Leinbniz-Institut für Meereswinssenschaften
der Christian-Albrechts Universität zu Kiel

# Effect of the food quality (taxonomy and biochemical composition of the microalgae) on the reproduction and survival of the copepod *A. tonsa*, from the Kiel Bight

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Der Dekan

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# 1. Introduction

To evaluate some of the effects of man's impingement on the oceans, a basic understanding of the organization of biological energy transfer in the seas is necessary. Calanoid copepods are the most abundant and probably the most ecologically significant animals at the first consumer level of the marine plankton, and are also prominent among the primary carnivores. Quantitative data on calanoid productivity and especially the effects wrought by changes in various environmental conditions on that productivity, form a basis from which evaluations of this vital link can be made (Parrish & Wilson, 1978).

In marine pelagic food web, zooplankton plays an important role in the energy transfer between primary producers and pelagic fish populations, and it is thus a key factor influencing fish production (Gowen *et al.*, 1999; Möllmann *et al.*, 2000; Biktashev *et al.*, 2003).

With more than 5,000 species, there are more copepods than any other kind of zooplankton and they dominate nearly all of the oceans and marginal seas. The crustacean order Copepoda is by far the largest group of crustaceans in the zooplankton. Most copepods are marine, but copepods are also found in freshwater, damp land or as parasites. They range in size from less than 1 mm to a few mm in length (Björnberg, 1981; Levinton, 1995).

Copepods are frequently considered as the major consumers of phytoplankton throughout the world's oceans and, as consequence, to play a key role in the transfer of phytoplankton carbon to higher trophic levels (Bartram, 1980; Gowen *et al.*, 1999). An understanding of quantitative trophic interactions between phytoplankton and herbivorous copepods is required to elucidate the nature of marine food webs in terms of the rates (Frost, 1972).

Copepods also contributed to the "microbial loop", by contributing to the pool of dissolved organic material (DOM) through excretion, leakage from fecal pellets and "sloppy feeding". The DOM from copepod is an important link in the trophic web, since only about half the carbon requirement of the bacterioplankton can be directly met by release of organic carbon from phytoplankton (Azam *et al.*, 1983; Baines & Pace, 1991; Moller & Nielsen, 2001).

Calanoid copepods and their naupliar stages are the typical natural food source of most marine fish larvae (Hunter, 1982). Their relative importance at the first consumer level in marine food chains has led to several attempts to cultivate copepods in the laboratory to obtain the degree of control necessary for reproducible experimental work (Stottrup & Munk, 1983). By weight, there are probably more calonoid copepods than any other planktonic animal group. Calanoid feed mainly on phytoplankton, some organic particles, and smaller zooplankton (Björnberg, 1981; Levinton, 1995).

*A. tonsa*, an important in many coastal and estuarine communities, often dominating the zooplankton, is a widely spread species present in the Indian Ocean, Malay Archipelago, Cayenne, Atlantic and Pacific coasts of the North and South Americas, Baltic Sea, up to the Gulf of Finland, Black, Azov and Mediterranean Seas. At present this species is present in the plankton of the Caspian Sea (Kurashova & Abdullaeva,1984).

*A tonsa* is lives in the water column, more frequent from the surface down to 200-m depth. It performs both horizontal and daily vertical migrations. During the nighttime it goes up to the surface layers while in the morning and daytime mature males and females sink to deeper layers of water. Young individuals undertake migrations only within surface layers (Brodsky, 1950).

It is a brackishwater euryhaline and eurythermic species, which occurs all the year round at a temperature from 0 to 29.50C. *A tonsa* is also an eurybathic species, it occurs sporadically (2 ind./m3) in the halistatic zone at depths ranging from 200 to 600 m. In relation to oxygen conditions, it is an oxyphilic species. Low concentrations of dissolved oxygen retard the development of eggs and result in death of all copepodite stages of *A. tonsa* (Kurashova & Abdullaeva,1984).

It is a prehensile copepod. Raking movements of maxillipaeds I and mandibles are the most important mode of food capture. Food is captured at frequent small jumps or smooth sliding. The former type is used when capturing large food organisms (30-100 μm), the latter when seizing small organisms (5-30 μm) (Petipa, 1959). It is an euryphagous species.

*A tonsa* consumes both phytoplankton and animal food. It prefers the largest single, spherical or elliptic, algae. *A tonsa* feeds actively on the algae *Chaetoceros socialis*, *Exuviaella cordata* (16x12 μm), *Gymnodinium* sp. (56x39 μm), *Prorocentrum micans* (40x30 μ), *Skeletonema costatum* (1-200μm; h – 6-10 μm), *Nitzschia closterium*

(30x40 μm), *Cyclotella caspia* (1-15-36 μm; h –10 μm). It can also consume actively minute *Flagellate* (6-8 μ) (Petipa, 1959).

Digestion of *A tonsa* lasts for 1-3 hours at 20-250C. Digestion rate can read 75-80%, but drop to 20-30% in conditions of excessive feeding. Daily food rations in different age groups vary from 6 to 20% of body weight. When using animal food or excess feeding, daily diet increases up to 26% of body weight. Average daily indices of stomach fullness range from 59 to 62 % (Pepita, 1959).

The fertilization is internal. A male can fertilize several females. The number of spermatophore attached to females varies from 1 to 3, and more. After the spermatophore has attached, fertilization takes place. *A tonsa* lays eggs into water. Eggs are heavier than water, therefore they descend to the bottom. Eggs are covered with 2 membranes: the outer is thick, and the inner is extremely elastic. A nauplius larva develops (Björnberg, 1981; Levinton, 1995).

There are no specific areas of reproduction. *A tonsa* reproduces throughout the year. The duration of reproduction period is 75 days. The spawning interval is 5-6 days. The maximum duration of sexual activity in females is 2.5 months. She can lay up to 20 eggs in each of 13 batches, maximal fecundity is 260 eggs (Sazhina, 1971).

Temperature, light factor, feeding, salinity, oxygen concentration can cause perturbation on the reproduction of *A tonsa* (Sazhina, 1987).

The life cycle of *A tonsa* has a long metamorphosis period (from egg to adult stage). There are twelve stages of development, of which six nauplial and 6 copepodite stages, their sequence are determined by molts. When the embryonic development is concluded, larvae hatch from eggs through the cracks in the thick egg membrane. The whole process of hatching lasts for 0.5-1.5 min. Copepodite stages follow the naupliar period. Duration of the full developmental cycle is 30-33 days (Björnberg, 1981; Levinton, 1995).

The most vigorous breeding activity occurs at a water temperature 20-220C (Sazhina, 1971). The life span of females is 70-80 days, 14 days - in males (Sazhina, 1987). Latent eggs hatch rapidly at 50C. When the temperature increases from 5 to 230C, 70-100% of the nauplii hatch in 20 days, and 40% - at 25-400C (Sazhina, 1987).

Mesozooplankton growth rate and reproduction are determined by temperature, food quantity and food quality (Jónasdóttir, 1994, Koski *et al.*, 1998). Egg production of copepod in nature is generally assumed to be food limited, while juvenile growth often

seems to be dependent on temperature alone (Mclaren, 1978; Huntley & Lopez, 1992). Nevertheless both increasing developmental stages, somatic growth may be food limited as well (Klein Breteler & Schogt 1994). Factors affecting food quality may include cell size (Berggreen *et al.* 1988), cell morphology (Burns, 1987), toxicity (Lampert 1981), mineral composition (Kiorboe, 1989) or content of some biochemical components, such as amino acids, polyunsaturated fatty acids (PUFAs) or vitamins (Jónasdóttir, 1994; Müller-Navara, 1995; Brown *et al.*, 1997).

There are two general problems that make interpretations of feeding studies difficult: 1. The large intraspecifc and interspecific variation in the biochemical composition of live diets grown under different levels of resource limitation, and 2. The large variation in responses of herbivores to diets of differing quality (Kilhan *et al.*, 1997a).

The food availability and risk of predation have been also reported as potential triggering components of copepod diel feeding rhythmus (Calbet *et al.*, 1999). Feeding implies higher motility and consequently higher probability of encounter rates with predators (Gerritsen & Strickler, 1977), and also increase the risk of predation by the creation of hydrodynamic signals which can be detected by carnivorous consumers (Tiselius *et al.*, 1996). When food is scarce, zooplankton must spend more time searching for food and this behavior increases the risk of predation and so, there is a balance between the necessity of feeding continuously to obtain the minimum food requirements and the risk of being predated (Calbet *et al.* 1999).

Food composition plays an important role in the fecundity and hatching success of copepod eggs even though food properties and characteristics responsible for these variations are not well established. Ianora and others have also hypothesized that diatoms might be toxic. Differences in the reproductive responses of copepods have been attributed to subtle differences between diatoms and flagellates such as an absence or presence of cell wall silica, shape and size of the cells, palatability or nutrient concentrations per unit cell volume (Ianora *et al.*, 1995a). Other studies have emphasized the importance of essential compounds for growth or reproduction such as fatty acids, proteins or vitamins, to explain the better quality of dinoflagellates or microzooplankton compared to diatoms in inducing higher fecundity (Kleppel *et al.*, 1991; Ianora & Poulet, 1993).

The ingestion rates and egg production of copepod are known from separate laboratory experiments to be related to the supply of available food (e.g. ingestion:

Mullin, 1963; Frost, 1972; egg production; Checkley, 1980a). Others studies suggest that the growth efficiency is a declining function of food concentration and is unrelated to the species of phytoplankton food (Checkley, 1980b).

Egg production and development rates in marine and freshwater copepods have been shown to depend on several factors, including food concentration and algal composition of the diet (Kiorboe *et al.*, 1985a; Arnott *et al.*, 1986; Ianora & Poulet, 1993; Jónasdóttir *et al.*, 1995). In addition, these production rates have been shown to be positively correlated with temperature, female body size, reproductive conditions and remating (Ianora & Poulet, 1993). Good egg quality, leading to vigorous nauplii, determines variability in the recruitment rate of natural populations (Ianora & Poulet, 1993) and increases the ability to survive in the first development stages (Laabir *et al.*, 1995a). It also indirectly influences fish recruitment, since the first naupliar stages of copepods are important prey to fish larvae (Laabir *et al.*, 1995b).

The study of copepod egg production quality is essential, since variability of copepod recruitment may depend largely on the rate of production of viable eggs rather than fecundity per se. This parameter also seems worthy of study because it can shed light on the secondary production, which has been defined as the biomass produced by a population in a given time interval, regardless of whether it survives to the end of the interval (Kimmerer, 1987).

Generation time and number of offspring are the most important determinant of the rate of population growth in species and thus, differences in generation time affect numerous ecological processes (e.g. competition, predation). These differences among species are sometimes attributed to body mass for diverse assortments of plants and animal (Gillooly, 2000).

Diversity and availability of food vary seasonally in marine environments (Kleppel & Hazzard, 2000), offering various alternative preys to phytoplankton in copepod diets. As a component of nano- and microzooplankton, protozoas are an important fraction of the food resource for copepod reproduction and growth (Kang *et al.*, 2000)

The high diversity of organisms (bacteria, phytoplankton and smaller zooplankton) and detritus used as food in commercial mariculture offered a balanced biochemical composition, that provides good chances for meeting all the nutritional requirements of larvae of fish and shellfish. Larviculture nutrition, more particularly for the start-feeding early larval stages, appears to be one of the major bottlenecks for the

industrial upscaling of the culture of many species of marine fish and shellfish (Coutteau & Sorgeloos, 1997). Often, only suboptimal nutrition is provided, as can be expected from the significant differences in physical and biochemical characteristics compared with the natural diet. This has resulted in various research for improving live feed and has encouraged the development of cheaper formulated diets, which can supplement or, in some cases, completely replace the live diets (Léger & Sorgeloos, 1992). Complete diet formulations can be incorporated in microbound and microencapsulated diets (Coutteau & Sorgeloos, 1997).

The existing literature considers two main limiting factors in the diet, i.e. phosphorus (stoichiometric theory) and fatty acids. Nevertheless, opinions and interpretations regarding the importance of these two factors is a subject of controversy in the literature. Aquaculture studies provide some direct evidence of the importance of long-chained polyunsaturated fatty acids (PUFA) for zooplankton that can affect the growth rates of zooplankton significantly. It is not yet clear if PUFA deficiency in the diet is in some way related to or caused by P deficiency (Gulati & Demott, 1997; Becker & Boersma, 2003).

Aquaculturists have emphasized the importance of knowing the nutritional requirements of individual consumers relative to the food available. Diatoms and flagellates are generally considered as good-quality foods because of their high eicosapentaenoic acid (EPA) content. On the contrary, cyanobacteria are low-quality food, having both low EPA and P content and also due to size- and form-related constraints on ingestion as well as toxicity (Gulati & Demott, 1997; Becker & Boersma, 2003).

Blooms of phytoplankton, generally dinoflagellates or flagellates (Smayda, 1991), are known to induce mass mortalities in fish and shellfish. The toxic potential of algae may cause neurological intoxication or paralytic intoxication of humans through accumulation in shellfish. Several species of dinoflagellates and flagellates also affect the feeding of copepods, the production of eggs and the survival of these organisms (Huntley *et al.*, 1987, Verity & Smayda, 1989). Diatoms (Bacillariophyceae), other important component of marine phytoplankton, were rarely reported as toxic to marine organisms until the identification of the high concentration of domoic acid in *Nitzschia pungens* f. *multiseries* by Douglas & Bates (1991). Diatoms, however, can have other harmful effects on zooplankton with important consequences for marine food webs (DeMott *et al.* 1991; Imada *et al.*, 1991; Kirk & Gilbert, 1992).

The first development phases of marine copepods generally exhibit higher mortalities in the plankton, than adults. Among development stages, nauplii seem to be the most sensitive, suffering higher mortality than copepodites and adults. Also, embryonic stages are subject to high mortality, around 60-70% of the eggs in certain times of the year. In field, one of the assumed main causes for the mortality of nauplii is predation. Laboratory experiments showed that food with certain characteristics, such as deficiencies in essential nutrients, have an important influence on the size of the eggs and, indirectly, their viability. Other factors have been suggested, such as viruses and diseases, genetic disorders, low health conditions and pollution (Jónasdóttir, 1989; Ianora *et al.*, 1992; Jónasdóttir *et al*., 1994).

The discovery of an inhibitory effect in certain species of diatoms (e.g. *Phaeodactylum tricornutum*) on the reproduction of copepods throws some doubt on the role of predation as the main factor that influences changes in the production of aquatic ecosystems (Shaw et al., 1994).

Egg production in some copepods species depends on the chemical composition and on the nutritive value of the foods, as well as on environmental factors such as temperature and recycling of nutrients (Kleppel *et al.*, 1991; Ianora, *et al.*, 1995b). Differences in the reproductive output of copepods fed diatoms or dinoflagellates have been attributed to differences in cell wall structure, cell size and shape, flavour, as well as concentration of essential nutrients (Poulet *et al*., 1994). These results suggest that the relative nutritional value of several species negatively affects egg production or embryonic development. Nutritional deficiency in the diet could explain the low viability of eggs observed under laboratory conditions or *in situ* under high diatom concentrations (Ianora & Poulet, 1993). Alternatively, the presence of inhibitory components in diatoms may be harmful to embryonic development and responsible for a low amount of viable eggs and production of abnormal nauplii (Poulet *et al.*, 1994; Ianora *et al.*, 1995ab).

Previous studies have shown that marine phytoplankton produces components that reduce or inhibit the grazing of several herbivore zooplankters. These observations, made in the field and in the laboratory, have focused on the feeding behavior of copepods in various phytoplankton assemblages (Shaw *et al.*, 1994). In laboratory experiments, the calanoid copepod *Calanus pacificus*, known not to feed on the dinoflagellate *Gymnodinium flavum* in its natural environment, did not feed on a dinoflagellate known to produce substances that inhibit its feeding (Huntley *et al.*,

1986). Based on these observations, the authors proposed that the production of feeding deterrents allows the slow growth of species obtaining bloom concentrations once that, producing such substances, the microalgae inhibit the grazing of the zooplankton. Feeding deterrents possess commercial and ecological importance. A number of phytoplankton species are used in mariculture. Production of these substances by the phytoplankton species will decrease the growth or increase the mortality in cultivated species, such as oysters.

The present study intends to analyze the influence of different phytoplankton algae on feeding behavior, survival, and reproduction of the copepod *Acartia tonsa* from the Kiel Bight. The work has as key-questions: Are the phytoplankton an appropriate food to *Acartia tonsa*? Do they interfere in survival and reproduction of *A. tonsa*? To answer these questions I measured the gross biochemical composition (protein, carbohydrate and lipid) of the phytoplankton species, belonging to different class of marine organisms.

# 2. Material and Methods

## 2.1.Phytoplankton species

The following phytoplankton species were used: *Alexandrium tamarense* (At); *Chrysocromulina acantha* (Ca); *Skeletonema costatum* (Sc); *Prorocentrum lima* (Pl); *Rhodomonas baltica* (Rb).

Diatom

•*Skeletonema costatum* (Greville) Cleve 1873

Order Biddulphiales

Suborder Coscinodiscineae

Family Thalassiosiraceae

Synonyms: *Melosira costata* Greville 1866.

Distribution: cosmopolitan (absent from the high Arctic and Antarctic).

This species was acquired in the Culture Collection of the IFM-GEOMAR Leibniz Institute of Marine Research (Kiel-Germany)

Marine Flagellates

•*Rhodomonas baltica* Karsten *sensu* Zimmerman 1925

Division Chromophyta

Class Cryptophyceae

Order Cryptomonadales

Family Cryptomonadaceae

Distribution: coastal, oceanic; Baltic, Atlantic.

Flagella: two, slightly, shorter than the cell.

Chloroplasts: one or two, lobed, poppy red or olive yellow.

Synonyms: *Cryptomonas baltica* (Karsten) Butcher; *Cryptomonas pseudobaltica* Butcher.

This species was acquired in the Culture Collection of the IFM-GEOMAR Leibniz Institute of Marine Research (Kiel-Germany)

•*Chrysocromulina acantha* Leadbeater & Manton, 1971

Division Chromophyta

Class Prymnesiophyceae

Order Prymnesiales

Family Prymnesiaceae

This species was acquired in the Culture Collection of the IFM-GEOMAR Leibniz Institute of Marine Research (Kiel-Germany)

•*Alexandrium tamarense* (Lebour) Balech 1992.

Order Gonyaulacales

Family Goniodomataceae

Synonyms: *Gonyaulax tamarensis* Lebour 1925; *G. tamarensis* var. *exavata* Braarud 1945; *G. exclavata* (Baarrud) Balech 1971; *Gessnerium tamarensis* (Lebour) Loeblich III and L. Loeblich 1979; *Protogonyaulax tamarensis* (Lebour) F. J. R. Taylor 1979; and *A. exclavatum* (Braarud) Balech and Tangen 1985.

Distribution: Coastal; western Europe from Norway to the Iberian peninsula including the British Isles, the Atlantic Ocean of the United States from Maine to 400N, Argentina, Japan, Korea, Gulf of Thailand, Canadian Pacific, Venezuela, Barents Sea, Kamchatka peninsula in the Soviet Union, and south of Taiwan.

This species was acquired from the CCAP (Culture Collection of Algae and Protozoa – Scotland, UK). The strain has the number CCAP 1119/5. Potentially toxic.

•*Prorocentrum lima* (Ehrenberg) Dodge 1975.

Order Prorocentrales Lemmermann 1910

Family Prorocentraceae Stein 1883

Synonyms: *Exuviaella lima* (Ehrenberg) Butschli 1885.

Distribution: Neritic and estuarine. Benthic/epiphytic; can be tychoplanktonic. Worldwide distribution.

This species was acquired from the CCAP (Culture Collection of Algae and Protozoa – Scotland, UK). The strain has the number CCAP 1136/9. Potentially toxic.

## 2.2.Maintenance of phytoplankton culture

The algae species were cultivated in Ostsee Medium in 12:12 light:dark cycle, in a volume of 50 ml (5 replicate), on 160C. Each ca. 10 days was replicated.

•Ostsee Medium

The Ostsee Medium is constituted by natural see water enriched with nutrients (Table 1).

Table 1 - Constitution of the Ostsee Medium.

| | **Chem. Substance** | **Quantity of Nutrient in 1 L distilled water** | **Concentration in the distilled water (mol.l-1).** | **Concentration in 1 L see water (μl).** | **Final concentration in seawater (μmol.l-1).** |
|---|---|---|---|---|---|
| **Macronutrients** | | | | | |
| N | NaNO3 | 8.499 g | 0.1 | 800 | 80 |
| P | NaH2PO4.H2O | 13.799g | 0.1 | 50 | 5 |
| Si | Na2O3Si.5H2O | 21.214g | 0.1 | 100 | 10 |
| **Micronutrients** | | | | | |
| EDTA (Titripex III) | Na2EDTA.2H2O | 0.372g | 0.001 | 1000 | 1 |
| Fe | FeSo4.5H2O | 0.242g | 0.001 | 1000 | 1 |
| Mn | MnCl2.2H2O + 2 drops of H2SO4 | 0.162g | 0.001 | 1000 | 1 |
| **Vitamin** | | | | | |
| Biotin (Vitamin H) | C10H16N2O3S | 0.050g | 0.0002 | 100 | 0.02 |
| Thiaminchlorid (Vitamin B1) | C12H18Cl2N4OS.H 2O | 0.050g | 0.0005 | 100 | 0.05 |
| Cyanocobalamin (Vitamin B12) | C63H88CoN14O14 P | 0.050g | 0.00004 | 100 | 0.004 |

## 2.3. Maintenance of zooplankton culture

The copepod *Acartia tonsa* Dana (1848) was maintained in ca. 20-liter seawater. The water was filtered by a Millipore filter (0,8 μm). They were fed with one mixture of the natural sea water containing detritus or/and organisms < 0,8 μm, from the Kiel Bight and *Rhodomonas baltica*.

Weekly, half of the water was replaced by filtered see water and more food (*Rhodomonas baltica*) was added.

During the experiments, more copepods were collected in front of the IFM-GEOMAR Leibniz Institute for Marine Research (Kiel) and Kiel Bight during the fall and winter. The copepods were acclimatized to the temperature of 160C and they were maintained in the same conditions as doing the experiments. This temperature was based on Jónasdóttir (1994), who found high female mortality as 200C, but survival close to 100% at 160C.

### 2.4.Biochemical analyses

The phytoplankton species were analyzed in relation to the contents of total protein, carbohydrate and lipid (gross composition).

For total protein Biuret method was used (Itzahki & Gill, 1964; Boechat & Giani, 2000). As standard substance a solution stock of bovine albumin was used.

For total carbohydrate the method of phenol-sulfuric acid was used (Herbert *et al.*, 1971; Boechat & Giani, 2000). As standard substance a solution stock of glucose was used.

For total lipid the method of vanilin was used (Zöllner & Kirsch, 1962; Boechat & Giani, 2000). As standard substance a solution stock of linoleic acid was used.

### 2.5. Experiments

To quantify the nutritive value of these algae for *A tonsa*, survival of adults, nauplii and copepodite, egg production and percentage of viable eggs were analyzed. The biochemical composition of macrocomponents (protein, lipids, carbohydrates) of the algae, used in experiments, was analyzed with the objective of knowing their nutritional characteristics.

#### 2.5.1. Experiment 1: Biochemical contents of algae species

This experiment was performed under the same condition of the maintenance of the phytoplankton and zooplankton species.

The phytoplankton species were cultivated in triplicate, in a 12:12 hours light:dark cycle in Ostsee Medium for four serial days. The start of the experiment was

in the 4th day of the phytoplankton growth (exponential phase). The algae were cultivated in a total volume of 600 ml with constant aeration. Daily, 250 ml of the cultivation was removed and it was substituted by new midium. 200 ml was filtered onton glass-fiber filter (GF/F) and frozen for the analysis (biochemical parameters and chlorophyll a). 50 ml was destined for the determination of the nutrients in the water (nitrate, phosphate and silicate) and for the determination of the number of cells. The cellular density was determined by the sedimentation method (Üthermohl, 1958). The results expressed in cell.ml-1. The pH of the samples was measured dialy. For chlorophyll *a* was used the spectrophotometric method (Parsons e Strickland, 1963; UNESCO, 1966; Teixeira, 1973). 30 individuals of each phytoplankton species were measured main to calculated the biovolume of the species. The cellular content of the biochemical constituents was expressed in pg.µm-3. The quantity of protein, carbohydrate and lipid that was offered as food to A. tonsa was expressed in µg.ml-1.

For this experiment there were two hypotheses:

Zero Hypothesis (H0): There is no difference in biochemical composition between the phytoplankton species.
Alternative Hypothesis (HA): There is a significant difference in biochemical composition between phytoplankton species.

Experimental outlines:

Objective: Production and maintenance of the algae to analyze the chemical composition and feeding of the copepod *A tonsa*. The biochemical components were measured during the first day of the experiment (survival and reproduction of *A tonsa*), when the phytoplankton species were in the 4th day of growth (exponential phase), and in during second day of the experiment, when the copepods were transferred for a new plate with new medium. The biochemical contents were measured only in the replicates without copepods.
Number of treatments: 5 treatments with 3 replicates.
Experimental time: several weeks.

Experimental replicates:

1. *Alexandrium tamarense* cultivated without copepods (replicates At I, II and III).
2. *Chrysocromulina acantha* cultivated without copepods (replicates Ca I, II and III).
3. *Skeletonema costatum* cultivated without copepods (replicates Sc I, II and III).
4. *Prorocentrum lima* cultivated without copepods (replicates Pl I, II, III).
5. *Rhodomonas baltica* cultivated without copepods (replicates Rb I, II, III).

### 2.5.2. Experiment 2: Feeding behavior, reproduction and survival of the copepod *A tonsa*.

It has been conducted under the same conditions as the maintenance of the phyto- and zooplankton species and the biochemical experiment. All the experiments were performed at the same time.

Experimental Conditions:

Grazer species: *A tonsa* Dana
Density of Grazer: for each replicated 5 females and 2 males. Sex ratio of 2.5:1.
Food: five phytoplankton species (*A. tamarense, C. acantha, S. costatum, P. lima, R. baltica*).
Density of food: The food concentration was established at ca. 200 µgC-ml-1. This concentration was based on the work of Berggreen *et al.*, (1988), where this concentration was sufficiently high to sustain ample egg production, but below typical saturation points for the numerical and functional responses of *A tonsa* in experimental conditions. In the mixed food the concentration of each specie was ca. 40 µgC-ml-1.
Duration: 24 h to several weeks, depending on the objective of the experiment and survival of the copepod.
Monitored Parameters: salinity (19 PSU), temperature (160C), light (12:12 dark:light cycle), cell density and nutrients. For the nutrients, cell density and chlorophyll *a* one sample for each replicated was obtained diary.

Step 1 – Determination of ingestion rates and food selectivity

Hypothesis zero (H0): *A. tonsa* does not interfere in the prey density after 24 hours. No significant ingestion occurs in the experiments.

Alternative Hypothesis (HA): *A. tonsa* exercises significant influence on food density. The food density in replicates with copepods is significantly lower than in control replicates without copepods.

Quantification of the ingestion rate:

After we verify that ingestion happened (HA is true), the difference in the food density, after 24 hours, between treatments with and without copepods, will be used for the calculation of the ingestion rate, by the following equations (Meyer-Harms, 1996):

Calculation of the algal growth (w)

$w = (\ln K24 - \ln K0)/t$

K0: Concentration of algae at the beginning of the experiment in the Control-Replicates (without copepods)
K24: Concentration of algae at the end of the experiment in the Control-Replicates (without copepods)
t: Incubation time

Calculation of the Grazing rate (g) under algal growth (w)

$g = w - [(\ln F24 - \ln F0)/t]$

F0: Algal concentration in the Feeding Replicates (with copepods) at the beginning of the experiment
F24: Algal concentration in the Feeding Replicates (with copepods) at the end of the experiment
t: Incubation time
Calculation of mean (average) algal concentration (K) in feeding replicates

Been w different of g will be K = VF0.F24

Been w = g will be K = F0 = F24 (no net growth)

Calculation of ingestion rates

$I = (g * K * V)/N$

V: Volume (ml)

N: Amount of copepod per container

Experiment A:

Objective: To quantify the ingestion rates and feeding selectivity of *A. tonsa*.

Number of treatments: 12 treatments with 3 replicates

Samples for cell counts: 42 (24 samples of initial density + 24 samples after 24 h)

Duration: 24 h

Experimental treatments:

1. *A. tonsa* fed by *A. tamarense* (replicates CAT I, II and III), to determine the ingestion of *A. tamarense* in 24 hours.
2. *A. tamarense* cultivated without copepods (replicates At I, II and III), as control for CAT.
3. *A. tonsa* fed by *C. acantha* (replicates CCA I, II and III), to determine the ingestion of *C. acantha* in 24 hours.
4. *C. acantha* cultivated without copepods (replicates CA I, II and III), as control for CCA.
5. *A. tonsa* fed by *S. costatum* (replicates CSC I, II and III), to determine the ingestion of *S. costatum* in 24 hours.
6. *S. costatum* cultivated without copepods (replicates SC I, II and III), as control for CSC.
7. *A. tonsa* fed by *P. lima* (replicates CPL I, II and III), to determine the ingestion of *P. lima* in 24 hours.
8. *P. lima* cultivated without copepods (replicates PL I, II and III), as control for CPL.
9. *A. tonsa* fed by *R. baltica* (replicates CRB I, II and III), to determine the ingestion of *R. baltica* in 24 hours.
10. *R. baltica* cultivated without copepods (replicates RB I, II and III), as control for CRB.

11. *A. tonsa* fed by a mixture of algae (replicates CAA I, II and III), to determine the ingestion of the species and the feeding selectivity (selectivity index) in 24 hours.
12. Mixture of the algae species cultivated without copepods (replicates AA I, II and III), as control for CAA.

Step 2 - Comparison of the nutritional quality of algae with the reproduction and survival of *A.tonsa*.

Zero Hypothesis (H0): There is no effect of the food on survival and reproduction of *A. tonsa*. The mortality of *A. tonsa* is the same in treatments without food or when offered any of the algae species.
Alternative Hypothesis (HA): The presence of food and food type has a significant effect on survival and reproduction of *A. tonsa*.

Experiment B:
Objective: To check the effect of the food on survival and reproduction of *A. tonsa*.
Number of treatments: 7 treatments with 3 replicates.
Experiment duration: The experiments of survival and reproduction lasted ca. 40 days.
Density of the copepod: 5 females and 2 males.

Experimental treatments:

1. Copepods (*A. tonsa*) maintained without food (reply CN I, II and III), to determine survival time without food.
2. *A. tonsa* fed by *A. tamarense* (replicates CAT I, II and III), to determine the mortality and reproduction.
3. *A. tonsa* fed by *C. acantha* (replicates CCA I, II and III), to determine the mortality and reproduction.
4. *A. tonsa* fed by *S. costatum* (replicates CSC I, II and III), to determine the mortality and reproduction.
5. *A. tonsa* fed by *P. lima* (replicates CPL I, II and III), to determine the mortality and reproduction.
6. *A. tonsa* fed by *R. baltica* (replicates CRB I, II and III), to determine the mortality and reproduction.

7. *A. tonsa* fed by a mixture of the algae species (replicates CAA I, II and III, to determine the mortality and reproduction.

#### 2.5.2.1. Determination of the Reproduction of the copepod *A tonsa*

Daily, the egg production was examined in each replicate and the adult copepods were transferred for a new plate with new food. The eggs were counted and they were transferred to tissue cell plates, where they were examined daily to determine the hatching and survival for nauplii and copepodite. As parameters we counted the number of total egg production in each replicate. For the statistical analysis we considered the total eggs production, total eggs production per day (effect of the time), total eggs production per female and total eggs production per female per day (effect of the mortality of female with the time).

#### 2.5.2.2. Determination of the survival of the copepod *A tonsa*

Daily the parental copepods were counted to determine the time of survival of the copepod in different treatments. In the tissue cell plates we counted the survival of nauplii and copepodite too to know the effect of the food on the survival of the early stages.

### 2.6.Statistical Analysis

The statistical test utilized was ANOVA one way to analyze the effect of the food quality on the reproduction, survival and feeding behavior of *A tonsa* and the Tukey or Ducan test for the comparison of means values.

Correlation Moment-product of Pearson was utilized to analyze the variation of the biochemical composition of the phytoplankton species in the medium during the cultivation time as so as, the effect of the different foods on the survival, reproduction and feeding behavior of *A tonsa*, considered the abiotic parameters too.

# 3. Results

## 3.1. Characteristic of the phytoplankton species.

### 3.1.1. Cell volume of the phytoplankton species.

In the table 2 find the cell volume of the phytoplankton species.

Table 2 – Cell volume of the phytoplankton species, which were used as food for the copepod *A tonsa.*

| Species | n | Dimension (μm) | Volume (μm-3) |
|---|---|---|---|
| *A tamarense* (At) | 30 | 31 | 15590 |
| *C. acantha* (Ca) | 30 | 10 | 523 |
| *S. costatum* (Sc) | 30 | 15-42l/7-9w | 7418 |
| *P. lima* (Pl) | 30 | 31-42 | 40362 |
| *R. baltica* (Rb) | 30 | 8-19 | 1216 |

### 3.1.2.Biochemical composition per cell volume of the phytoplankton species (pg.μm-3).

#### 3.1.2.1. *Alexandrium tamarense*

The medium content of carbohydrate per cellular volume was 193 pg.μm-3 (124-242 pg.μm-3). Lipid showed a value of 103 pg.μm-3 (96-108 pg.μm-3) and protein a value of 472.5 pg.μm-3 (312-613.5 pg.μm-3).

Of the biochemical constitutes measured, carbohydrate per volume represented 25% of the biochemical constitution, lipid 13% and protein 62% (Figure1).

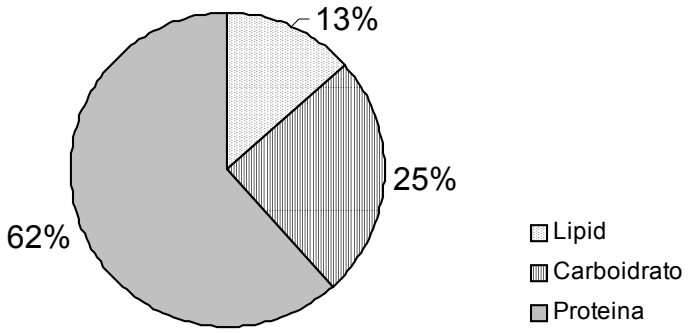


Figure 1 – Relative lipid, carbohydrate and protein composition of *Alexandrium tamarense*.

### 3.1.2.2. *Chrysocromulina acantha*

The content medium of carbohydrate per cellular volume was 4,062.5 pg.µm-3 (2,880-5,059.5 pg.µm-3). Lipid was of 3,407 pg.µm-3 (2,947-4,103 pg.µm-3) and protein of 16,763.5 pg.µm-3 (12,972-20,784.5 pg.µm-3).

Of the biochemical constitutes measured, carbohydrate per volume represented 17% of the biochemical constituents, lipid 14% and protein 69% (Figure 2).

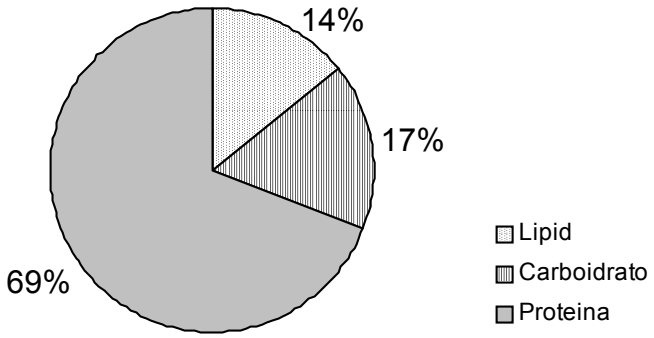


Figure 2 – Relative lipid, carbohydrate and protein composition of *Chrysocromulina acantha.*

### 3.1.2.3. *Skeletonema costatum*

The content medium of carbohydrate per cellular volume was 722 pg.µm-3 (643-781 pg.µm-3). Lipid was 321.5 pg.µm-3 (280-347 pg.µm-3) and protein was 1,250 pg.µm-3 (1,169-1,326b pg.µm-3).

Of the biochemical constitutes measured, carbohydrate per volume represented 31%, lipid 14% and 55% respectively (Figure 3).

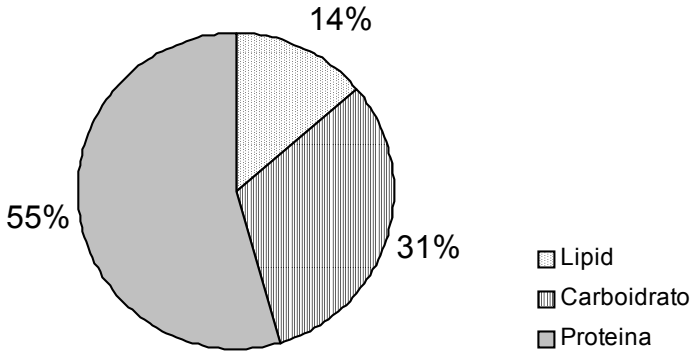


Figure 3 – Relative presentation of the biochemical composition of *Skeletonema costatum*.

### 3.1.2.4. *Prorocentrum lima*

The content medium of carbohydrate per cellular volume was 88 pg.µm-3 (63-138.6 pg.µm-3). Lipid was 55.5 pg.µm-3 (45-77 pg.µm-3) and protein was 182 pg.µm-3 (124-284 pg.µm-3).

Of the biochemical constitutes measured, carbohydrate per volume represented 27% of the cellular volume, lipid 17% and protein 56% (Figure 4).

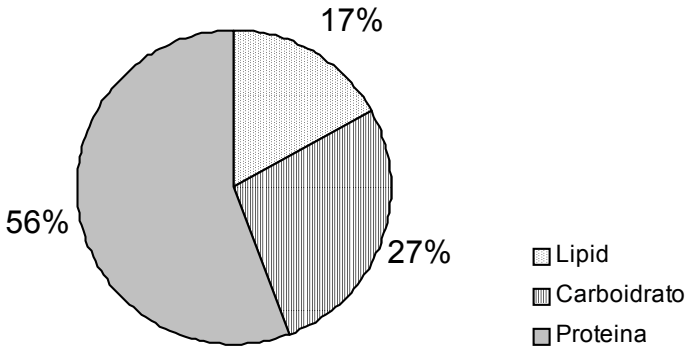


Figure 4 – Relative lipid, carbohydrate and protein composition of *Prorocentrum lima.*

**3.1.2.5. *Rhodomonas baltica***

The content of carbohydrate per cellular volume was 3,087 pg.µm-3 (2,672-3,736 pg.µm-3). The valor of lipid was 2,219 pg.µm-3 (2,050-2,550.5 pg.µm-3) and protein was 6,999.5 pg.µm-3 (5,502-9,304.5 pg.µm-3).

Of the biochemical constitutes measured, carbohydrate per volume represented 25% of the cellular volume, lipid 18% and protein 57% (Figure 5).

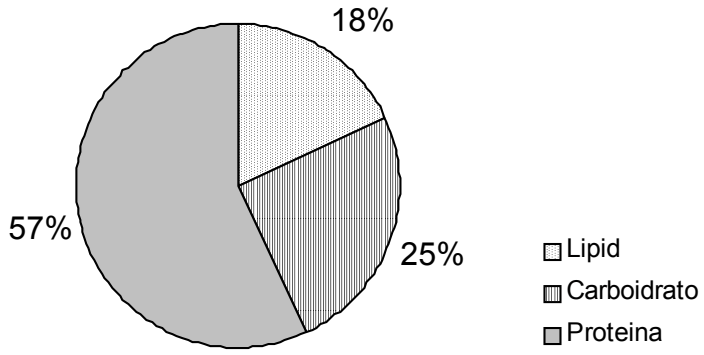


Figure 5 – Relative lipid, carbohydrate and protein composition of *Rhodomonas baltica.*

**3.1.2.6. Variance Analysis (ANOVA one-way).**

The ANOVA of the lipid content showed highly significant interspecific differences ($p < 0.01$) (Table 3).

Table 3 - ANOVA summary of the lipid content per cellular volume of the five phytoplankton species used as food to the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (**) |
|---|---|---|---|---|
| Between treatments | 0.002757 | 4 | 0.000689 | |
| Within treatments | 9.2E-05 | 10 | 9.2E-06 | 74.95562 |
| Total | 0.002849 | 14 | | |

** – highly significant ($p < 0.01$)

The Tukey test showed a significant difference the mean value of lipid among *C. acantha* and the other species and between *C. acantha* and *R. baltica*. *R. baltica* was significantly different from *S. costatum*, *A tamarense* and *P. lima*. The latter species did not show significant differences (Figure 6).

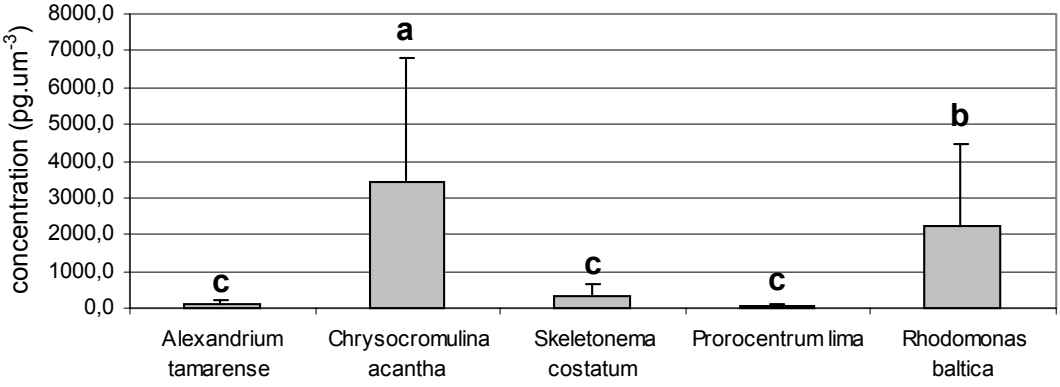


Figure 6 - Content of lipid in the five phytoplankton species, per cellular volume, used as food to the copepod *A tonsa*. Comparison among the phytoplankton species. (Tukey test, same letters mean a not significant difference among the medium value; n = 3).

The variance analysis for carbohydrate showed highly significant interspecific differences ($p < 0.01$) (Table 4).

Table 4 - ANOVA summary of the carbohydrate content per cellular volume of the five phytoplankton species used as food to the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (**) |
|---|---|---|---|---|
| Between treatments | 0.003992 | 4 | 0.000998 | |
| Within treatments | 0.00031 | 10 | 3.1E-05 | 32.23445 |
| Total | 0.004302 | 14 | | |

** – highly significant ($p < 0.01$)

The Tukey test demonstrated that the carbohydrate content of *C. acantha* and *R. baltica*. were not significantly different and they were significantly different from the others species. *S. costatum, A tamarense* and *P. lima.* did not differ among them (Figure 7).

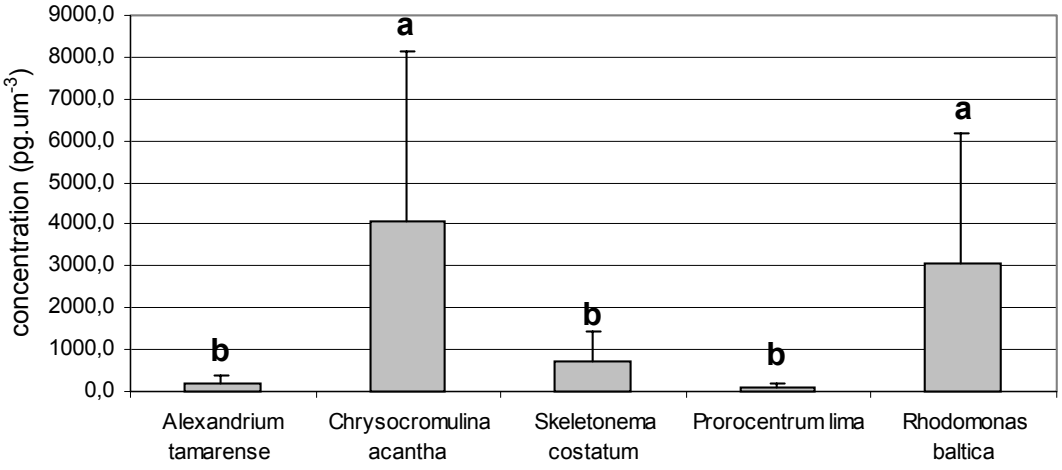


Figure 7 - Content of carbohydrate in the five phytoplankton species per cellular volume used as food to the copepod *A tonsa*. Comparison among the phytoplankton species. (Tukey test, same letters mean a not significant difference among the medium value; n = 3).

The ANOVA for the protein content per cellular volume showed highly significant interspecific difference ($p < 0.01$) (Table 5).

Table 5 - ANOVA summary of the protein content per cellular volume of the five phytoplankton species used as food to the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (**) |
|---|---|---|---|---|
| Between treatments | 0.060018 | 4 | 0.015005 | |
| Within treatments | 0.003888 | 10 | 0.000389 | 38.59307 |
| Total | 0.063906 | 14 | | |

** – highly significant ($p < 0.01$)

The Tukey test showed that the protein content of *C. acantha* and *R. baltica* were significantly different between them and they were significantly different from the other species. *S. costatum, A tamarense* and *P. lima* did not differ among them (Figure 8).

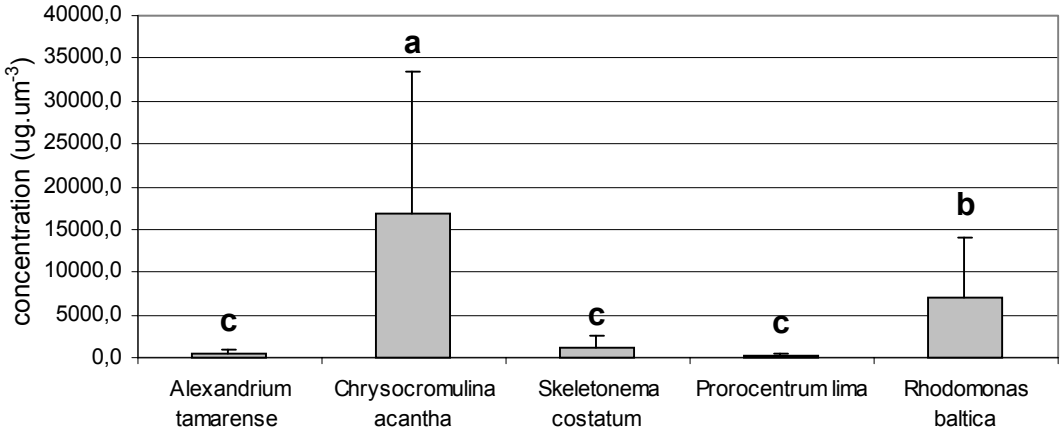


Figure 8 - Content of protein in the five phytoplankton species per cellular volume used as food to the copepod *A tonsa*. Comparison among the phytoplankton species. (Tukey test, same letters mean a not significant difference among the medium value; n = 3).

### 3.2. Biochemical contents of the food offered to *A tonsa*

#### 3.2.1 Biochemical composition of the food. Biochemical composition per dry weight.

The food offered to *A* tonsa constituted of the species A *tamarense* was made up of 30.26% (14.42-51.18%) of protein, 11.96% (2.69-19.99%) of carbohydrate and 6.50% (4.92-8.67%) of lipid per dry weight. *C. acantha* food was constituted of 35.03% (20.50-60.26%) of protein, 8.50% of carbohydrate (1.89-14.83%) and 7.23% (4.85-10.04%) of lipid. The food constituted of *S. costatum* shown 35.75% (11.74-58.91%) of protein, 20.37% (12.50-29-26%) of carbohydrate and 9.17% (6.75-12.52%) of lipid. *P. lima*s food is constituted of 29.27% (13.28-46.96%) of protein, 14.09% (6.33-20.56%) of carbohydrate and 8.99% (7.50-11.81%) of lipid. *R. baltica* as food to *A tonsa* was constituted of 32.48% (14.41-51.24%) of protein, 14.50% (13.01-17.85%) of carbohydrate and 10.45% (7.03-13.56%) of lipid (Figure9).

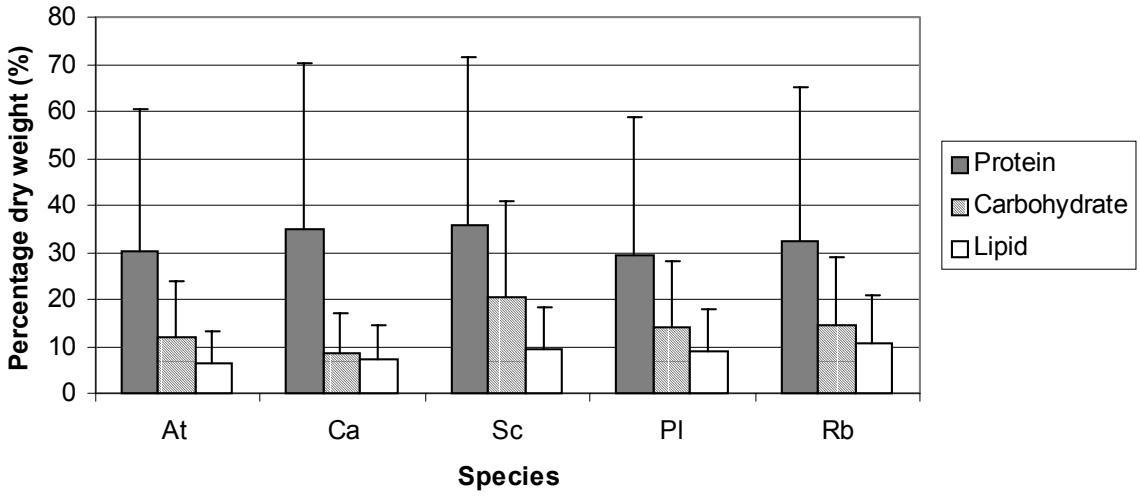


Figure 9 - Representation of the biochemical constitutes in % dry weight in five phytoplankton species offered as food to the copepod *A tonsa*( *A tamarense* (At); *C. acantha* (Ca); *S. costatum* (Sc); *P. lima* (Pl); *R. baltica* (Rb)

**3.2.1.1. Variance analysis (ANOVA one-way).**

### 3.2.1.1.1. Contents of lipid in the food

The analysis of the data variance of the lipid content per dry weight (%) offered as food showed highly significant interspecific differences ($p<0.01$) (Table6).

Table 6 - ANOVA summary of the lipid content per dry weight (%) of the five phytoplankton species used as food to the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (**) |
|---|---|---|---|---|
| Between treatments | 70.45173 | 4 | 17.61293 | |
| Within treatments | 123.9058 | 30 | 4.130193 | 4.264433 |
| Total | 194.3575 | 34 | | |

** - statistically significant ($p < 0.01$)

The Tukey and Duncan test were not significant, there was not significant difference among the lipid content per dry weight (%) among the phytoplankton species (Figure10)

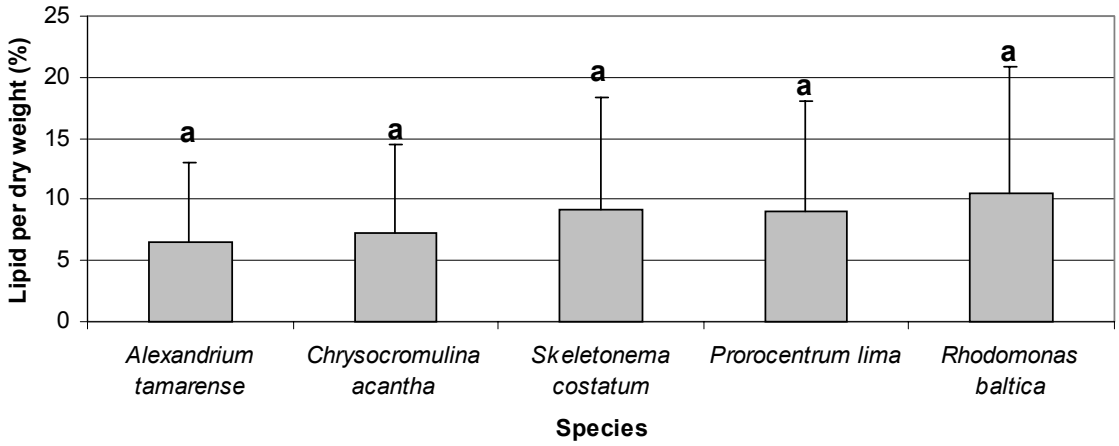


Figure 10 - Content of lipid per dry weight (%) in the five phytoplankton species used as food to the copepod *A tonsa* (Tukey and Duncan test, same letters mean a not significant difference among the medium value; n = 3).

### 3.2.1.1.2. Contents of carbohydrate in the food

The ANOVA for the carbohydrate content per dry weight (%)in the food offered to *A tonsa* showed significant interspecific difference ($p<0.01$) (Table 7).

Table 7 - ANOVA summary of the carbohydrate content per dry weight (%) of the five phytoplankton species used as food to the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (**) |
|---|---|---|---|---|
| Between treatments | 526.6642 | 4 | 131.6661 | |
| Within treatments | 817.7558 | 30 | 27.25853 | 4.83027 |
| Total | 1344.42 | 34 | | |

** - statistically significant ($p < 0.01$)

The Duncan test showed significant difference between *S. costatum* and *C. acantha*. However, *S. costatum* and *C. acantha* were not significantly different from the others species. There was not significant difference among *R. baltica*, *P, lima*, and *A tamarense* (Figure 11).

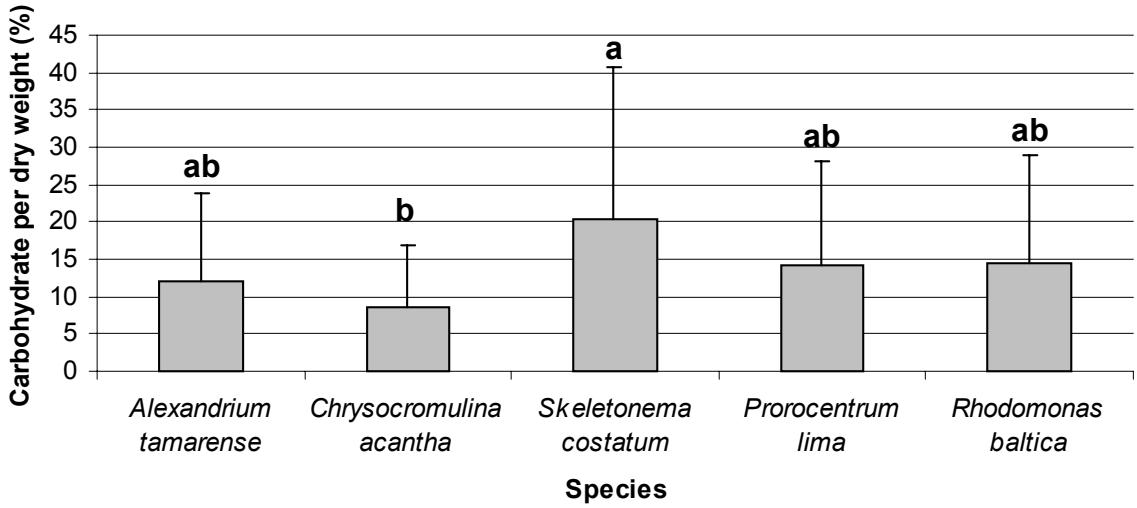


Figure 11 - Content of carbohydrate per dry weight (%) in the five phytoplankton species used as food to the copepod *A tonsa* (Duncan test, same letters mean a not significant difference among the medium value; n = 3).

### 3.2.1.1.3. Contents of protein in the food

The analysis of variance of the contents of protein per dry weight (%) in the food showed not significant interspecific differences (p≥0.05). The Tukey and Duncan tests did not identify a significant difference among the contents of protein per dry weight (%) of the phytoplankton species used as food to the copepod *A tonsa* (Table 8; Figure 12).

Table 8 - ANOVA summary of the protein content per dry weight (%) of the five phytoplankton species used as food to the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (NS) |
|---|---|---|---|---|
| Between treatments | 226.6847 | 4 | 56.67118 | |
| Within treatments | 7192.098 | 30 | 239.7366 | 0.236389 |
| Total | 7418.783 | 34 | | |

NS – no statistically significant (p ≥ 0.05)

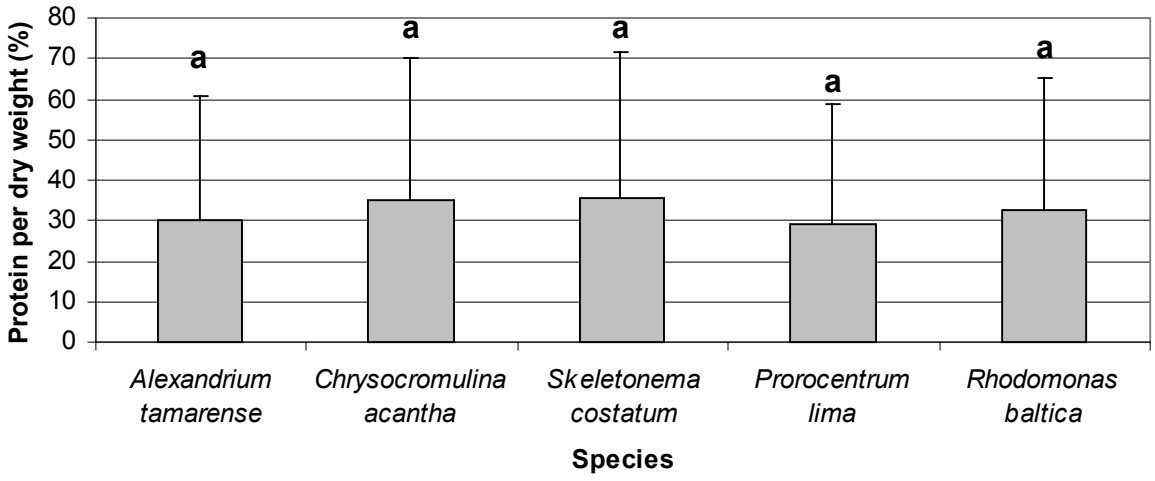


Figure 12 - Content of protein per dry weight (%) in the five phytoplankton species used as food to the copepod *A tonsa* (Tukey and Duncan tests, same letters mean a not significant difference among the medium value; n = 3).

### 3.2.2. Biochemical composition of the food. Quantity of protein, carbohydrate and lipid offered to the copepod *A tonsa* (μg.ml-1).

#### 3.2.2.1. Lipid

The mean value of lipid in *A tamarense* offered to *A tonsa* was 16 μg.ml-1 (15-17 μg.ml-1). The mean values of lipid in *C. acantha* was 18 μg.ml-1 (15.5-21.5 μg.ml-1) In *S. costatum* the mean content of lipid was 24 μg.ml-1 (21-26 μg.ml-1). The mean values of lipid in *P. lima* was 22.5 μg.ml-1 (18-31 μg.ml-1). The mean values in *R. baltica* was 27 μg.ml-1 (25-31 μg.ml-1).

#### 3.2.2.2. Carbohydrate

The mean values of carbohydrate in *A tamarense* offered as food to the copepod *A tonsa* was 30 μg.ml-1 (19-38 μg.ml-1). In *C. acantha* the mean content was 21 μg.ml-1 (15-26.5 μg.ml-1). The mean values in *S. costatum* was 53.5 μg.ml-1 (48-58 μg.ml-1). The medium value of carbohydrate in *P. lima* was 35.5 μg.ml-1 (25.5-56 μg.ml-1). The medium content of carbohydrate in *R. baltica* was 37.5 μg.ml-1 (32.5-45.5 1μg.ml-1).

#### 3.2.2.3. Protein

The medium value of protein in *A tamarense* offered as food to the copepod *A tonsa* was 73.5 μg.ml-1 (48.5-95.5 μg.ml-1). In *C. acantha* this content was 88 μg.ml-1 (68-109 μg.ml-1). The medium values of protein in *S. costatum* was 93 μg.ml-1 (87-98.5 μg.ml-1). In *P. lima* the medium content of protein offered to *A tonsa* was 73.5 μg.ml-1 (50-114.5 μg.ml-1) and in *R. baltica* the medium value was 85 μg.ml-1 (67-113 μg.ml-1).

### 3.2.2.4. Biochemical composition. Comparison among the different species.

The food offered to *A* tonsa constituted of the species A *tamarense* was made up of 62% of protein, 25% of carbohydrate and 13% of lipid, when the comparison was done among the three biochemical constituents. *C. acantha* food was constituted of 69% of protein, 17% of carbohydrate and 14% of lipid. The food constituted of *S. costatum* shown 55% of protein, 31% of carbohydrate and 14% of lipid. *P. lima*s food is constituted of 54% of protein, 27% of carbohydrate and 17% of lipid. *R. baltica* as food to *A tonsa* was constituted of 57% of protein, 25% of carbohydrate and 18% of lipid (Figure13).

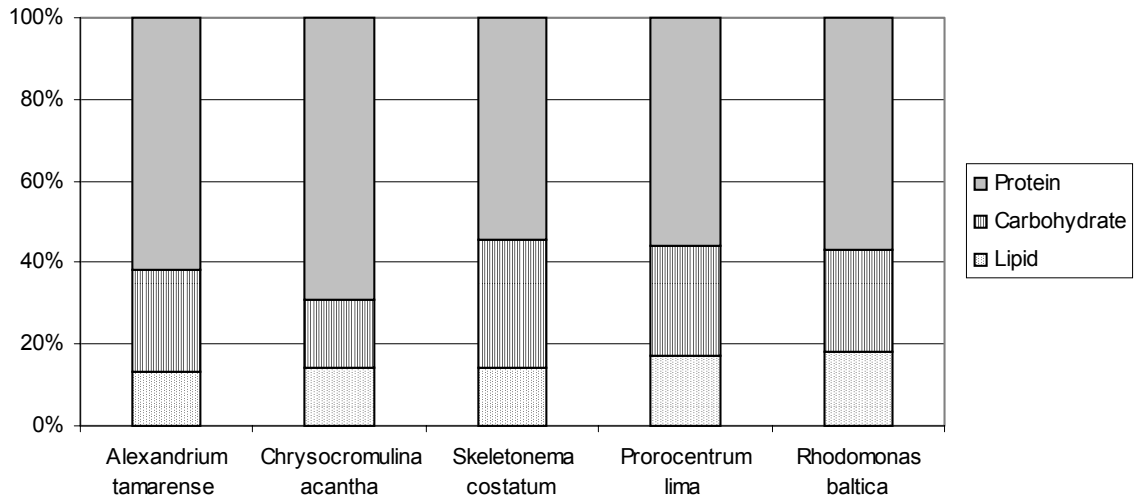


Figure 13 - Representation of the biochemical constitutes in % in five phytoplankton species offered as food to the copepod *A tonsa.*

### 3.2.2.5. Variance analysis (ANOVA one-way).

3.2.2.5.1. Contents of lipid in the food

The analysis of the data variance of the lipid content offered as food showed significant interspecific differences ($p<0.05$) (Table 9).

Table 9 - ANOVA summary of the lipid content of the five phytoplankton species used as food to the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (*) |
|---|---|---|---|---|
| Between treatments | 239.6003 | 4 | 59.90008 | |
| Within treatments | 171.2432 | 10 | 17.12432 | 3.497954 |
| Total | 410.8435 | 14 | | |

* - statistically significant ($p < 0.05$)

The Tukey test showed that the value of lipid in the food constituted for *R, baltica*, *S. costatum*, *P. lima* or *C. acantha* was not significant however, the value of *R. baltica* was significantly different to *A tamarense*. The food constituted for *A tamarense* was not different in lipid contents as *S. costatum*, *P. lima* and *C. acantha* (Figure14)

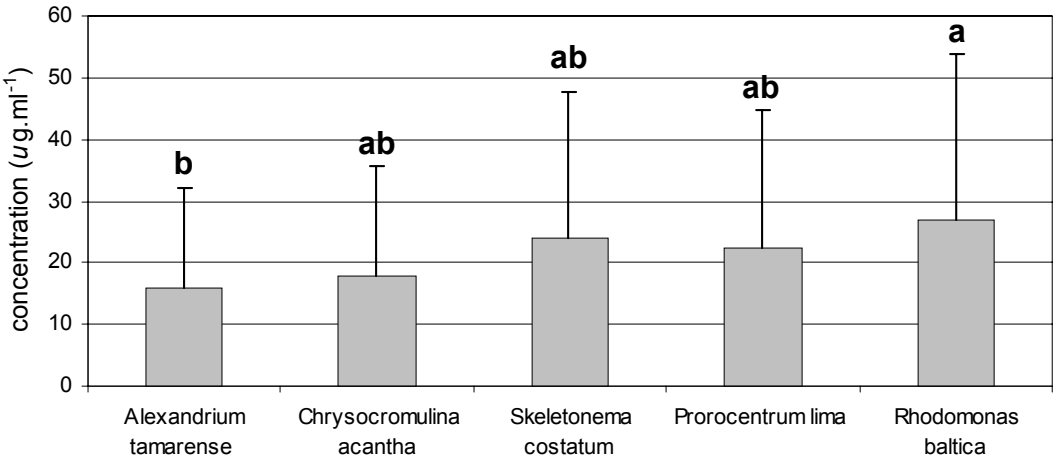


Figure 14 - Content of lipid in the five phytoplankton species used as food to the copepod *A tonsa* (Tukey test, same letters mean a not significant difference among the medium value; n = 3).

3.2.2.5.2. Contents of carbohydrate in the food

The ANOVA for the carbohydrate content in the food offered to *A tonsa* showed significant interspecific differences ($p<0.05$) (Table 10).

Table 10 - ANOVA summary of the carbohydrate content of the five phytoplankton species used as food to the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (*) |
|---|---|---|---|---|
| Between treatments | 1686.574 | 4 | 421.6435 | |
| Within treatments | 1020.113 | 10 | 102.0113 | 4.133301 |
| Total | 2706.687 | 14 | | |

* - statistically significant ($p < 0.05$)

The Tukey test showed that there was a significant difference between *C. acantha* and *S. costatum*, but these species were not significant different as the other. There was not significant difference among *R. baltica*, *P, lima*, and *A tamarense* (Figure 15).

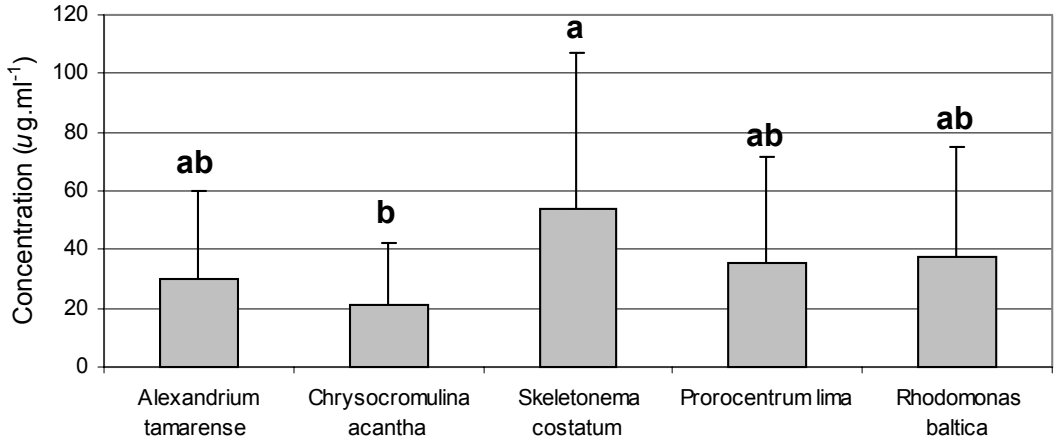


Figure 15 - Content of carbohydrate in the five phytoplankton species used as food to the copepod *A tonsa* (Tukey test, same letters mean a not significant difference among the medium value; n = 3).

## 3.2.2.5.3. Contents of protein in the food

The analysis of variance of the contents of protein in the food showed not significant interspecific differences ($p \geq 0.05$). The Tukey test did not identify a significant difference among the contents of protein of the phytoplankton species used as food to the copepod *A tonsa* (Table 11; Figure 16).

Table 11 - ANOVA summary of the protein content of the five phytoplankton species used as food to the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (NS) |
|---|---|---|---|---|
| Between treatments | 895.2557 | 4 | 223.8139 | |
| Within treatments | 5783.2557 | 10 | 578.3586 | 0.386981 |
| Total | 6678.842 | 14 | | |

NS – no statistically significant ($p \geq 0.05$)

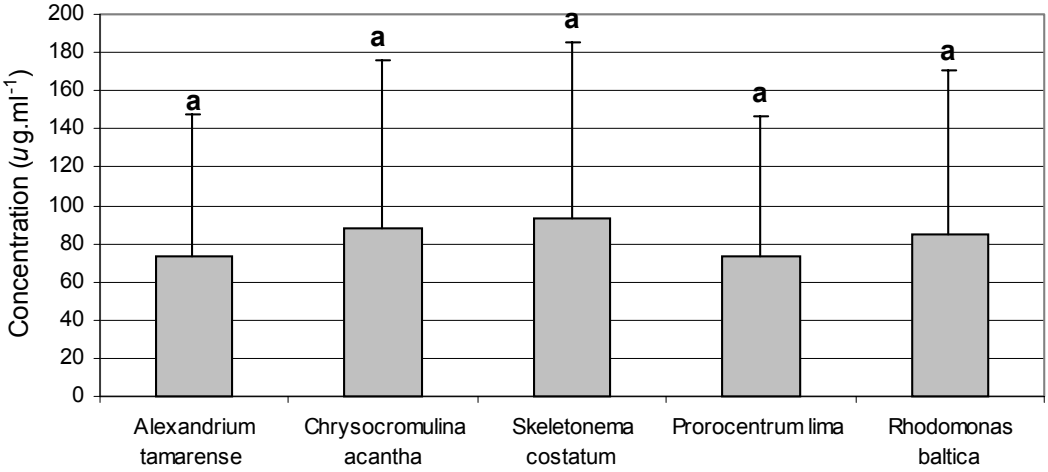


Figure 16 - Content of protein in the five phytoplankton species used as food to the copepod *A tonsa* (Tukey test, same letters mean a not significant difference among the medium value; n = 3).

### 3.3. Feeding behavior of the copepod *A tonsa*

#### 3.3.1. Ingestion rate

The ingestion rate in the treatment with *A tamarense* was 683 cell.cop-1.h-1. The copepod *A tonsa* fed on *A acantha* in a rate of 139,513 cell.cop-1.h-1. The ingestion rate in the treatment with *S. costatum* was of 33,977 cell.cop-1.h-1. *R. baltica* was eaten in a rate of 94,063 cell.cop-1.h-1 and *P. lima* in a rate of 7.5 cell.cop-1.h-1. In the mix-food (treatment mix) the ingestion rate was 96,862.5 cell.cop-1.h-1.

Into the mix-food, *A tamarense* was ingested in a rate of 2,732 cell.cop-1.h-1. The ingestion rate of *C. acantha* was 13,517 cell.cop-1.h-1 and *S. costatum* was of 35,220.5 cell.cop-1.h-1. The ingestion rate of *P. lima* and *R. baltica* were 8 cell.cop-1.h-1 and 34,245 cell.cop-1.h-1, respectively.

In the mixed food these species were 1/5 in biomass of the single food. The ingestion rate of these species increased in the mixed food. Only the ingestion rate of *C. acantha* decreased in the mixed food (Table 12).

Table 12 – Comparison of the ingestion rate (IR) of the phytoplankton species as mixed and single food.

| Species | IR in the single diet | Expect IR in the mixed diet* | IR in the mixed diet | Result |
|---|---|---|---|---|
| A. tamarense | 683 | 136 | 2,732 | > |
| *C. acantha* | 139,513 | 27,906 | 13,517 | < |
| *S. costatum* | 33,977 | 6,795 | 35,220 | > |
| *P. lima* | 7.5 | 1.5 | 8 | > |
| *R. baltica* | 94,063 | 18,812 | 34,245 | > |

*IR in the mixed food 1/5 of the single food (same concentration)

The variance analysis of the ingestion rate among the treatments showed highly significant interspecific differences ($p < 0.01$) (Table 13).

Table 13 - ANOVA summary of the ingestion rate in the treatments with the five phytoplankton species used as food to the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (**) |
|---|---|---|---|---|
| Between treatments | 3.42E+13 | 6 | 5.7E+12 | |
| Within treatments | 1.98E+12 | 14 | 1.41E+11 | 40.40426 |
| Total | 3.62E+13 | 20 | | |

** – highly significant ($p < 0.01$)

The Tukey test showed that the treatment with *C. acantha* did not differ significantly to the mix-food treatment however, it was significantly different to the other one. The treatment with *R. baltica* did not show significant difference between itself and the mix-food and, it was significantly different to the other one. The mix-food treatment was different of the other treatment (Figure 17).

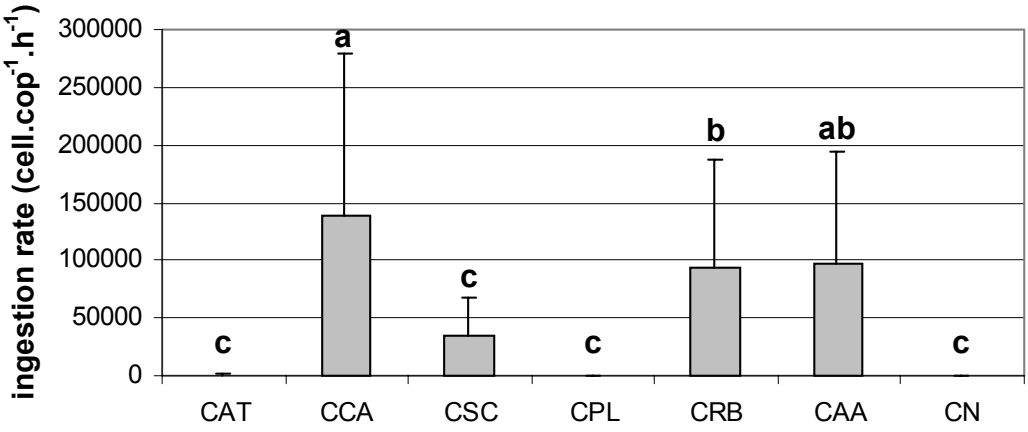


Figure 17 - Average of the ingestion rate in the treatments with five phytoplankton species (cell.copepod-1.h-1), used as food to the copepod *A tonsa*. Comparison among the treatments. (Tukey test, same letters mean a not significant difference among the medium value; n = 3) (Copepod + *A tamarense* (CAT); Copepod + *C. acantha* (CCA); Copepod + *S. costatum* (CSC); Copepod + *P. lima* (CPL); Copepod + *R. baltica* (CRB); Copepod + Mix-food (CAA); Copepod without food (CN)).

In the mix-food treatment the variance analysis showed highly significant interspecific differences ($p < 0.01$) (Table 14).

Table 14 - ANOVA summary of the ingestion rate of the five phytoplankton species used as food to the copepod *A tonsa.* Mix-treatment (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (**) |
|---|---|---|---|---|
| Between treatments | 5.44E+08 | 4 | 1.36E+08 | |
| Within treatments | 80445066 | 10 | 8044507 | 16.91134 |
| Total | 624618643 | 14 | | |

** – highly significant ($p < 0.01$)

The Tukey test did not show significant difference between the ingestion rate of *S. costatum* and *R. baltica* however, these phytoplankton species differed significantly from the other one. Among *C. acantha*, *A tamarense* and *P. lima* there was not significant difference (Figure 18).

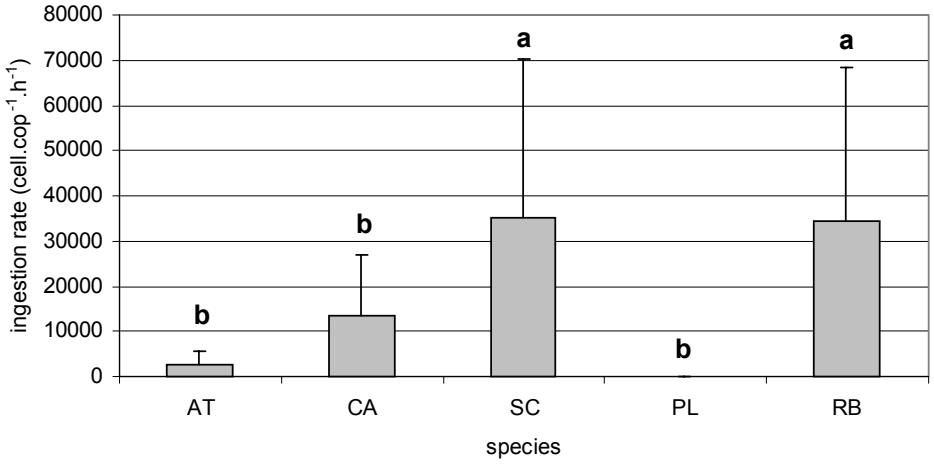


Figure 18 - Average of the ingestion rate in the mix-treatments of the five phytoplankton species (cell.copepod-1.h-1), used as food to the copepod *A tonsa*. Comparison into the treatment. (Tukey test, same letters mean a not significant difference among the medium value; n = 3) (*A tamarense* (AT); *C. acantha* (CA); *S. costatum* (SC); *P. lima* (PL); *R. baltica* (RB)).

### 3.4. Reproduction and survival of the copepod *A tonsa*.

#### 3.4.1. Reproduction of the copepod *A tonsa*.

##### 3.4.1.1. Total production of eggs

The total egg production ranged from 0.3 eggs in the treatment without food to 402.5 eggs in the treatment with the mixed food. The treatment with *C. acantha* provided an average eggs production of 42.5. In the treatments with *A tamarense* and *P. lima* as food, the average production of eggs was 29 and 13.5 eggs respectively. In the treatment with *S. costatum*, *A. tonsa* did not produce eggs.

The variance analysis showed significant differences among the treatments ($p<0.05$). (Table 15).

Table 15 - ANOVA summary of the total eggs production of the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (*) |
|---|---|---|---|---|
| Between treatments | 375962.5 | 6 | 62660.4127 | |
| Within treatments | 237215.3 | 14 | 16943.95238 | 3.698098961 |
| Total | 613177.8 | 20 | | |

* – statistically significant ($p < 0.05$)

The Tukey test showed that the treatment with the mix-food, *R. baltica* and *C. acantha* were identical, however the treatment with *C. acantha* was identical as the treatments with *A tamarense, S. costatum, P. lima* and the treatment without food (Figure 19).

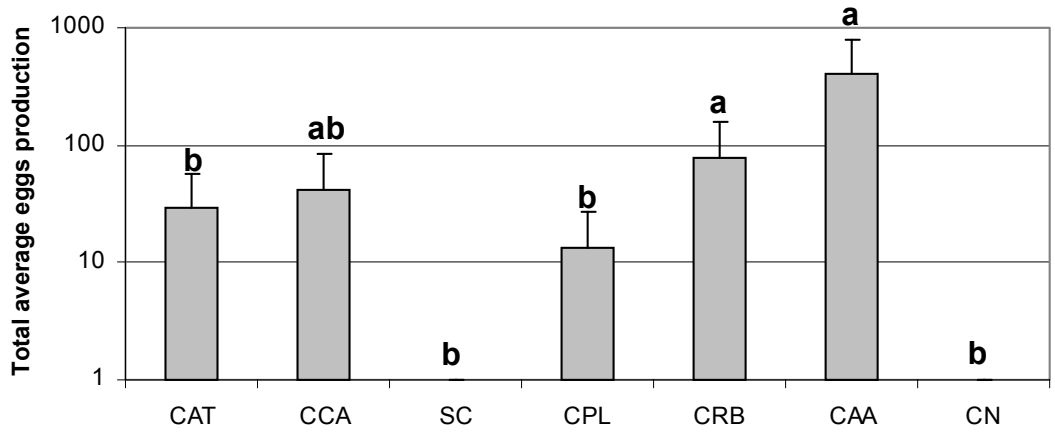


Figure 19 - Average of the total egg production of the copepod *A tonsa*. Comparison of the treatments. (Tukey test, same letters mean a not significant difference among the medium value; n = 3) (Copepod + *A tamarense* (CAT); Copepod + *C. acantha* (CCA); Copepod + *S. costatum* (CSC); Copepod + *P. lima* (CPL); Copepod + *R. baltica* (CRB); Copepod + Mix-food (CAA); Copepod without food (CN)).

### 3.4.1.2. Eggs production per day

The egg production per day in the treatment with the mix-food was of 1-51 eggs.day-1 following for the treatment with *R. baltica* (1-50 eggs.day-1). The treatment with *A tamarense, C. acantha* and *P. lima* produced 1-23, 1-26 and 1-31 eggs.day-1 respectively. The treatment without food produced only 1 egg in a unique day. The treatment with *S. costatum* did not produce eggs.

The ANOVA (one-way) showed highly significant differences among the treatments ($p < 0.01\%$) (Table 16).

Table 16 - ANOVA summary of the eggs production per day of the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (**) |
|---|---|---|---|---|
| Between treatments | 3564.525 | 6 | 594.0875 | |
| Within treatments | 15669.75 | 111 | 141.169 | 4.208344 |
| Total | 19234.28 | 117 | | |

** – highly significant ($p < 0.01$)

The Duncan test demonstrated a significant difference between the mix-food treatment and the treatment with *S. costatum*, which did not produce eggs. The treatments with *S. costatum*, on which eggs were not produced, were not significantly different from the others (Figure 20).

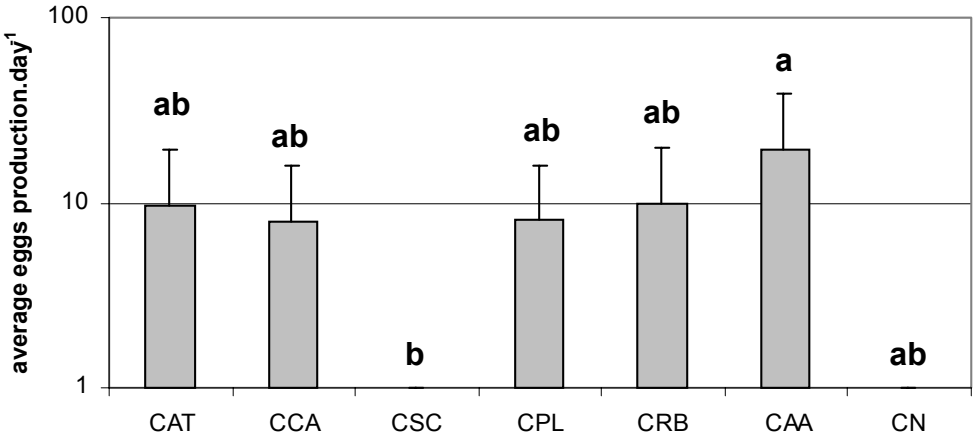


Figure 20 - Average of the eggs production per day of the copepod *A tonsa*. Comparison of the treatments. (Duncan test, same letters mean a not significant difference among the medium value; n = 3) (Copepod + *A tamarense* (CAT); Copepod + *C. acantha* (CCA); Copepod + *S. costatum* (CSC); Copepod + *P. lima* (CPL); Copepod + *R. baltica* (CRB); Copepod + Mix-food (CAA); Copepod without food (CN)).

### 3.4.1.3. Total eggs production per female

The average of the total eggs production per female was 373 eggs.female-1 on the treatment with the mixed food. The treatments with *R. baltica, C. acantha* and *A tamarense* had a average of total eggs production per female of 36, 21 and 11 eggs.female-1 respectively. *P. lima* provided 3.5 eggs.female-1 and the treatment without food only 0.1 eggs.female-1.

The variance analysis showed significant differences among the treatments ($p<0.05\%$) (Table 17).

Table 17 - ANOVA summary of the total eggs production per female of the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (*) |
|---|---|---|---|---|
| Between treatments | 338404.5 | 6 | 56400.74 | |
| Within treatments | 265346.5 | 14 | 18953.32 | 2.975771 |
| Total | 603751 | 20 | | |

* – statistically significant ($p < 0.05$)

The Duncan test demonstrated that only the treatment with the mix-food was different from the others (Figure 21).

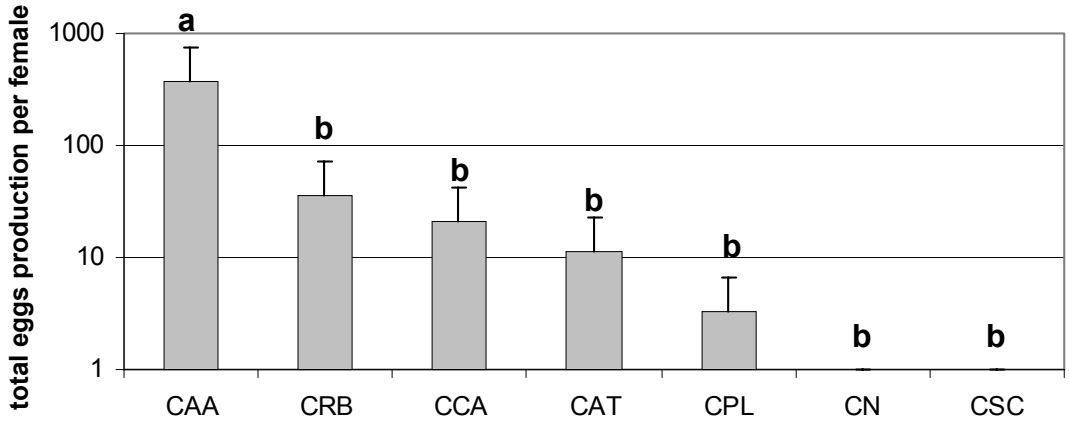


Figure 21 - Average of the total eggs production per female of the copepod *A tonsa*. Comparison of the treatments. (Duncan test, same letters mean a not significant difference among the medium value; n = 3) (Copepod + *A tamarense* (CAT); Copepod + *C. acantha* (CCA); Copepod + *S. costatum* (CSC); Copepod + *P. lima* (CPL); Copepod + *R. baltica* (CRB); Copepod + Mix-food (CAA); Copepod without food (CN)).

### 3.4.1.4. Eggs production per female per day

The egg production per female per day on the treatment with mix-food ranged from 0.2 to 51 eggs.female-1.day-1. The food with *R. baltica* provided from 0.2 to 25 eggs.female-1.day-1 and with *C. acantha* from 0.5 to 13 eggs.female-1.day-1. The egg production on the treatment with *A tamarense* and *P. lima* were 0.2-18 and 0.5-6 eggs.female-1.day-1. The treatment without food produced 0.3 eggs.female-1.day-1. There was not egg production in the treatment with *S. costatum*.

The variance analysis showed highly significant differences between the treatments($p < 0.01$) (Table 18).

Table 18 - ANOVA summary of the eggs production per female per day of the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (**) |
|---|---|---|---|---|
| Between treatments | 5991.683 | 6 | 998.6138 | |
| Within treatments | 11804.53 | 111 | 106.3471 | 9.390135 |
| Total | 17796.21 | 117 | | |

** – highly significant ($p < 0.01$)

The Duncan test demonstrated a significant difference between the treatment with the mixed food and the treatment with *S. costatum*. The latter treatment did not differ from the others (Figure 22).

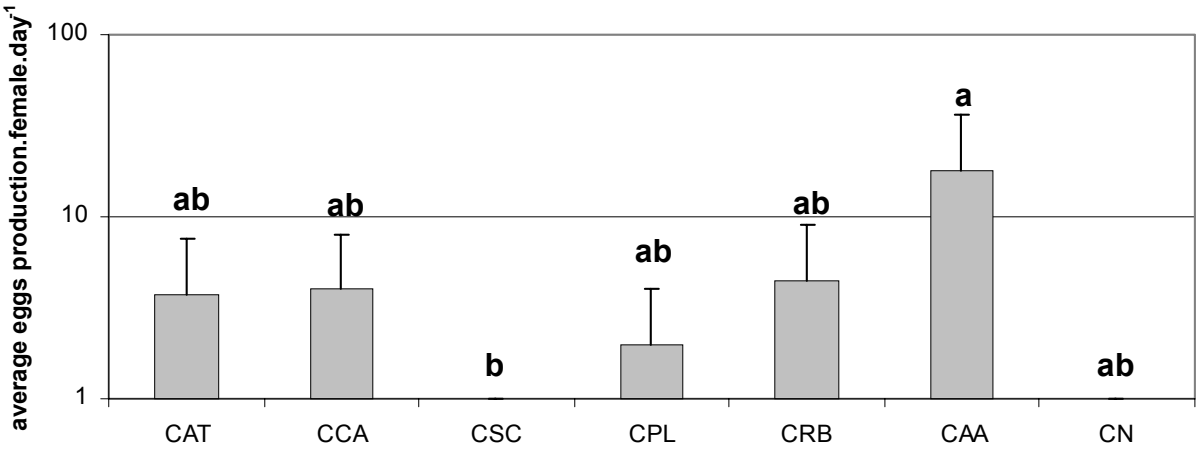


Figure 22 - Average of the eggs production per female per day of the copepod *A tonsa*. Comparison of the treatments. (Duncan test, same letters mean a not significant difference among the medium value; n = 3) (Copepod + *A tamarense* (CAT); Copepod + *C. acantha* (CCA); Copepod + *S. costatum* (CSC); Copepod + *P. lima* (CPL); Copepod + *R. baltica* (CRB); Copepod + Mix-food (CAA); Copepod without food (CN)).

### 3.4.1.5. Hatching success

The average of the hatching success that was found in the treatment with *R. baltica* was 97.15% (66-100%). In the treatment with the mix-food, the hatching was 77.02 % (6.25-100%). With *C. Acantha* as food, the mean value of hatching was 60.53% (20-100%). In the treatment with *A tamarense* and *P. lima* the mean hatching success was 7.28 % (5.88-8.69%) and 56.45% (12-100%) respectively. There was no hatching on the treatment without food.

The ANOVA showed highly significant differences between the different food types ($p<0.01\%$) (Table19).

Table 19 - ANOVA summary of hatching success of the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (**) |
|---|---|---|---|---|
| Between treatments | 28598.53 | 6 | 4766.422 | |
| Within treatments | 35863.44 | 45 | 796.9654 | 5.980741 |
| Total | 64461.98 | 51 | | |

** – highly significant ($p < 0.01$)

The Tukey test demonstrated that the treatment with *R. baltica* as food was identical in hatching success with the treatments with *C. acantha*, *P. lima* and the mix-food. Only *R. baltica* treatment was not equal to the treatment with *A tamarense*. The treatments whit *S. costatum* and without food were identical but they were different from the others (Figure 23).

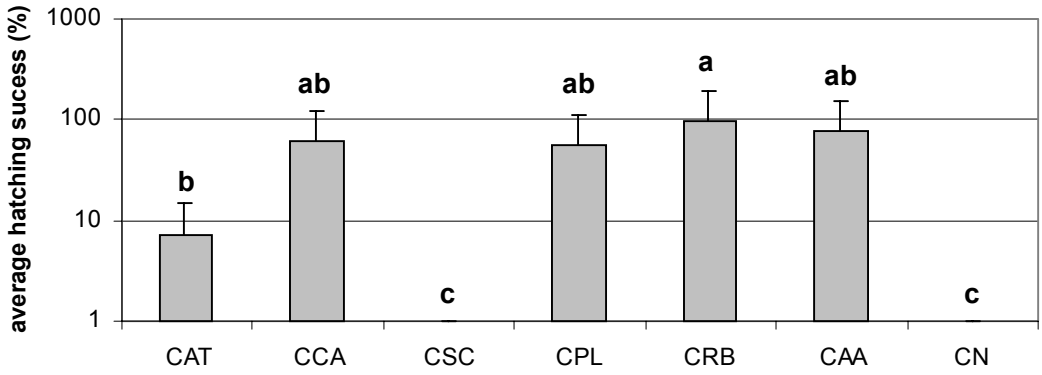


Figure 23 - Average of the hatching success of the copepod *A tonsa*. Comparison of the treatments. (Tukey test, same letters mean a not significant difference among the medium value; n = 3) (Copepod + *A tamarense* (CAT); Copepod + *C. acantha* (CCA); Copepod + *S. costatum* (CSC); Copepod + *P. lima* (CPL); Copepod + *R. baltica* (CRB); Copepod + Mix-food (CAA); Copepod without food (CN)).

### 3.5. Survival of the copepod *A. tonsa*.

#### 3.5.1. Survival of the adults

The experiment lasted 35 days. Among the replicates (n=3), the treatment with the mixed food provided the biggest mean survival time (100%) followed by the treatment with *C. acantha* (90%) and *R. baltica* (80%). The treatments with *P. lima* and *S. costatum* showed a survival of 40%. *A tamarense* and the treatment without food demonstrated a survival time of 35% each (Figure 24).

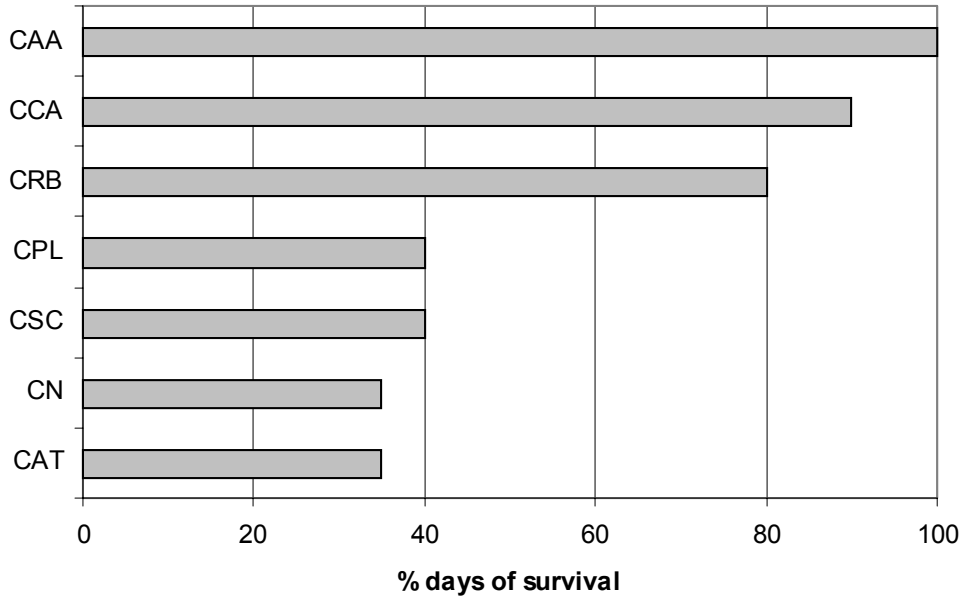


Figure 24 – Percent of survival in the treatments about the survival and reproduction of the copepod *A tonsa* (Copepod + *A tamarense* (CAT); Copepod + *C. acantha* (CCA); Copepod + *S. costatum* (CSC); Copepod + *P. lima* (CPL); Copepod + *R. baltica* (CRB); Copepod + Mix-food (CAA); Copepod without food (CN)).

The average of survival time of the copepod *A tonsa* ranged from 7 days in the treatments without food and with *A tamarense* to 19.5 days in the treatment with mix-food. The treatments with *C. acantha* and *R. baltica* showed an average of 18 and 16.5 days respectively, and the treatments with *S. costatum* and with *P. lima* demonstrated an average survival day of 8 and 8.5 respectively.

The variance analysis showed highly significant differences between the different food types ($p<0.01$) (Table20).

Table 20 - ANOVA summary of survival day of adults of the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (**) |
|---|---|---|---|---|
| Between treatments | 156.5371 | 6 | 26.08951 | |
| Within treatments | 845.0615 | 272 | 3.108844 | 8.397432 |
| Total | 1001.599 | 278 | | |

** – highly significant ($p < 0.01$)

The Tukey test did not demonstrate significant difference among the treatments (Figure 25).

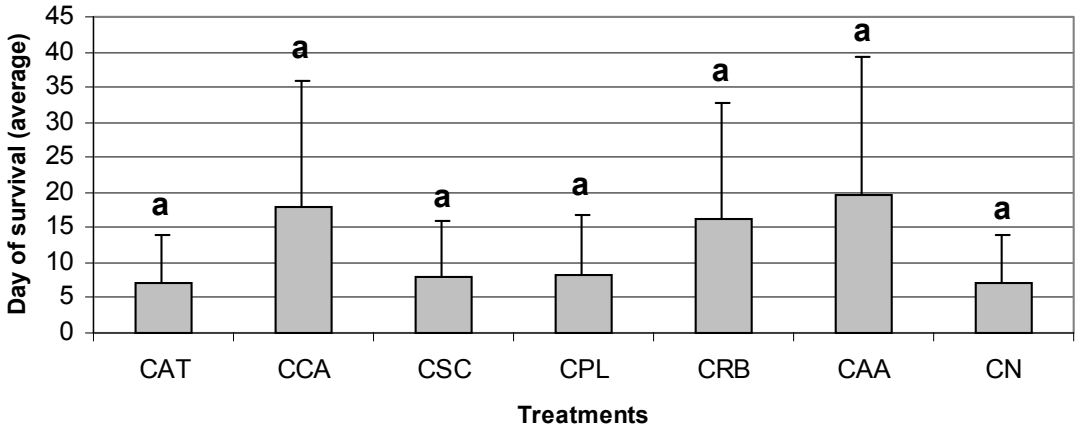


Figure 25 - Average of the survival time of adults of the copepod *A tonsa*. Comparison of the treatments. (Tukey test, same letters mean a not significant difference among the medium value; n = 3) (Copepod + *A tamarense* (CAT); Copepod + *C. acantha* (CCA); Copepod + *S. costatum* (CSC); Copepod + *P. lima* (CPL); Copepod + *R. baltica* (CRB); Copepod + Mix-food (CAA); Copepod without food (CN)).

### 3.5.2. Survival of nauplii and copepodite

The mean survival time of nauplii ranged from less than a 1 day in the treatments with *A tamarense* (0.41 day) and *P. lima* (0.33) to 7 days in the treatment with the mixed food. With *C. acantha*, the nauplii survival was on the average of 2 days and with *R. baltica* was 5.05 days on average (Figure 26).

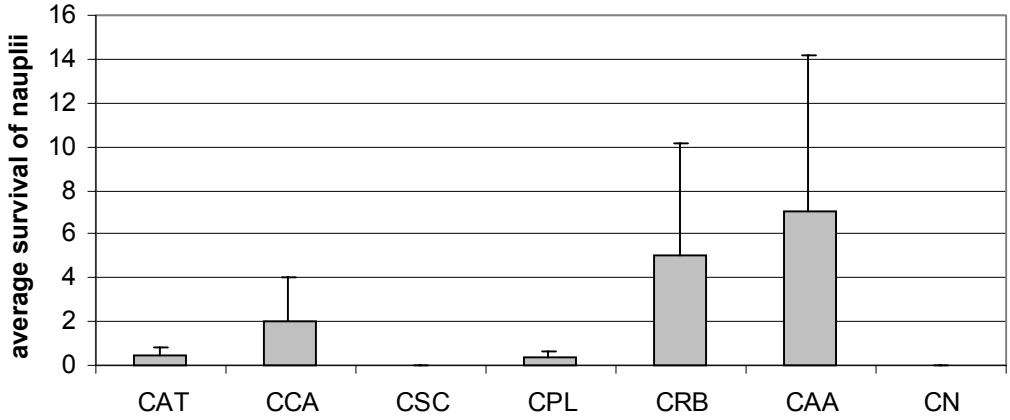


Figure 26 - Average of the survival days of nauplii of the copepod *A tonsa*. Comparison among the treatments (Copepod + *A tamarense* (CAT); Copepod + *C. acantha* (CCA); Copepod + *S. costatum* (CSC); Copepod + *P. lima* (CPL); Copepod + *R. baltica* (CRB); Copepod + Mix-food (CAA); Copepod without food (CN)).

The ANOVA showed not significant differences between the different food types (p≥0,05) (Table21).

Table 21 - ANOVA summary of survival day of nauplii of the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (NS) |
|---|---|---|---|---|
| Between treatments | 144.4916 | 6 | 24.8194 | |
| Within treatments | 153.1808 | 14 | 10.94149 | 2.200975 |
| Total | 297.6724 | 20 | | |

NS – no significant (p≥0,05)

The survival time of copepodite ranged from 1.08 days in the treatment with *C. acantha* to 3.76 days in the treatment with *R. baltica*. The treatment with the mix-food promoted a survival time of 3.06 days on average. In the other treatments did not occur copepodite (Figure 27).

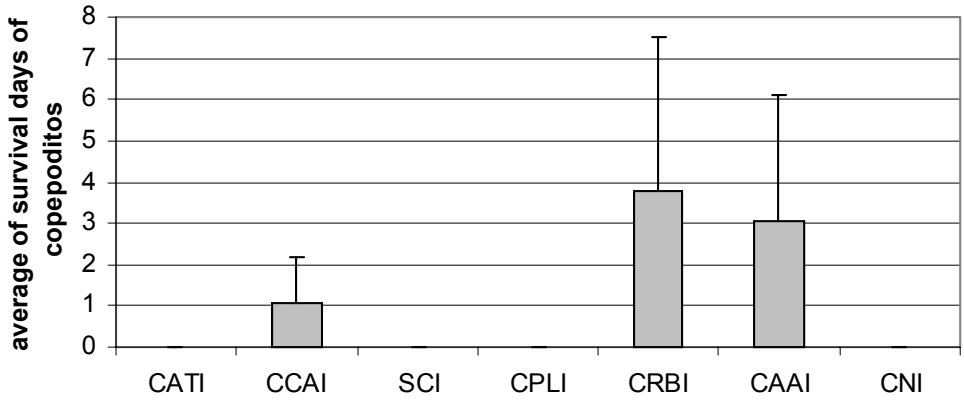


Figure 27 - Average of the survival days of copepodite of the copepod *A tonsa*. Comparison among the treatments (Copepod + *A tamarense* (CAT); Copepod + *C. acantha* (CCA); Copepod + *S. costatum* (CSC); Copepod + *P. lima* (CPL); Copepod + *R. baltica* (CRB); Copepod + Mix-food (CAA); Copepod without food (CN)).

The variance analysis did not showed significant difference among the treatments (p≥0,05) (Table22).

Table 22 - ANOVA summary of survival day of copepodite of the copepod *A tonsa* (V = variability; SS = sum of squares; DF = Degrees of freedom; MS = mean squares; F = F-Test).

| V | SS | DF | MS | F (NS) |
|---|---|---|---|---|
| Between treatments | 47.37353 | 6 | 7.895588 | |
| Within treatments | 45.93368 | 14 | 3.280977 | 2.406474 |
| Total | 93.30721 | 20 | | |

NS – no significant (p≥0,05)

**3.6. Comparison among the nutritional value of the phytoplankton species, abiotic parameters and the survival and reproduction of *A tonsa*.**

The concentration of the biochemical constituents per volume medium (µg.ml-1) offered to *A tonsa* had not significant influence on the survival and reproduction of the copepod. Only the lipid concentration was poorly correlated to the hatching success (r = 0.58). The contents of lipid, carbohydrate and protein per ml were positive related to the dry weight (protein r = 0.97; lipid r = 0.94; carbohydrate r = 0.83) (Figure 28).

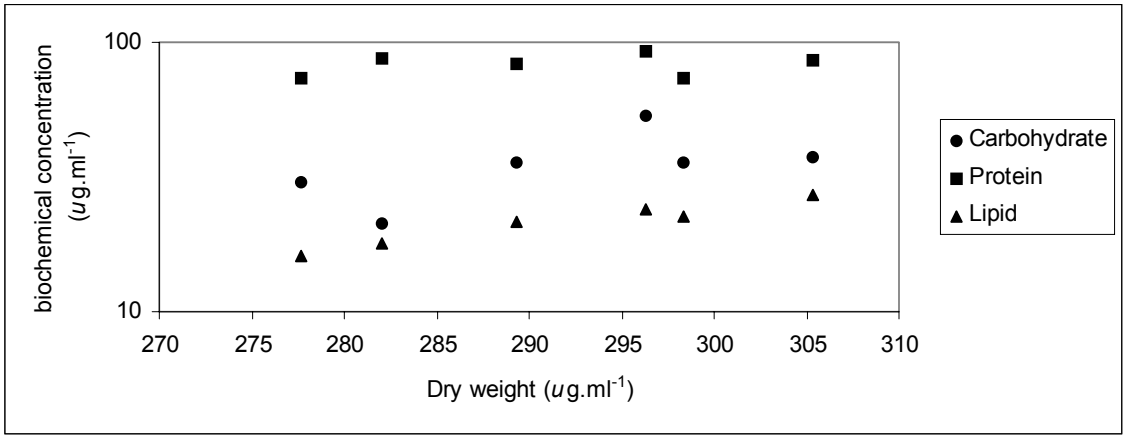


Figure 28 – Correlation between dry weight and the biochemical concentration per volume medium (protein r = 0.97; lipid r = 0.94; carbohydrate r = 0.83).

However, the content of the biochemical parameters per cell volume had a positive effect in the hatching success (lipid r = 0.65; carbohydrate r = 0.67; protein r = 0.54) (Figure 29).

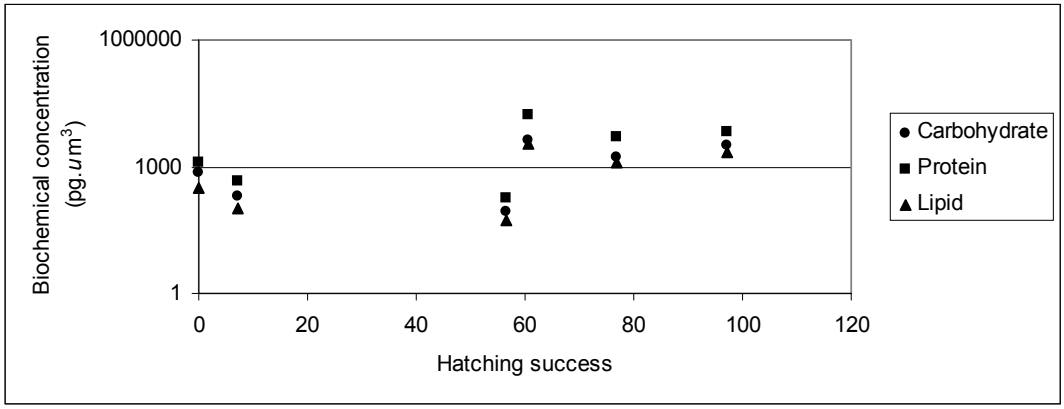


Figure 29 – Correlation between hatching success of *A. tonsa* and the biochemical concentration per cell volume (lipid r = 0.65; carbohydrate r = 0.67; protein r = 0.54).

The values of lipid and carbohydrate per cell volume were poorly direct related to last day on which hatching occurred (lipid = 0.52; carbohydrate r = 0.53) and, to the average survival time of copepodite (lipid = 0.58; carbohydrate r = 0.63) but the content of lipid, protein and carbohydrate showed a highly direct influence on the average survival time of adults (lipid r = 0.82; carbohydrate r = 0.83; protein r = 0.77) (Figure 30).

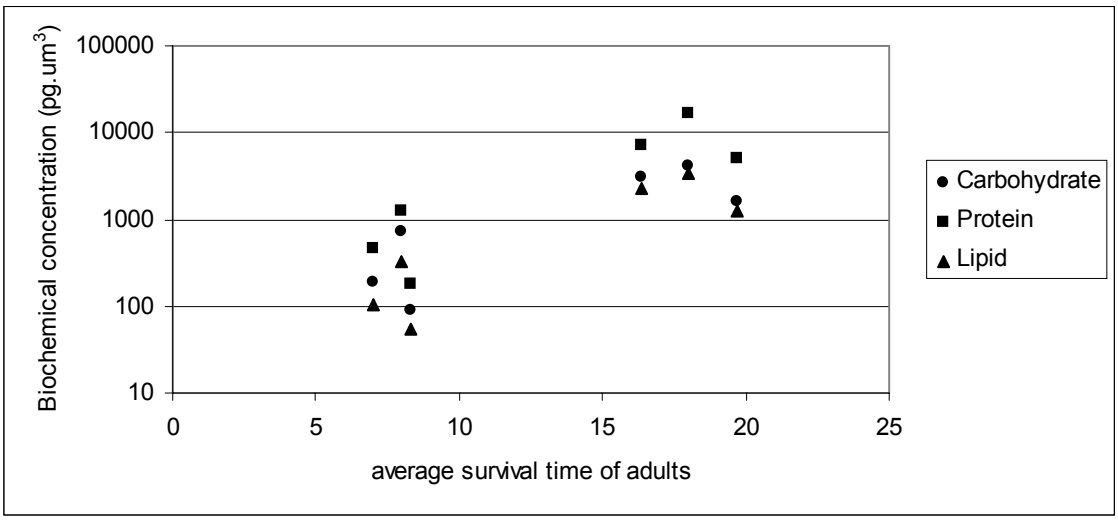


Figure 30 – Correlation between the mean survival time of adults and the biochemical concentration per cell volume (lipid r = 0.82; carbohydrate r = 0.83; protein r = 0.77).

The ingestion rate of *A tonsa* was directly correlated to the hatching success (r = 0.53) and to the average survival time of adults (r = 0.68) (Figure 31 and 32).

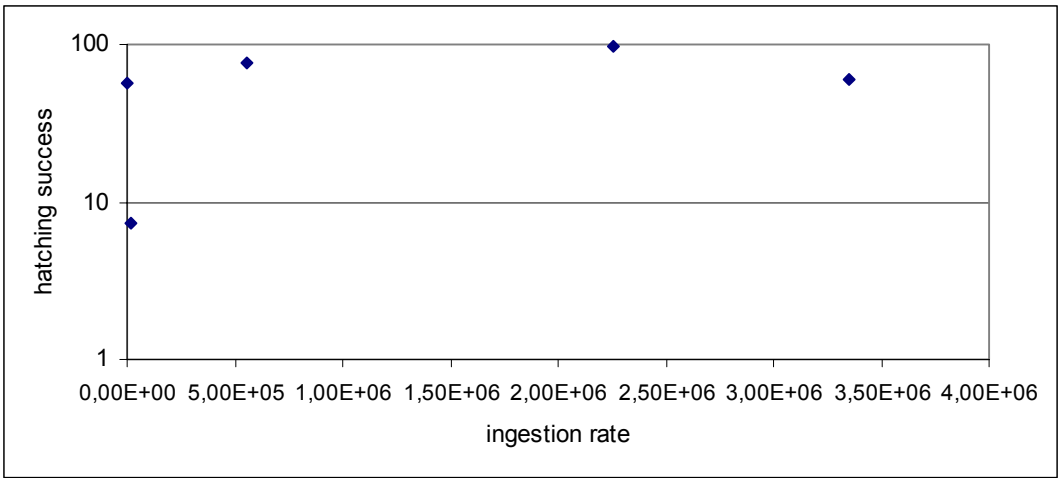


Figure 31 – Correlation between ingestion rate and hatching success of *A. tonsa* (r = 0.53).

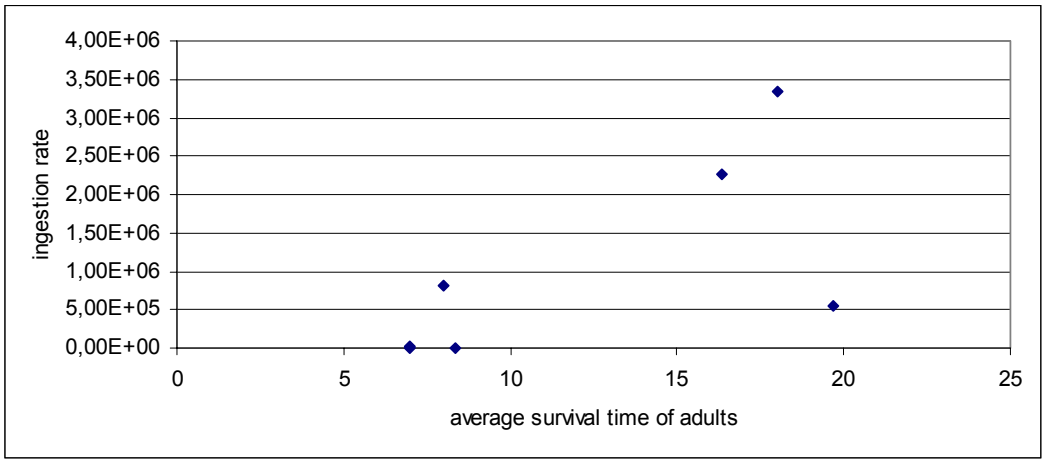


Figure 32 – Correlation between ingestion rate and average survival time of adults (r = 0.68).

The total eggs production in the treatments was directly related to the average eggs production per female per day (r = 0.98), total number of nauplii (r = 0.99) and copepodite (r = 0.93), the last day on which hatching was observed (r = 0.81) and hatching success (r = 0.52). The parameter was also directly correlated to the average survival time of nauplii (r = 0.87) and copepodite (r = 0.66).

The total egg production was correlated to the mean survival time of adult copepods (r = 0.70) (Figure 33).

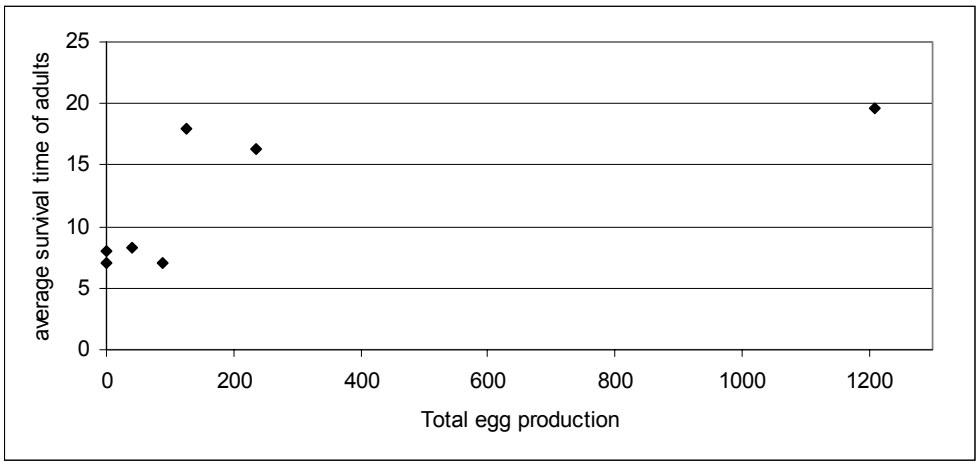


Figure 33 – Correlation between total egg production and average survival time of adults copepods (r = 0.70).

The average eggs production per female per day was directly related to the total number of nauplii (r = 0.96), total number of copepodite (r = 0.91), last day on which hatching occurred (r = 0.87).

The mean of egg production per female per day was poorly correlated to hatching success (r = 0.55) (Figure 34).

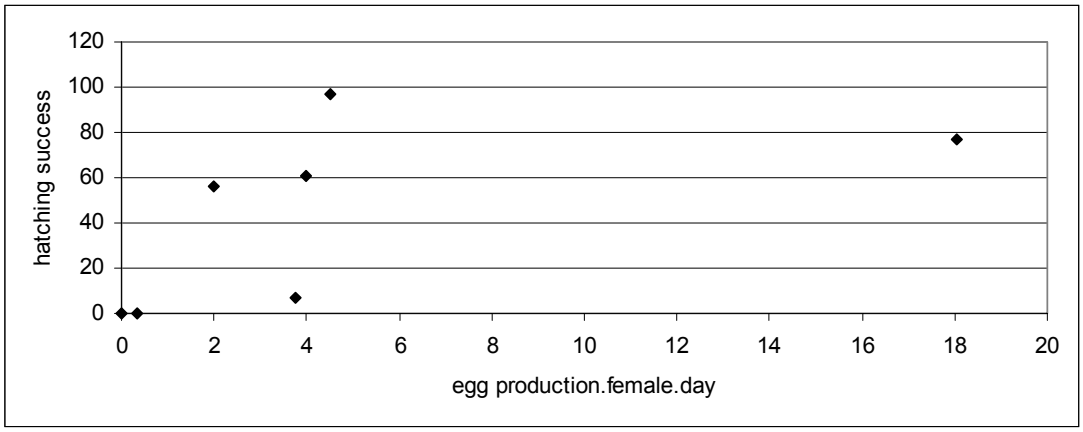


Figure 34 – Correlation between egg production per female pre day and hatching success (r = 0.55).

The first day on which hatching was observed (r = 0.58). It was also directly related to average survival time of nauplii (r = 0.86), copepodite (r = 0.66) and adults (r = 0.72).

The hatching success was highly related to the average survival time of nauplii (r = 0.80), copepodite (r = 0.84) and adults (r = 0.82) (Figure 35), and also it was directly correlated to the last day on which hatching occurred (r = 0.69) and the total number of nauplii (r = 0.55) and copepodite (r = 0.66).

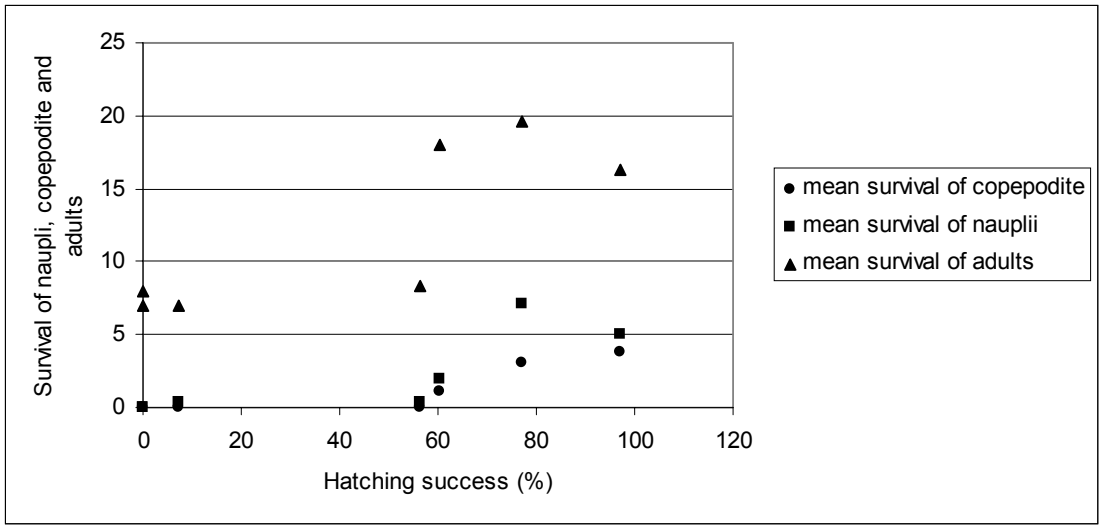


Figure 35 – Correlation of hatching success and mean survival time of nauplii (r = 0.80), copepodite (r = 0.84) and adults (r = 0.82).

Hatching success was directly related to the dry weight (r = 0.50) (Figure 36).

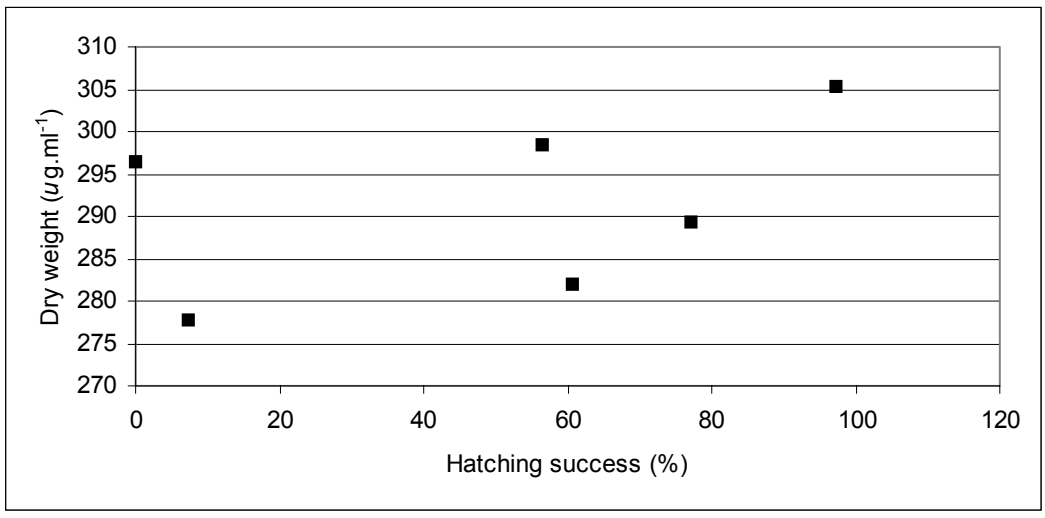


Figure 36 – Correlation between hatching success and dry weight (r = 0.50).

The total number of nauplii and copepodite was directly related to the average survival time of nauplii (nauplii r = 0.90; copepodite r = 0.96), copepodite (nauplii r = 0.73; copepodite r = 0.85) and adults (nauplii r = 0.70; copepodite r = 0.73) and also to the last day on which hatching occurred (nauplii r = 0.79; copepodite r = 0.81). The total number of nauplii was highly direct related to the total number of copepodite (r = 0.97).

In despite on the first and last day on which hatching was observed, they were directly related to the average survival time of nauplii (first r = 0.53; last r = 0.88) and adults (first r = 0.50; last r = 0.82). The first day on which hatching occurred was directly correlated to the last day on which hatching occurred (r = 0.84) and dry weight (r = 0.51) and, the last day on which hatching occurred was direct related to the average survival time of copepodite (r = 0.78).

The survival time of nauplii and copepodite was also directly related to the survival time of adults (nauplii r = 0.87; copepodite r = 0.83) (Figure 37). The average survival time of copepodite was highly correlated to the average survival time of nauplii (r = 0.94).

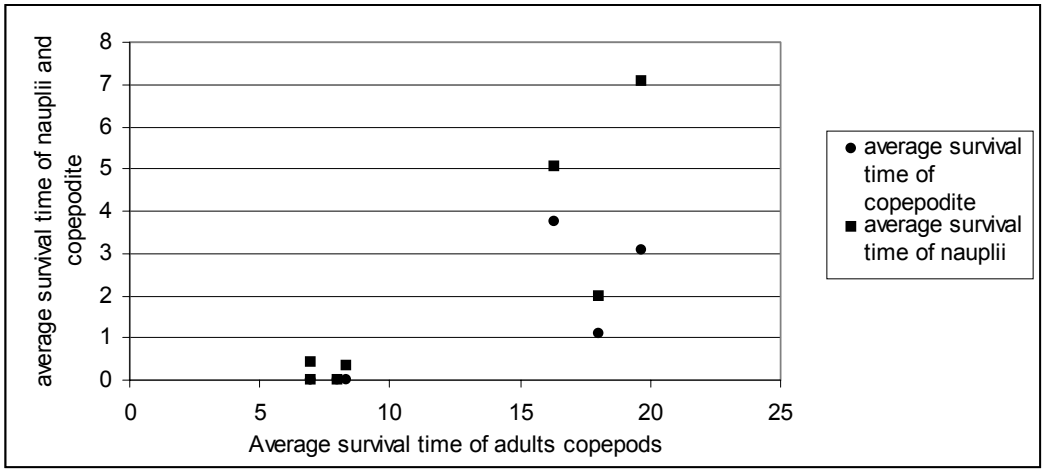


Figure 37 – Correlation between the mean survival time of nauplii and copepodite and the mean survival time of adult copepods (nauplii r = 0.87; copepodite r = 0.83).

The content of chlorophyll a was not significantly correlated to the others parameters.

# 4. Discussion and Conclusion

## 4.1. Interspecific differences and their effect on the aquatic organisms. Biochemical composition

In the second half of the 1980ies, new perspectives have guided the research on the biochemical composition of the plankton. Most of this interest emerged from studies on applied physiology, mainly in areas as aquiculture and the determination of the nutritional quality of the algae for aquatics organisms (Walne, 1974; Webb & Chu, 1982; Whyte, 1987; Sicko-Goad & Andersen, 1991; Brown & Jeffrey, 1992). In most of these studies the food value of the phytoplankton was judged by the larval growth rate, percentage of survival, prediveliger production (mollusks), and settling success. It was suggested that various phytoplankton are not equal in nutritional value and that an algal diet composed of two or more species provides a better feeding source than any individual species (Epifanio, 1979; Chu *et al*. 1982; Brown, 1991; Brown & Jefrey, 1992). And these differences might reflect different culture conditions, growth phase and genetic differences (Fabregas *et al.*, 1986; James *et al.*, 1989; Whyte *et al*., 1989; Brown *et al*., 1997).

In the research of Brown *et al.* (1997), microalgae varied in their proportions of protein (6-52%), carbohydrate (5-23%) and lipid (7-23%). In my study, less variability of the biochemical components (relation among protein, carbohydrate and lipid) was found. Protein ranged from 54% in *P. lima* to 69% in *C. acantha*. Carbohydrate varied from 17% in *C. acantha* to 31% in *S. costatum* and lipid ranged from 13% in *A tamarense* to 18% in *R. baltica*. Therefore, these differences between the proportion found in the present study can be due to the different conditions, on which the microalgae were cultivated.

The importance of gross composition (relative proportions of protein, carbohydrate, lipid and minerals) in determining the nutritional quality of microalgae is poorly understood, with different workers claiming either total carbohydrate or protein to be more important, dependent on the animal species and life stage (Brown & Jeffrey, 1992). However the levels of specific amino acids, fatty acids, sugars, sterols, vitamins and mineral, within the fractions may be more important (Kristensen, 1972; Brown *et al*, 1997). In this context, the knowledge of the biochemical composition of microalgae

obtained in the laboratory, with a specific culture medium, should not be extended to field conditions if another culture medium is to be employed. This fact restrains the applicability of laboratory data about biochemical composition of microalgae for use in outdoor systems. On the others hand, this limitation emphasizes the need for simple and cheap culture media in order to obtain more predicable data that may be useful for tank cultures.

### 4.1.1. Importance of the lipid, carbohydrate and protein.

Several projects have approached the importance of the biochemical composition of the algae, basically protein, carbohydrate and lipids in the processes of growth, development and reproductive success of several species of zooplankton and fish (Boechat, I., 2000). In each case the chemical composition of the algal species were qualitatively similar, although there were some quantitative differences in certain chemical components (Parson *et al*.; 1961; Chu *et al.*, 1982).

The importance of lipid quality in food was first discovered in fish aquaculture. Specifically, the long-chained polyunsaturated fatty acids (PUFA) of the ω3 and ω6 type, eicosapentaenoic acid (EPA), docosaheyaenoic acid (DHA), and arachidonic acid (AA) were found to be crucial for the growth and survival of early larval stages of many fish and shellfish (Watanabe *et al.*, 1983). These long-chain PUFA are vital components of cell membranes and have important regulatory functions in all living cells, particularly in reproduction (Ahlgren *et al*., 1997). They are also useful biomarkers since they are essential components of all living cells and display a high structural diversity coupled with higher level taxonomic specificity. Microalgal species with low levels of polyunsaturated fatty-acids (PUFAs) have frequently been proven to be inadequate as unialgal diets, whereas species with high concentrations of the PUFAs have generally proven to be good to moderate food as unialgal diets (Enright *et al.*, 1986; Sargent *et al.*, 1987; Desvilettes *et al.*, 1997; Reuss & Poulsen, 2002). Total lipid contents probably does not explain very much the variation in responses of aquatics organisms to different diets. Flagellates have usually high concentration of DHA and low concentration of EPA and diatoms are usually low in DHA and high in EPA (Sommer, 1998).

In this study it was found that the treatment with a mixed food provided a positive effect on the reproduction and survival of *A tonsa*. The effect of the mixed food

was compared to the treatment with *R. baltica* and *C. acantha*, which appeared to be the best of the studied monoespecific diets.

The concentration of the specific components of lipid, as EPA, DHA and PUFA, were not measured in the present study. The flagellates *C. acantha* and *R. baltica* provided a good effect on the reproduction and survival of the copepod *A tonsa*, as monospecific diet. Probably, *R. baltica* has a high content of essential fatty acids.

The value of total lipid (% dry weight) of the phytoplankton species used, grown actively, was relatively low when compared to the results of Sargent & Falk-Petersen (1998), who had found that the total amount of lipids accounts for 10-20% of the dry weight in actively growing algae.. The levels of lipid ranged from 4.85% of the dry weight in the flagellate *C. acantha* to 13.56 in the flagellate *R. baltica.*

Microalgae of the class Dinophyceae have been documented extensively as producers of an impressive array of lipophilic chemicals not commonly found in other classes of eukaryotic microalgae. These include a substantial number of toxins, pigments, sterols and steroidal compounds and fatty acids. Of these classes of compounds, toxins, particularly brevetoxins, are perhaps the most studied, because of their often lethal effects on aquatic animals and the hazards to human health associated with consumption of toxin-contaminated shellfish (Leblond *et al.,*2003).

The two species of dinoflagelattes used as food for *A tonsa* have shown a low level of lipid. *P. lima* had a good effect on the hatching success of *A tonsa* but the survival of adults and the early stages of the copepod, as well as, the egg production was lower than with the flagellates *C. acantha* and *R. baltica*. The survival and reproduction of *A tonsa* fed with *A tamarense* was reduced and the results were comparable to the treatment without food.

Of the environmental factors that influence phytoplankton growth, the major inorganic nutrients (N, P and Si) have the greatest documented effect on lipid content and synthesis according to studies of algal cultures (Parrish & Wangersky, 1987; Wainman & Smith, 1997). In the ocean, the taxonomic composition of the phytoplankton (particularly the relative abundance of certain flagellates vs. Diatoms) may influence lipid allocation significantly (Fernàndez *et al.*, 1994). However, at the present moment, there is no evidence that general prediction of lipid synthesis in marine phytoplankton could be predicted successfully solely from the taxonomic composition (Wainman & Smith, 1997).

The taxonomic comparison in the present study demonstrated interspecific differences in lipid content under identical culture conditions. The flagellates *C. acantha* and *R. baltica* had a higher value of lipid (volume-based) than the flagellates of the Class Dinophyceae (*A tamarense* and *P. lima*). The diatom *S. costatum* showed a medium content of lipid in comparison with the flagellates.

Comparisons of biochemical data from the literature can be complicated due the different methods of analysis. This is particularly true for protein analysis. Different protein values have been obtained from samples by different methods (Clayton *et al.*, 1988). According to Brown (1991) *Chaetoceros calcitrans* and *Phaeodactylum tricornutum* contained 34 and 30% of protein, but in the works of Whyte (1987) and Fèrnandez-Reiriz *et al.* (1989) these species contained only 11 and 1.4% of protein. According to Myklestad (1974), the protein content also varied from 52.4% (*Thalassiosira gravida*, logarithmic phase) to 11% for *S. costatum* (stationary phase).

The nutritional value of the algal protein content (protein plus free amino acids) is considered to be high if its essential amino-acid composition is close to that of the feeding animal (Brown, 1991), but the level for optimum growth are not known (Teshima *et al.*, 1986). The protein content of the algae is almost constant during exponential growth while accumulation of carbohydrate and lipid are observed only in the stationary phase (Handa, 1969; Brown *et al.*, 1993).

The content of protein in this study was always higher (2-4 times) per cell volume than the content of carbohydrate and lipid. The levels of protein in the food (μg.ml-1 and % dry weight) showed no significant difference among the phytoplankton species. However a highly significant difference was found in the content of protein per cellular volume. Protein per cellular volume had a direct weak influence on the hatching success but a high influence on the survival of *A tonsa*. The content of lipid and carbohydrate showed similar results as protein concentration. Only *S. costatum* showed a higher concentration of carbohydrate per dry weight and volume of medium than the others species.

Differences in the polysaccharides of microalgae may be of nutritional significance, since the efficiency with which marine animals digest polysaccharides is dependent on the polysaccharide type (Kristensen, 1972). According to Brown (1991), the sugar composition of the algal polysaccharides and the nutritional value of the algae were not directly related, the results demonstrate large differences in the classes of polysaccharides in the different algal species.

The production of cellular carbohydrate proved to vary radically from species to species under the defined conditions and seems to be governed by the limitation of nitrate in the culture medium. Chu *et al.* (1982) studied the polysaccharide composition of five algal species demonstrated that the production of total carbohydrate increased with the age of culture, probably due the production of acid-soluble glucan, the main common polysaccharide reserve of diatom. According to Myklestad (1974), the response of the cell composition was very pronounced, as demonstrated by a carbohydrate content of 12.8% in rapidly growing cells of *Corethron hystriy*, and of 90% in the stationary growth phase cells of *Skeletonema costatum*.

According to Whyte (1987), in general the diatoms contained a higher total carbohydrate than the phytoplagellates and, the content of polysaccharide was similar at both growth stages (exponential and stationary) for all species of diatoms except *Thalassiosira*.

As single alga, *S. costatum* contained a higher levels of carbohydrate (μg.ml-1 and % dry weight) than the flagellates as single food. However the diatom did not have the highest content of carbohydrate per cellular volume. Higher levels were found in the flagellates *C. acantha* and *R. baltica*. *S. costatum* in the present study, in exponential phase of growth, showed a mean of 20.37% of carbohydrate per dry weight.

Phytoplankton as food can supply both energy and essential nutrients (Langdon, 1982). Differences in the composition of protein, lipid, and particular fatty acids in the algal diet were associated with different growth rates and different biochemical composition of the larvae (D´Souza & Loneragan, 1999). The studies on the nutritional requirements of crustaceans indicate that fertility and development may require specific fatty acids, also for *A tonsa* (Stottrup & Jensen, 1990). The lipid content of copepod eggs and gonads of females is higher than that of whole individual, suggesting the potential importance of lipid in egg production by copepods (Jónasdóttir, 1994). Kiorboe *et al.* (1985a) suggested that lipids were important for egg production, while protein was important for somatic growth of nauplii and copepodite.

The importance of the nutritional value of the carbohydrate is controversial and it is possible that carbohydrate may not be as important as lipid in determining food quality of algae for the oyster larvae (Chu *et al*., 1982), with was asserted for Pearsons *et al*. (1961).

Copepods are typically rich in lipid (Gatten *et al.*, 1980) and essential fatty acids are therefore potentially limiting nutritional components of diatom diets, as

demonstrated by Jónasdóttir & Kiorboe (1996). The best results were found with high lipid algae, (*R. baltica*). Lipid (expressed in μg.ml-1 or per cellular volume) seems to be the biochemical compound that was best correlated with the hatching success of *A. tonsa*. But, when the biochemical parameters are expressed per cellular volume, carbohydrate and protein were also important. The hatching success in *A tonsa* was directly correlated with these three biochemical components

Part of the differences in nutritional value that effects on the reproduction and survival of *A. tonsa* can be better explained by the interspecific differences in the biochemical contents (lipid, carbohydrate and protein) per cell volume than for the concentration of the biochemical constitutes per dry weight and per volume of the medium.

The highest values of egg production and survival of *A tonsa* were found with the algae *R. baltica*, *C. acantha* and mixed food. The highest lipid value in the food was offered in the treatment with *S. costatum* and *R. baltica*, as single food.

In the mixed food, the high ingestion rate of *R. baltica* and *S. costatum* could be resulted from an increase on the levels of DHA, found in *R. baltica*, and EPA, found in *S. costatum*, also as for the increase of protein supplied for *C. acantha*, major content of protein per cell volume. But for a better understanding of the relation of the biochemical contents with survival and reproduction of aquatic animals makes necessary a analysis of vitamins, minerals and of the constitutes of each gross biochemical composition, such as fatty acids, amino acids, sterols, sugars.

### 4.2. Interspecific differences and their effect on the aquatic organisms. Diatom x Flagellates.

Food composition plays an important role in the fecundity and hatching success of copepod eggs. But, the effects of such compositional changes of phytoplankton on zooplankton growth and reproduction are not well understood. Different food types elicit different reproductive responses in copepod (Marschall & Orr, 1952; Checkley 1980ab; Enright *et al.*, 1986; Brown, 1991; Brown *et al.*, 1997).

In the nature, results demonstrated that suspended particulate matter is a much higher-quality food resource than sinking matter. While diatoms are considered a high-quality food and the primary food for herbivorous or suspensions-feeding meso- and macrozooplankton such as copepods (Parsons *et al.*, 1984; Uye, 1996; Ahlgren *et al*. 1997). Other studies demonstrated that a diatom-based food chain leads to higher

growth rate and better conditions of fish larvae, which feed on copepods, compared to a dinoflagellate-based food chain (Kioboe & Nielsen, 1994; Hansen, 1998).

Differences in the reproductive responses of copepods have been attributed to subtle differences between diatoms and flagellates such as an absence or presence of wall cell silica, shape and size of the cells, palatability or nutrient concentrations per unit cell volume (Ianora *et al.*, 1995ab). Other studies have emphasized the importance of essential compounds for growth or reproduction such as fatty acids, proteins or vitamins, to explain the better quality of dinoflagellates or microzooplankton compared to diatoms in inducing higher fecundity (Kleppel *et al.*, 1991; Ianora & Poulet, 1993).

It has long been known that many copepod species feed on diatoms in the sea. However, the importance of diatoms as a dominant and high quality food source for copepod production has recently been questioned (Kleppel *et al.*, 1991). Kleppel (1993) has argued that copepods in nature feed mainly on other microplankters (dinoflagellates, ciliates) which primarily account for reproduction, and that diatom alone provide an insufficient diet for reproduction. New studies have reported that some diatom species induce copepod egg mortality by blocking embryogenesis (Poulet *et al.*, 1995; Ianora *et al.*, 1996; Uye, 1996; Ban *et al*. 1997).

The implication of these results challenges the traditional view of the role of diatoms in the pelagic food web dynamics and our understanding of the basis for fish production in the oceans. (Ban *et al.*, 1997; Jónasdóttir *et al*., 1998). One example is the feeding response of krill to the diatom *Pseudo-nitzchia*. This diatom is considered to produce the toxin domoic acid (DA) (Bargu *et al.*, 2003). *S. costatum* had a bad effect on the survival of adults and principally on the reproduction of the copepod *A tonsa*. But this effect seems to be caused for its biochemical composition and not for the presence of toxic compounds, because *Skeletonema* has not been reported as a toxic alga. The survival time of adults fed on *S. costatum* was similar to the survival time on the replicates with the toxic alga *A tamarense* and *P. lima* and also, to the control (without food).

The paradox about the interaction diatom-herbivores, particularly copepods, often a discussion about the true value of the diatoms for the food web. The working group of Ianora believe that diatoms produce toxic metabolites, which can cause mortality and/or inhibit the reproduction of copepods, whereas the group of Jónasdóttir claims that diatoms are organisms with a poor nutritional value. Recently, Ianora *et al* (2003) asserted that the negative effect of diatom on hatching success of copepod can be

reversed quickly by shift to another diet. Copepods can feed on diatoms, as long as they need energy for somathic growth. But before producing eggs, they should eat other organisms, such as flagellates and ciliates.

Several studies have demonstrated that some diatom diets result in lower egg production and/or hatching success and/or a higher frequency of malformed nauplii than do, for example, dinoflagellate and flagellate diets (Stottrup & Jensen, 1990; Jónasdóttir & Kiorboe, 1996; Ban *et al.*, 1997).

*S. costatum* did not produce a good effect on the reproduction of *A tonsa*. *A tonsa* fed on *S. costatum* as single food did not produce eggs. With *A tamarense* or *P. lima* as food, *A tonsa* produced eggs. The hatching success with *P. lima* was similar, when *A tonsa* had *C. acantha* or *R. baltica* as single food. However, the hatching success with *A tamarense* as food was the lowest as single food.

Potential toxic compounds, on the other hand, have not yet been identified, with the exception of a few diatom species that have toxins similar to those isolated from many dinoflagellates (Bates *et al.*, 1993) The use of phytoplankton extracts, where the eggs are exposed to phytoplankton compounds at increasing extract concentrations, has been applied to test for possible toxicity of diatoms. These experiments suggest a toxic or deleterious effects of diatom extracts on embryonic development and hatching (Poulet *et al.*, 1994; Ianora *et al.*, 1995ab; Ianora *et al*, 1996; Uye, 1996; Ban *et al.*, 1997; Lee *et al.*, 1999; Ianora *et al.*, 1999; Miralto *et al.*, 1999) .

Jónasdóttir & Kiorboe (1996) reported that the negative effects of diatom extracts on hatching disappeared when oxygen was bubbled through the extract solutions, suggesting that the effect was due to the anoxia that developed in this rich organic soup. Components of the food that are potentially toxic to egg hatching are mediated by the female and, thus, need to be examined by feeding experiments. Likewise, potential toxic effects of diatom exudates need to be examined by applying exudates, not extracts (Jónasdóttir *et al.*; 1998). In the present study, oxygen was not limiting. Daily, the copepods were transferred to a new plate with new medium and food. The period of 24 h has been not sufficient to affect the levels of oxygen in the culture. Also, the plates were not totally closed, what could cause anoxia. The results found with the others phytoplankton could demonstrate that anoxia was not a limiting factor.

According with Jónasdórttir *et al*. (1998), all field studies are consistent with nutritional insufficiency of diatoms if one assumes that the diatoms provide the basic

food and that alternative food sources (ciliates, dinoflagellates) complement the diet to make it nutritionally complete. The field evidence may also, however, be consistent with the idea that diatoms are toxic, but only if one assumes that the copepods feed mainly or solely on the non-diatom components of the microplankton.

Our results may also be consistent with the assertion of Jónasdóttir *et al*. (1998), If the diatom *S. costatum* was toxic, why should the ingestion of this species in the mix-food increase? The value of *S. costatum* as food seemed to be related with its nutritional quality. In the laboratory, diatoms have been shown to have a detrimental effect on egg viability (Ianora & Poulet, 1993; Poulet *et al.*, 1994, 1995; Ianora *et al.*, 1995ab; Miralto *et al.*, 1995) either when adults were fed algae at saturation levels, or when freshly spawned eggs were exposed to diatom extracts (Laabir *et al.*, 1995ab). In the field, Ianora & Poulet (1993) reported that in wild *Temora stylifera* a succession of low and high egg viability coincided with periods of high and low diatom biomass, respectively.

Jónasdóttir *et al.* (1998) maintain that reduced hatching rates are due to absence of essential nutritional requisites in the diet rather than to the presence of toxic compounds affecting embryogenesis. According with Ianora *et al* (1999), this deduction is questionable, because it has never really been demonstrated in copepods. Moreover, there is mounting evidence that diatoms possess anti-mitotic properties similar to the cytotoxic compounds isolated from numerous benthic marine algae.

In recent study of Miralto *et al.* (1999), the diatom *Thalassiosira rotula* can also inhibit cell cleavages in copepod, sea urchin and tunicate embryos whereas control dinoflagellate (*Prorocentrum minimum*) extracts, at the same concentrations, have no effect on cell division.

Initial studies on diatom-copepod interactions have shown that egg viability decreased dramatically when female copepods were fed with a diatom diet (Ianora & Poulet, 1993). This inhibition was reversible when a diatom diet was substituted with a dinoflagellate diet (Laabir *et al*., 1995ab; Uye, 1996; Ianora *et al*, 2003).

According to Ianora *et al*. (1999), the chemical nature of the toxic compounds that induce inhibition of cell division during copepod embryogenesis is known. Zimmerman *et al.* (1995) isolated 3 aldehydes of low molecular weight. Aldehydes are a class of compounds that arrests cell division and induces apoptosis (programmed cell death) in cultured cell lines. These aldehydes were, recently isolated from *Thalassiosira rotula* (Miralto *et al.* unpubl., quoted in Ianora *et al.*, 1999). The mechanism through

which aldehydes affect copepod reproduction is still unknown. Probably, inhibitory compounds are transferred to the gonads via feeding, followed by diffusion through the gut epithelium and accumulation in the developing oocytes during oogenesis.

An extract of *T. rotula* induced aberrations in tubulin organization similar to those reported for other toxicants classically defined as anti-mitotic drugs ( Ianora *et al.*, 1999). The diatom *Skeletonema costatum* inhibits proliferation of human bronchopulmonary tumoral cells (Bergé *et al.*, 1997). Cell growth is blocked in the G1 phase of the cell cycle and this growth arrest is irreversible.

Experiments conducted by Lee *et al.* (1999) on *Pseudocalanus newmani* showed that with mixed diets of the diatom *Chaetoceros gracilis* and the Prymnesiophyceae *Pavolova* spp. hatching success was lower than with *Pavolova* spp. alone but higher than with *C. gracilis* alone.

According to Ianora *et al.* (1999), evidence that compounds, like long-chained polyunsaturated fatty acids and amino acids, are lacking in the food grazed by copepods is still uncertain. Laabir *et al.* (1999) showed that hatching success was not due to the lack of any amino acids. With a diatom diet, there was a drastic increase in the amino acid pool in developing embryos notwithstanding reduced hatching rates.

Jónasdóttir *et al.* (1998) conclude that if the toxicity hypothesis were true, an entirely new interpretation of copepod feeding ecology would be required. Ianora *et al.* (1999) agree with the authors. The mounting evidence of the negative impact of diatoms on the reproductive biology of copepods (Poulet *et al.*, 1995; Ban *et al.*, 1997; Lee *et al.*, 1999) clearly indicates that it is time to revisit the classic view on diatom-copepod interactions.

As Huntley *et al*. (1986) once pointed out, the compounds that make a food "bad" or "toxic" are specific metabolites other than nutritious substances such as proteins, lipid and carbohydrates. It is time to look for such specific molecules and to understand their role in the pelagic realm.

As far back to the 16th century, Paracelus recognized that "all substances are poisons; there is none that is not a poison. The right dose separates the poison and the remedy" (Walker *et al.*, 2001). Accordingly, the Jónasdóttir *et al.* (1998) framework assumes that a toxic effect is dose dependent, which in turn is given by the amount of toxin ingested. Therefore, in the context of the experiments presented by Colin & Dam (2002), toxic effects should depend on algal ingestion regardless of whether the alga was offered as a single food or in a food mixture.

The effect of the mixed food on the reproduction of *A tonsa* was satisfactory and comparable to the effect of the flagellates *C. acantha* and *R. baltica.* But the mixed food had a better effect on the survival of adults, nauplii and copepodite, more important for the survival of the future generations. The better biochemical condition in the mixed food proportioned for major variation of organisms seemed to mask the effect of the dinoflagellates, or this result is in agreement with the assertion of Paracelus. The dinoflagellate ate as single food prevented any possibility of choice to the copepod, resulted in a major quantity of toxin ingested for the copepod.

The effect of toxins or nutritional components (protein, carbohydrate, lipid, amino acid, fatty acid, and vitamin) can vary among and within species. The assertion that an organism is toxic and/or has a low nutritional value must be not generalized. In this context, a generalization about an organism or group of organism is hasty. The knowledge about the biology of the grazer is also very important and assertion about the effect of toxic or nutritional compounds must be considered with care, as also must be considered the conditions, under which the experiments were realized.

### 4.3. Feeding Behavior of copepods

Although omnivory is a common strategy among zooplankton (Dam *et al.*, 1994; Laabir *et al.*, 1995; Kang *et al.*, 2000), information is scarce about the relative chemical input of plant and animal diets. Diversity and availability of food vary seasonally in marine environments, offering various alternative preys to phytoplankton in copepod diets (Kang *et al,* 2000).

A considerable amount of data has been compiled on the feeding behavior of copepod (Kiorboe *et al.*, 1985; Dagg & Walser, 1987; Haney, 1988; Roman *et al*. 1988; Uye & Takamatsu, 1990; Calbet *et al.*, 1999). Recent studies show that copepods are discriminating feeders. *Acartia clausi* is reported to select phytoplankton cells over plastic beads or similar size and equal availability (Donaghay & Small, 1979). Friedman & Strickler (1975) suggested the importance of the chemical composition of particles.

*A tonsa* consumes both phytoplankton and animal food. It prefers the largest single, spherical or elliptic, algae. *A tonsa* feeds actively on the algae *Chaetoceros socialis*, *Exuviaella cordata* (16x12 μm), *Gymnodinium* sp. (56x39 μm), *Prorocentrum micans* (40x30 μ), *Skeletonema costatum* (1-200μm; h – 6-10 μm), *Nitzschia closterium*

(30x40 µm), *Cyclotella caspia* (1-15-36 µm; h –10 µm). It can also consume actively minute *Flagellate* (6-8 µ) (Petipa, 1959).

The copepod *A tonsa* had a selective feeding behavior. The ingestion rates of *A tonsa* were significantly different between the treatments. There was no difference between the ingestion rates of *A tamarense*, *S. costatum*, and *P. lima*. But there were significant difference among the treatments with *C. acantha*, *R. baltica* and the mixed food. In case of the mixed food, *A tonsa* fed on the species with significantly different ingestion rates showed a feeding preference for *R. baltica* and *S. costatum*. The ingestion rate of *R. baltica* and *S. costatum* was similar in the mixed food treatment, but it was significantly different from the others species.

Terrestrial plants and marine algae are known to contain a variety of toxic secondary compounds whose function, is often poorly understood (Bargu, *et. al.*, 2003; Watson & Cruz-Rivera, 2003). Marine herbivores have a variety of responses to blooms of phytoplankton species (Turner and Tester, 1997). Turner and Teste (1997) reported that effects of toxin on grazers can be sublethal, inducing changes in the behavior or reduction in food intake, or can be lethal if grazers starved rather than eat toxic phytoplankton. Field observations and laboratory tests have shown a variety of responses to toxic algae, ranging from avoidance to ingestion of the algae (Teegarden & Cembella, 1986; Shaw *et al.*, 1994; Tester *et al.*, 2000; Turne *et al.*, 2000; Bargu *et al.*, 2003).

An example is the feeding behavior for *Acartia hundsonica, A tonsa* and *Calanus* during a bloom of *Olisthodiscus luteus* in the Narragansett Bay, USA, where this flagellate was rejected by the copepods (Tomas & Deason, 1981). Studies show further rejected species, including *Gonyaulax tamarensis, Gyrodinium aureolum, Protoceratium reticulatum, Ptychodiscus brevis and Scrippsiella trochoidea* (Turner & Anderson, 1983; Huntley *et al.*, 1986; Van Alstyne, 1986; Gill & Harris, 1987; Ives, 1987; Sykes & Huntley, 1987).

Blooms of phytoplankton, generally dinoflagellates or flagellates (Smayda, 1991), are known to induce mass mortalities in fish and shellfish. The toxic potential of algae may cause neurological intoxication or paralytic intoxication in humans through accumulation in shellfish. Several species of dinoflagellates and flagellates also affect the feeding of copepods, the production of eggs and the survival of these organisms (Huntley *et al.*, 1987, Verity & Smayda, 1989). Diatoms (Bacillariophyceae), other important component of marine phytoplankton, were rarely reported as toxic to marine

organisms until the identification of the high concentration of domoic acid in *Nitzschia pungens* f. *multiseries* by Douglas and Bates (1991). Diatoms, however, can have other harmful effects on zooplankton with important consequences for marine food webs (DeMott *et al.* 1991; Imada *et al.*, 1991; Kirk and Gilbert, 1992).

The feeding deterrents produced by some dinoflagellates caused marked behavioral changes in some marine organisms. These substances have both commercial and ecological significance. Production of deterrent substances by such species may decrease growth rates or increase mortality in mariculture species. In the natural environment, production of these compounds may control grazing and determines which species will bloom and how long the bloom will persist (Shaw *et al.*, 1994).

Phytoplankton food quality depends on both morphological and biochemical features. Parameters like size, algal hardness, possession of gelatinous sheaths, cell wall structures and toxicity may influence the ingestibility and digestibility of algae and, consequently, affect zooplankton growth rates (Vanni & Lampert, 1992; Lürling & Van Donk, 1997).

Other than toxic effect on egg production could be due to nutritional deficiency (Schmidt & Jónasdóttir, 1997) or to reductions in feeding (Colin & Dam, 2002). Ingestion rates can provide information on both of these causes; however, they are often not measured or reported (Uye, 1996; Ban *et al.*, 1997). By comparing ingestion rates to egg production, Colin & Dam (2002) could estimate the amount of ingested carbon that is contributed to growth. This is an index of the nutritional quality of the diet. By this criterion, the authors did not observe significant differences in the nutritional quality of the diets.

A reduction in feeding can result from behavioral selection against a food item (acting as a feeding deterrent) or presumably by physiological incapacitation after ingestion (Van Alstyne, 1986: Ives, 1987). In the last case, the total ingestion should decrease as the concentration of the toxic food increased. Thus, changes in the total ingestion rates across the various mixtures must be examined to assess whether the copepod are physiologically incapacitated. This was observed by Colin & Dam (2002) for the high-toxin *Alexandrium* sp. diets., and it is likely that this strain of *Alexandrium* to incapacitate copepods. Ives (1987) found *Alexandrium tamarense* to incapacitate copepods. Likewise, *A tonsa* has been found to be severely incapacitated by unialgal diets of toxic *A tamarense*, but not by mixed diets containing the toxic alga (Teegarden, 1999).

Differences among studies examining the effects of *Alexandrium* on *A tonsa* could be due to variations in algal toxin content or in the resistance of different *A tonsa* populations to the toxins. In the study of Colin & Dam (2002), the low-toxin *Alexandrium* strain had no effect on *A tonsa´*s ingestion or egg production.

According to Colin & Dam (2002), a more plausible explanation for the observed feeding patterns of *A tonsa* feeding on *P. tricornutum* is behavioral prey selection. Apparent selections against *P. tricornutum* reduced *A tonsa´*s egg production to a level that might lead one to conclude that *P. tricornutum* is harmful to *A tonsa*. (Ban *et al*. 1997). However, when one compares *A tonsa´*s egg production with the amount of *Tetraselmis* sp. ingested, Colin & Dam (2002) conclude that the addition of *P. tricornutum* to the *Tetraselmis* sp. diet does not decrease egg production. Additionally, it did not decrease *A tonsa´*s ingestion rates.

In the present study was used two dinoflagellates were used, who are potentially toxic (*A tamarense* and *P. lima*). The total cell volume of the mixed diet was not the sum of the volumes of the single species treatments. The ingestion rate of these species was higher in the mixed food than the ingestion rate of *P. lima* and *A. tamarense* as single diet. The addition of these species did not decrease the ingestion rate of *A. tonsa* in the mixed food.

According to Colin & Dam (2002), the egg production does not appear to be due to the nutritional quality of the food, but rather to total feeding reduction. However, these negative effects of monoalgal diets on *A tonsa* were not observed when these algal species were mixed with even a small fraction of the control diet. More studies are necessary to examine the toxic effect of a diet as single food assays.

The toxic dinoflagellates *P. lima* and *A tamarense* were ingested for *A tonsa* as food isolated or as a component of the mixed food. But the ingestion rate of the copepod on these species as single food was lower than the other species, principally when compared with the ingestion rate of *C. acantha* and *R. baltica* as single diet. A negative effects on the survival and/or reproduction of *A tonsa* occurred on the treatment with *A tamarense*, who provided the lower survival time of nauplii, adults and lower egg production rate and hatching success than the other species. But *A tamarense* did not provide a negative effect when this specie was offered mixed.

The ingestion rate with *C. acantha* in the mixed food was lower than the ingestion rate of this specie as single diet. However this decrease on the ingestion rate

of *A. tonsa* on *C. acantha* in the mixed food occurred, this phytoplankton as isolated food provided a good effect on the reproduction and survival of the copepod.

Microalgae used for larval nutrition usually are in the nanoplankton size range (2-20 μm). Exceptions are the diatom chains and pennate diatoms in mariculture of prawn larvae and abalone (Brown *et al.*, 1997). The success as food species depend on the nutritional quality, as well as, their tolerance to temperature, salinity and light, especially if grown in outdoor tanks or ponds (Brown *et al.*, 1997).

The results of Jónasdóttir (1994) reported that the similar copepod production rates (eggs.female-1.d-1, Er) measured for *Acartia* spp. fed on particles of 226 μm3 (*Rhodomonas lens*) and 3650 μm3 (*Prorocentrum minimum*) excludes the importance of cell size as an indicator of food quality. This result was consistent with the findings of Ahlgren *et al*. (1990) and Stottrup & Jersen (1990), but contrasts with the assumptions of the others investigators (Verity & Smayda, 1989; Sommer, 2003). However, the lower size limits of edibility reported in the literature were smaller than the smaller species used in this study.

In the present study was used as food two nanoplanktonic algae (*C. acantha* and *R. baltica*) and three microplanktonic algae (*S. costatum*, *A tamarense* and *P. lima*). The ingestion rates of C. acantha as isolated food was high, also as, the ingestion rate of *R. baltica*.

The size of the phytoplankton was not important to *A tonsa* within the offered size range. The food offered to the copepod varied from 523.33 μm3 to 40,362 μm3. The effect of the cell size is oft related to the capacity of the copepod on capture and feed on an organism. The advent of the biochemistry demonstrated that the level of the biochemical constitute per cellular volume is more important than the size of the cell, provided that the cell size is within the edible range. The quality of a cell lies is not in its size but in the quantity of nutrient that has this cell. *A tonsa* seemed not to choose the phytoplankton for its size but for its biochemical content. The low ingestion rate found to *P. lima* could indicate the preference for size due the low ingestion rate but, *S. costatum* was not smaller than *P. lima*. The ingestion rate to *S. costatum* was higher than the one to *P. lima*, principally in the mixed food.

One severe limitation of work with a single food is the possibility that the results obtained do not represent the complete spectrum of feeding behavior of copepods in nature, where the compositions of their food is diverse and variable (Bartram, 1980).

There are two general problems that make interpretations of feeding studies difficult: 1 – the large intraspecific and interspecific variation in the biochemical composition of live diets grown under resource limitation and, 2 – the large variation in response of herbivores to diets of differing quality (Kilham *et al.*, 1997ab). Moreover, the food quality of algae varies intraspecifically. Growing cells at intermediate growth rates using continuos or semi-continuos cultures reduce this variation and produce cells that are growing at rates similar to natural algae (Sommer, 1989).

### 4.4. Survival and Reproduction of copepods

The study of copepod egg production quality is essential, since variability of copepod recruitment may depend largely on the rate of production of viable eggs rather than fecundity per se. This parameter also seems worthy of study because it can shed light on the secondary production, which has been defined as the biomass produced by a population in a given time interval, regardless of whether it survives to the end of the interval (Kimmerer, 1987).

*Acartia* spp. are good copepod species to use in such studies because of their rapid response (< 24h) to the ambient food environment (Tester & Turner, 1990; Jónasdóttir *et al.*, 1995). *Acartia tonsa* is an opportunistic copepod that does not build up energy reserves but rather invests its entire metabolic production in egg production as soon as food conditions become favorable (Kiorboe *et al*. 1985). *A tonsa* has been reported to spawn eggs just 9.5 h after ingestion of food (Tester & Turner, 1990). *A tonsa* and *A hudsonica* rapidly decrease egg production rates when food conditions deteriorate (Kioboe *et al.*, 1985; Durbin *et al.*, 1992).

Production of eggs is energetically expensive. The female has to synthesize genetic material and oocytes and provide nutritionally adequate egg yolk. The embryo must obtain all its nutrition from the egg yolk within the egg. Sufficient nutrition is essential for the female to meet the increased energy expenditure of egg production (Harrison, 1990; Jónasdóttir, 1994), that was correlated with protein and specific fatty acids (16:1ω7, negative, 20:5ω3, 22:6ω3 and 18:0 positive) and, specially, the fatty acid composition of the algae expressed as the ω3: ω6 and 20:22 fatty acid ratios. In the literature is showed that carbon, nitrogen and protein content of the cells all exhibited significant correlation with the egg production of *A tonsa*. The highest egg production rate (Er) was found on the youngest diatom cultures and exponentially growing

flagellates and, the lowest Er was recorded for female fed the senescent diatom cultures. The eggs production rates of *Acartia* spp. were observed to be a function of both food concentration and algal type. The Er increased with the food concentration until a critical concentration was reached.

Starting from the fact that no egg production was produced in the replicates with *S. costatum* as food, we could suppose that the egg production was related to the algal type. This assertion is premature, because only one diatom was used in the present study. The concentration of the food can be discarded, since the concentration of the food offered to *A tonsa* was constant for all algal type as single food and mixed food. The major Er found for the copepod was due the best biochemical concentration in the food, why the best Er was found with the species with the high levels of lipid (*R. baltica*, *C. acantha* and mixed food). Also, the biochemical contents measured were correlated positively with the hatching success of the copepod. Hatching success was related directly with the egg production (Er) of *A tonsa*.

No correlation was found for Jónasdóttir *et al*. (1995) between egg production and the concentration of chlorophyll, ciliates and nitrogen and the C:N ratio of the seston, and the major assumption in this study is that the females from the different sampling dates would have equal Er, if they were at the same food conditions. The levels of chlorophyll *a* did not show any influence on the reproduction and survival of *A tonsa*. Chlorophyll *a* did not have correlation to any parameter analyzed.

For Stottrup & Jesen (1990) the egg production of *A tonsa* was related in the flagellate *Isochrisis galbana* and the lowest in *Ditylum brightwelli* and it was related with the ratio of the 20:22 fatty acid. The combination flagellate-diatom seems to be the best for reproduction of copepod. Flagellates have usually high concentration of DHA and low concentration of EPA and diatoms are usually low in DHA and high in EPA (Sommer, 1998).

Protein, lipid and carbohydrate per cell volume did not show a correlation to the Er of *A tonsa*, but with the hatching success of this copepod. This demonstrated that biochemical content was important for the egg production. Specific compounds, such as fatty acid and amino acid, were not measured. Maybe with the analysis of these compounds, a positive correlation could be demonstrated.

According to Jónasdóttir (1994), the Er of *A tonsa* was highest when were fed *Rhodomonas lens*. Lower but similar values were recorded for females fed *Thalassiosira weissflogii* and *Prorocentrum minimum*. However, the interaction of algal

species and culture age was highly significant for the Er of *A tonsa*, while the interaction between algal age and food concentration was non-significant. For this copepod, egg-production rates with the early-exponential stage of *R. lens* culture were significantly lower than those with the two older cultures. No difference in Emax (egg production on the maximum level of food concentration) was observed for *A hudsonica* fed *R. lens* of different ages.

In the present study, the Er of *A tonsa* was twice as high in the mixed treatment than *R. baltica*. *R. baltica* as single provided a good result, with the maximum egg production per female twice a high as with *C. acantha*. *A tamarense* provided a maximum Er higher than *C. acantha*, however the hatching success was low with *A tamarense*.

The poor result with *A tamarense* as food can be due the presence of toxin in this species. *P. lima* is also potentially toxic but *A tamarense* supported a higher egg production rate than *P. lima* and, while the hatching success was lower than in *P. lima*.

Good egg quality, leading to vigorous nauplii, determines variability in the recruitment rate of natural populations (Ianora & Poulet, 1993) and increase the ability to survive in the first developments stages (Laabir *et al.*, 1995ab). It also indirectly influences fish recruitment, since the first naupliar stages of copepods are important prey to fish larvae (Laabir *et al.*, 1995ab).

The female's capacity to produce viable eggs is one aspect of the reproductive biology that has been poorly investigated. The definition of viable eggs is one that has been fertilized and develops to hatching, giving rise to a viable nauplius. By contrast, a non-viable egg has either not been fertilized or, if fecund, fails to develop to hatching. Thus, the hatching success and survival through the initial naupliar stages may be more appropriate measures of the actual recruitment to the population and, hence, may be more important parameters in estimating population growth than is the number of egg produced (Ianora *et al.*, 1992; Jónasdóttir, 1994).

*P. lima* provided low eggs production but, the hatching success of this specie was high and not significantly different of the mixed food, *R. baltica* and *C. acantha*. However, the importance of this species to *A tonsa* is questionable. The survival of the first stages was very low (mean of 0.33 day). The survival of nauplii with *P. lima* was comparable to the survival with *A tamarense* (0.41 day), where the hatching success was extremely low (mean of 7.28%).

Factors affecting egg development times are egg size, with larger eggs requiring longer development times than smaller eggs of the same copepod species (Lonsdale & Levinton, 1985) and temperature (McLaren, 1965), however in the study of Jónasdóttir (1994) no correlation was found between development time and egg size. This factor is due the temperature was kept the same prior to and during all experiments and also, the parental acclimation was constant.

In the research of Jónasdóttir (1994) about the effects of the food quality on the reproductive success of *A tonsa* and *A hudsonica*, the development time of eggs was affected perhaps by the age of the phytoplankton culture fed to the female and the hatching success of eggs decreased with the culture age, but no correlation was found with the measured chemical component of the food. The ANOVA test showed that microalgal species (*Thalassiosira weissflogii* and *Rhodomonas lens*) did not significantly affect the development times of *A tonsa* eggs. However, there were highly significant differences between the development times of eggs of copepod fed algae of different age.

The age of the culture and the temperature can be discarded as influence on the development time of *A tonsa´*s eggs in my experiment. The biochemical content seemed to influence the development time of the eggs. The best results were found, when *A tonsa* fed on the mixed food, *R. baltica* and *C. acantha*, as single diets. These three food types led to the high egg production, hatching success and survival of adults, nauplii and copepodite. The diatom *S. costatum* did not prove eggs production, but this specie contributed certainly to the best results found with the mixed food.

Jónasdóttir (1994) had found that the hatching success of eggs from *A tonsa* females fed *T. weissflogii* decreased with increasing age of the culture, but the age of *R. lens* cultures did not affect the hatching success. Hatching success of *A tonsa* eggs appeared to be a function of growth rate of the phytoplankton cultures used to feed the females, it increased with increasing growth rate. However, when the hatching success of the eggs was correlated with the different chemical components of the cell, no significant correlation were evident for the *Acartia* species. Also, there was no correlation between hatching success and naupliar survival or the chemical contents of food and naupliar survival, generally, once an egg hat hatched, the nauplius had a good chance of surviving through the non-feeding stages.

Parrish & Wilson (1978) reported that egg viability was related to time since last mating, and associated non-viability with non-fertilized eggs. Ianora *et al*. (1989)

suggested that low hatching success in *Temora stylifera* eggs was due to poor fertilization during broadcast spawning. In the study of Jónasdóttir (1994) males copepods were present in all batch cultures and throughout the acclimation period, therefore, it is reasonable assume that all eggs were fertilized. Moreover, non-fertilized eggs usually disintegrate fairly soon after being spawned and are easily distinguished from fertilized eggs, which did not be noted in any of the experiments. So, the observed difference in hatching success probably resulted from differences in the chemical composition of the food fed to the females and/or the conditions of the female while producing eggs. However, none of the correlation between hatching success and the individual chemical components measured was significant.

The hatching success of *A tonsa´*s eggs, in the present study, was directly and positively correlated with the biochemical compounds of the food (volume-based). The hatching success of this copepod was directly correlated with the ingestion rate. A high correlation was found between the hatching success and the survival time of nauplii and copepodite.

A good diet must provide not only a good egg production and hatching success, but also a high survival of the first stages, leading to the survival of the organism in the environment. The species *A tamarense* and *P. lima* provided eggs production and hatching success but, the survival time of the first stages was so low that make these algae a not suitable food for *A tonsa*.

The males of *A tonsa* were present in all time of the experiments. Therefore a lade of fertilization seems highly unproblable. Non-fertilized eggs were not observed. The difference on the hatching success in the foods for *A tonsa* was correlated directly with the biochemical content measured of the food (volume-based). The biochemical components of the food (volume-based) were highly correlated with the survival time of adults. This observation can make true that the condition of the female while producing eggs is also important to prove an optimal hatching success of the copepod *A tonsa*.

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# 6. Abstract

Studies of food quality in aquatic environment have been increasing with the advance of the biochemistry. The effect of the different diets on marine and freshwater organisms is very important, because the food can affect directly the reproduction and the survival of the individual. Principally reproduction, the egg production, eggs viability and survival of the early stages can affect the future generation and then, affect the survival of the animal in the environment. Copepods are an important animal in aquatic ecosystems. They are an important link between the phytoplankton and the superior's level of the food web. Calaniod are the most important group of copepods. *A. tonsa*, an important copepod in many coastal and estuarine communities, often dominating the zooplankton is a common species used in laboratory experiments, because its rapidly response to chances of the culture conditions. The present study aimed to know the influence of five phytoplankton species on the feeding behavior, reproduction and survival of the copepod *A. tonsa*. Biochemical parameters (total lipid, carbohydrate and protein) were measured to know the possible nutritional value of the diet. The species were offered to *A tonsa* as single and mixed diet. The species used were the flagellates *C. acantha* and *R. baltica*, the potentially toxic dinoflagellates *A. tamarense* and *P. lima* and the diatom *S. costatum*. The food was offered in a constant concentration (ca. 200 ug.ml-1). The phytoplanktonic species and the copepod were cultured in a temperature constant of 160C, 19 PSU and a 12:12 light:dark cycle. The Ostsee Medium was used to culture of phytoplankton. Two main experiments were performed: 1 – Maintenance and biochemical analysis (total content of protein, lipid and carbohydrate); 2 – Feeding behavior (ingestion rate), survival (parental copepod and the early stages) and reproduction (egg production, hatching success) of the copepod. The second experiment had two steps: 24h experiment (ingestion rate); and a long experiment (ca. 45 days) to analyze the survival and reproduction of the copepod. The experiments were carried out under the same conditions of the maintenance of the phytoplankton species and copepod. During the experiment, pH was dialy measured, and samples were take for measure the level of Chlorophyll *a*, nutrients (N, P, Si) and cell density (cell.ml-1). The biochemical parameters were measured at the first and second day of the main experiments, in the replicates without copepod. Protein (55-69%) was the major biochemical components per cell volume, following for carbohydrate (17-31%) and lipid (13-18%). The concentration of the biochemical components measured per cell volume influenced the hatching success and survival of the adults. The hatching success was correlated with the egg production and ingestion rate of the copepod and survival of the early stages. No correlation was found between egg production and the biochemical concentration per cell volume. The biochemical components per cell volume showed a high interspecific difference between the species and seemed to better explain part of the nutritional value of the phytoplankton, as the concentrations per dry weight and volume medium. The copepod *A tonsa* had a selective feeding behavior feeding preferable on *S. costatum* and *R. baltica* in the mixed food. The ingestion rate of the specie *C. acantha* was reduced into the mixed food. The size range of the species offered to *A. tonsa* did not influence its ingestion rate.The flagellates *R. baltica* and *C. acantha* and the mixed food provided the best effects on the reproduction and survival of *A. tonsa*. The best results among the single foods treatments were found with the alga, with the higher lipid content (*R. baltica* and *C. acantha*). The dinoflagellates *P. lima* all showed eggs production and good hatching

but, the survival of adults and early stages was affected. *S. costatum* prevented egg production. The survival and reproduction of *A. tonsa* fed on *A. tamarense* was reduced and comparable to the control (without food). In spite of, no influence was seen when *S. costatum*, *P. lima* and *A. tamarense* were offered mixed with the two flagellates. The mixed food was the best food. This result could demonstrate that the negative effect of these species on the reproduction and survival of *A. tonsa* was due their insufficient biochemical content as the presence of toxin. A good food muss proved not only a good egg production and hatching success, but a high the survival of the first stages, guaranteed the survival of the organism in the environment. The negative effect of the dinoflagellates on the survival of the early stages of the copepod indicated that these species were a bad food for *A. tonsa*. Therefore, *A. tamarense*, *S. costatum* and *P. lima* were no a good diet as single food to *A. tonsa*. The best results with the mixed food could be result from an increase on the levels of DHA, provided for *R. baltica*, and EPA, provided for *S. costatum*, also as for the increase of protein supplied for *C. acantha*, major content of protein per cell volume. But for a better understand of the relation of the biochemical contents with survival e reproduction of aquatic animals makes necessary a analysis of vitamins, minerals and of the constitutes of each gross biochemical composition, such as fatty acids, amino acids, sterols, sugars.

# 7. Zusammenfassung

Forschungen über Futterqualität in aquatischer Umgebung haben mit dem biochemischen Schritt zugenommen. Der Einfluss von verschiedenem Futter auf marine und süßwasser Tiere ist sehr wichtig, weil das Fressen die Reproduktion und das Überleben der Organismen beeinflussen kann. Eierproduktion, Eierfähigkeit und Überleben des Nachwuches können die zukünftige Generation betreffen und so die Überlebenschancen der Tiere in der Umwelt verringern. Copepoden sind wichtige Tiere in aquatischen Gebieten. Sie sind eine wichtige Bindung zwischen dem Phytoplankton und den hohen Stufen der Nahrungskette. Calanoiden sind die wichtige Gruppe von den Copepoden. *A. tonsa* ist oft dominant und ein wichtiges Tier in vielen Küstengebieten und Estuary. Sie ist die übliche Art experimenteller Forschung, weil sie eine schnelle Antwort auf Veränderung hat. In der Arbeit geht es um den Einfluss von fünf Phytoplanktonarten auf das Ernährungsverhältnis, Reproduktion und Überleben von dem Copepod *A. tonsa*. Biochemische Parameter (Total Protein, Lipid und Kohlenstoffe) werden gemessen, um den nahrhaften Wert des Futters kennen zu lernen. Die Arten wurden *A. tonsa* alleine und als Mischung angeboten. Die Phytoplanktonarten waren die Flagellaten *Chrysocromulina acantha* und *Rhodomonas baltica*, die potentielle gifte Dinoflagellaten *A.tamarense* und *Prorocentrum lima* und die Diatom *Skeletonema costatum*. Das Futter war in einer konstanten Konzentration (ca. 200 µg.ml-1) angeboten. Die Phytoplanktonarten und Copepoden sind in einer Temperatur von 16oC, 19USP und 12:12 hell/dunkel Zicle gezüchtet. Das Ostsee Medium wurde für die Phytoplanktonzucht benutzt. Zwei Hauptexperimente wurden gemacht: 1– Verpflegung und Messung der biochemischen Zusammensetzung der Phytoplanktonarten; 2-Ernährungsverhältnis (Einnahmerate), Überleben (Erwachne Copepoden und Nachwuchs) und Reproduktion (Eierproduktion; Eierfähigkeit) von *A. tonsa*. Das zweite Experiment war in 2 Teile verteilt: ein 24 Stunden Experiment (Einnahmerate); ein langes Experiment (ca. 45 Tage), um das Überleben und die Reproduktion von dem Copepod zu analysieren. Die Experimente wurden zu den gleichen Bedingungen wie die Verpflegung der Phytoplanktonarten und Copepoden durchgeführt. Der pH-Wert wurde täglich gemessen. Täglich wurden Proben genommen, um Chlorophyll a, Nährstoffe (N, P, Si) und Zelldichte (Zell.ml-1) kennen zu lernen. Die biochemischen Zusammensetzungen wurden am ersten und zweiten Tag gemessen, ohne Copepoden darin festzustellen. Protein (55-69%) war der grössere biochemische Teil der Zellmasse, gefolgt von Kohlenhydraten (17-31%) und Lipid (13-18%). Die Konzentration der gemessenen biochemischen Zusammensetzungen pro Zellmasse haben die Eierfähigkeit und das Überleben der erwachsenen Copepoden beeinflusst. Die Eierfähigkeit hat der Eierproduktion, der Einnahmerate und dem Überleben des Nachwuches entsprochen. Keine Entsprechung wurde zwischen der Eierproduktion und der biochemischen Zusammensetzung pro Zellmasse gefunden. Die biochemische Zusammensetzung pro Zellmasse hat einen hohen Unterschied zwischen den Phytoplanktonarten gezeigt. Sie hat einen besseren Einfluss auf die Reproduktion und Überleben von *A. tonsa*, als die Konzentration pro Trockengewicht bzw. pro Mediuminhalt. *A. tonsa* hatte ein selektives Ernährungsverhalten gezeigt. Sie hat bevorzugt *S. costatum* und *R. baltica* aus dem Mischfutter gefressen. Die Einnahmerate von *C. acantha* hat in dem Mischfutter abgenommen. Der Größenintervall der Phytoplanktonarten hat keinen Einfluss auf die Einnahmerate von *A. tonsa* gehabt. Die Flagellaten *R. baltica und C. acantha* und das Mischfutter haben den besseren Effekt

auf die Reproduktion und das Überleben von *A. tonsa* gegeben. Die besseren Ergebnisse hatten die Einzelfutterarten, die die höchste Konzentration von Lipid (*R. baltica* und *C. acantha*) aufzeigten. Der Dinoflagellate *P. lima* hat Eierproduktion und eine gute Eierfähigkeit gegeben, aber das Überleben der erwachsenen Copepoden war betroffen. *S. costatum* hat die Eierproduktion verhindert. Das Überleben und die Reproduktion von *A. tonsa*, die mit *A. tamarense* gefüttert wurde, hat abgenommen, ähnlich waren die Ergebnisse ohnen Fressen. Trotzdem, keinen Einfluss hatte es, wenn *S. costatum*, *P. lima* und *A. tamarense* in das Mischfutter getan wurde. Das Mischfutter war das beste Fressen. Dieses Ergebins hat gezeigt, dass der negative Einfluss dieser Arten auf die Reproduktion und das Überleben von *A. tonsa* seinen Grund in der biochemichen Zusammensetzung und nicht wegen ihrer Giftstoffe hatte. Ein gutes Fressen muss fördern, nicht nur eine gute Eierproduktion und Eierfähigkeit, sondern auch das Überleben des Nachwuches, der das Überleben der Organismen in der Umwelt sichert. Der negative Effekt der Dinoflagellaten auf das Nachwuchsüberleben hat gezeigt, dass diese Arten ein schlechtes Futter für *A. tonsa* gewesen sind. Trodzdem, *A. tamarense*, *P. lima* und *S. costatum* allein waren kein gutes Fressen für *A. tonsa*. Das beste Ergebnis mit dem Mischfutter kam vielleicht aus der Zunahme von DHA (aus *R. baltica*) und EPA (aus *S. costatum*), aber auch wegen der Zunahme von Protein, das aus *C. acantha* geliefert wurde. *C. acantha* war die Art mit der größten Konzentration von Protein pro Zellmasse. Aber, für ein besseres Verständnis über das Verhältnis zwischen der biochemischen Zusammensetzung und der Reproduktion und dem Überleben von aquatischen Tieren ist es nötig, dass Vitamine, Mineralien und kleine Teile der biochemischen Zusammensetzung wie Fettsäure, Aminosäure, Sterol und Zucker analysiert werden.

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