., Penry, D. L., and Glibert, P. M. (1995) The impact of trophic interactions on rates of nitrogen regeneration and grazing in Chesapeake Bay. Limnol. Oceanog., 40 (5): 1005-1011.

Miralto, A., Ianora, A., Guglielmo, L., Zagami, G., and Buttino, I. (1998) Egg production and haching success in the peri Antarctic copepod *Calanus simillimus*. J Plankton Res.: Vol; 20, No; 12: 2369-2378.

Murray, J. W., Top, Z., and Özsoy, E. (1991): Hydrographic properties and ventilation of the Black Sea. Deep-Sea Res. 38 (2): 663-689.

Niehoff, B. and 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. (1998) The gonad morphology and maturation in arctic *Calanus* species., J. Mar. System 15: 53-59.

Nybakken, J. W. (1982) Marine biology: An ecological approach. Harper and Row, Publishers, New York. 446p.

Oğuz, T., La Violette, P. E. and Ünlüata, Ü. (1992) The Upper Layer circulation of the Black Sea: Its variability as inferred from hydrographic and satellite observations. Journal of Geophysical Research. 97 (C8): 12569-12584.

Oğuz, T., Latun, V. S., Latif, M. A., Vladimirov, V. V., Sur, H. I., Markov, A. A., Özsoy, E., Kotovshchikov, B. B., Eremeev, V. V., Ünlüata, U. (1993) Circulation in the surface and intermediate layers of the Black Sea. Deep-Sea Res-I. 40 (8): 1597-1612.

Oğuz, T., Aubrey D. G., Latun V.S., Demirov E., Koveshnikov, L., Sur, H. I., Diaconu, V., Beşiktepe, Ş., Duman, M., Limeburner, R. and Eremeev, V. V.

(1994) Mesoscale circulation and thermohaline structure of the Black Sea observed during Hydro Black 91. Deep-Sea Res-I. 41 (4): 603-628.

Oğuz, T. and Beşiktepe, Ş. (1999) Observation on the Rim Current structure, CIW formation and transport in the western Black Sea Deep-Sea Res-I. 46: 1733-1753.

Oğuz, T. (2002) Role of physical processes controlling oxycline and suboxic layer structures in the Black Sea. Global Biochemical Cycles, 16 (2): 1-13.

Omori, M. and Ikeda, T. (1992) Methods of Zooplankton Ecology. Krieger Publishing Company Malabar, Florida, 332 p.

Özsoy, E. and Ünlüata, Ü. (1997) Oceanography of the Black Sea: A review of some recent results. Earth Sci. Rev. 42 (4): 231-272.

Paffenhöfer, G. A. (1988) Feeding rates and behavior of zooplankton. Bulletin of Marine Science, 43 (3): 430-445.

Pasternak, A., Arashkevich, E., and Tande, K. (2001) Seasonal changes in feeding, gonad development and lipid stores in *Calanus finmarchicus* and *C. hyperboreus* from Malangen, northern Norway. Mar. Biol., 138: 1141-1152.

Peters, R. H., and Downing, J. A. (1984) Empirical analyses of zooplankton filtering and feeding rates. Limnol. Oceanog., 29: 763-784.

Poulet, S. A., Ianora, A., Laabir, M. and Klein Breteler, W. C. M. (1995) Towards the measurements of secondary production and recruitment in copepods. ICES J. Mar. Sci., 52: 359-368.

Richardson, A. J. Verheye, H. M. (1998) The relative importance of food and temperature to copepod egg production and somatic growth in the southern Benguela upwelling system. J Plankton Res., 20 (12): 2379-2399.

Roman, M. P. and Rublee, P. A. (1980) Containment effects in copepod grazing experiments: a plea to end the back box approach. Limnol. Oceanog., 25: 982-990.

Rothhaupt, K. O. (1990) Changes of functional response of the rotifers *Brachionus rubens* and *Brachionus calyciflorus* with particle sizes. Limnol. Oceanog., 35 (1): 24-32.

Runge, J. A. (1985) Relationship of egg production of *Calanus pasificus* to seasonal changes in phytoplankton availability in Puget Sound, Washington. Limnol. Oceanog., 30 (2): 382-396.

Runge, J. A. and Plourde, S. (1996) Fecundity characteristics of *Calanus finmarchicus* in coastal waters of eastern Canada. Ophelia, 44: 171-187.

Saiz, E., Calbet, A., Irigoien, X., and Alcaraz, M. (1999) Copepod egg production in the western Mediterranean: response to food availability in oligotrophic environments. Mar. Ecol. Prog. Ser., 187: 179-189.

Sazhina, L. I. (1996) Fecundity of Black Sea copepods in 1992. Oceanology, 34 (4): 516-522.

Saydam, C., Tuğrul, S., Baştürk, O., and Oğuz, T. (1993) Identification of the oxic / anoxic interface by isopycal surface in the Black Sea. Deep-Sea Res., I 40: 1405-1412.

Stearns, D. E., Tester, P. A. and Walker, R. L. (1989) Diel changes in the egg production rate of *Acarcia tonsa* (Copepoda, Calanoida) and related environmental factors in two estuaries. Mar. Ecol. Prog. Ser., 52: 7-16.

Støttrup, J. G. and Jensen, J. (1990) Influence of algal diet on feeding and egg production of the calanoid copepod *Acarcia tonsa* Dana. J. Exp. Mar. Biol. Ecol., 1462: 87-105.

Sorokin, Y. I. (2002) The Black Sea Ecology and Oceanography. Backhuys Publishers, Leiden, The Netherlands, 2002. 875 pp.

Spindler, K. D. (1971) Der Einfluß von Licht auf die Eiablage des Copepoden *Cyclops vicinus*. Z. Naturforsch. Teil B 26: 953-955.

Sur, H., Ozsoy, E., Ilyin, Y. P. and Unluata, U. (1996) Coastal / deep interactions in the Black Sea and their ecological / environmental impacts. J. Mar. System 7: 293-320.

Tande, K. S. and 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.

Tester, P. A. and Turner, J. T. (1990) How long does it take copepods to make eggs? J. Exp. Mar. Biol. Ecol., 141: 169-182.

Tuğrul, S., Özden, B., Saydam, C. and Yılmaz, A. (1992) Changes in the hydrochemistry of the Black Sea inferred from water density profiles. Nature, 359: 137-139.

Uysal, Z. and Sur, H. I. (1995) Net phytoplankton discriminating patches along the Southern Black Sea coast in winter 1990. Oceanologica Acta 18 (6): 639-647.

Ünal, E. (2002) Seasonality of zooplankton in the southern Black Sea and genetics of *Calanus euxinus*. M.S. Thesis. METU – Institute of Marine Science. Erdemli, 202 pp.

Vinogradov, M. E., Musayeva, E. I. and Semenova, T. N. (1990) Factors determining the position of the lower of mezoplankton concentration in the Black Sea. Oceanology, 30 (2): 217-224.

Vinogradov, M. E. and Nalbandov, Y. R. (1990) Effects of changes in water density on the profiles of physiochemical and biological characteristics in the pelagic ecosystem of the Black Sea. Oceanology, 30: 567-573.

Vinogradov, M. E., Arashkevich, E. and Iichenko, S. V. (1992) 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.

Vinogradov, M. E. and Shushkina, E. A. (1992) Temporal changes in community structure in the open Black Sea. Oceanology, 32 (4): 485-491.

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

Vidal, J. (1980) Physioecology of zooplankton. I. Effects of Phytoplankton concentration, temperature and body size on growth rate of *Calanus pasificus* and *Pseudocalanus* sp. Mar. Biol., 56: 111-134.

Vladimirov, V. L., Mankovsky, V. I., Solovev M.V. and Mishonov A.V. (1997) Seasonal and Long-Term Variability of the Black Sea optical parameters. In NATO ASI Series. Ozsoy E. and Mikaelyan A. (eds.), Sensitivity to change: Black Sea, Baltic Sea and North Sea, 33-48. Kluwer Academic Publishers. Printed in Netherlands.

Yayla, M. K. (2001) Primary production, availability and uptake of nutrients and photoadaptation of phytoplankton in the Black Sea, the Sea of Marmara and the Eastern Mediterranean. M.S. Thesis. METU – Institute of Marine Sciences, Erdemli, Turkey, 128 pp.

Yunev, O. A., Vedernikov, V. I., Baştürk, Ö., Yılmaz, A., Kıdeyş, A. E., Moncheva, S., and Konovalov, S. K. (2002) Long term variations of surface chlorophyll-a and primary production in the open Black Sea. Mar Ecol. Prog. Ser., 230: 11-28.

Yuneva, T. V., Svetlichny, L. S., Yunev, O. A., Romanova, Z. A., Kıdeyş, A. E., Binlgel, F., Uysal, Z., Yılmaz, A., and Shulman, G. E. (1999) Nutritional condition of female *Calanus euxinus* from cyclonic and anticyclonic regions of the Black Sea. Mar Ecol. Prog. Ser., 89: 195-204.

Zaitsev, Y. P. and Alexandrov, B. G. (1997) Recent man made changes in the Black Sea ecosystem. In NATO Asi Series. Ozsoy E. and Mikaelyan A. (eds.), Sensitivity to change: Black Sea, Baltic Sea and North Sea, 25-31. Kluwer Academic Publishers. Printed in Netherlands.

Zar, J. H. (1984) Biostatical Analyses. A Simon and Schuster Company, Englewood Cliffs, New Jersey USA, 718 pp.