The quality of microalgae as a food for the rotifer *Brachionus plicatilis*: effects of temperature and algal strain

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by

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To

Kalthom Bushank and Abdullah Sayegh

My Parents

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Chapter 6. Does this study only applied to aquaculture?

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Abstract

Today, microalgae are used extensively in aquaculture and biotechnology. The rotifer *Brachionus plicatilis* is the most important live food used in aquaculture especially for early developmental stages of small-mouthed larvae. Knowledge of the differences between microalgal strains and the temperature at which they grow is far from complete. This study investigates this matter and tests the effect of microalgal quality on rotifer culture. The study was designed to assess if microalgal strain and their growth-temperature need to be considered by aquaculturists, in order to optimize their production. The evaluation was made on both microalgae and rotifers, based on factors that may affect the survival and growth of marine larvae such as biomass and nutritional value.

The study tested the dietary value of four strains, all identified as *Isochrysis galbana*, which were compared with another species *Nannochloropsis* sp. These strains are widely used in the commercial mariculture industry. The research indicated differences between these clones in growth rate, cell size, production, dry weight, and biochemical compositions. The influence of these on the dietary value of the rotifer *B. plicatilis* was also investigated using individuals and mass culture methods. Significant differences in rotifer growth, fecundity, and biochemical composition parameters were estimated. However, no differences in rotifer survival were indicated.

These strains were also studied at different temperatures from 15-30°C, these being typical of the temperatures used in aquaculture. There were significant differences between the strains in: cell size, production, and dry weight; but no differences were recorded in their growth rates. The influence of temperature on their biochemical composition (protein, carbohydrate, and total lipid) was also investigated and the differences were as high as those between species at such temperatures.

The rotifer was fed the four strains of *I. galbana* and *Nannochloropsis* sp. grown at different temperatures to test the affect of these diets on rotifer growth. Parameters tested were volumes, growth rate, life history, and several survival and fecundity parameters. The results showed differences in rotifer growth and no differences in rotifer survival between treatments, but overall these were not marked and may therefore have insufficient influence on rotifer culture and production.

The study also investigated the influence of these microalgal strains/species on the rotifers nutritional value. The results revealed biochemical composition content differences between rotifer treatments. However, no clear trend or strong relationship in biochemical composition between rotifer and its prey content were found. Nevertheless, the variation could largely be due to prey strain and prey temperature which might affect the transformation of biochemical components to fish larvae. Furthermore, the study tested other factors that might be involved in predicting the biochemical composition of rotifers. These analyses used the data from this study and from other literature but only one was significant, lipid content per dry weight, and this was not a strong relationship.

It was concluded that temperature could influence the choice of microalgal strains as mariculture food in aquaculture and biotechnology. It is recommended that researchers should be aware of which strains and temperature they are using as they could affect the quality of their microalgal product and the quality of their rotifers.

Preface

The structure of this thesis follows recommendations made by Stanisstreet (1996): it is a sequential series of linked chapters, bracketed by a general introduction and general conclusion. Each chapter was conceived as a paper that follows the style of Aquaculture, an appropriate target journal for this subject matter. For this reason, the reader should not be surprised if some sections (e.g. Material and Methods) are repetitive. One advantage of the chosen structure is that it facilitates the preparation of sections of the work for publication (Stanisstreet 1996) and thus provides the training in key-skills that I will need to obtain an academic position.

Stanisstreet M (1996) Writing your thesis. Suggestions for planning and writing theses and dissertations in science-based disciplines. The University of Liverpool, 14 pp.

Chapter1.

Introduction to the area of study

1.1. Live food role in aquaculture

1.1.1. Historical perspective

The fishery crisis in European and North American seas in the late 1800s stimulated the start of marine aquaculture (Bengtson, 2003). The purpose was to provide fertilised eggs, developing embryos and larvae for distribution back into the ocean. Because of the high mortality rates of fish in their early stages, these ocean stocking efforts were doomed to fail. The reason for releasing them at such an early stage of development was because no convenient live feed was available for their post-larval survival and growth. Studies that began in the 1880s used natural zooplankton as a diet to raise cod larvae (Rognerud, 1887).

The culture of algae also seems to have its origins in the late 1800s as a results of methods developed by bacteriologists (Bold, 1950). A significant advance in marine algal culture was reported by (Gross, 1937). Methods of marine algal culture continued to advance during the middle of the twentieth century with the development of artificial media (Provasoli et al., 1957), and the development of 'f' medium for the enrichment of seawater (Guillard and Ryther, 1962). Improved methods for monospecific algal cultures allowed the expansion of hatcheries for molluscan aquaculture and enabled culture of live invertebrates as feed for larval fish and crustaceans.

In the 1960s another important advance was made when Japanese researchers discovered that the rotifers *Brachionus plicatilis* could be used as a first food for larvae of both freshwater and marine fish (Hirata, 1974). This advance clearly allowed the culture of many more species whose larvae hatched at such a small size

that their mouth gapes were insufficient for the ingestion of the typically used larger prey *Artemia* (Fukusho, 1985; Su et al., 1994; Lubzens and Zmora, 2003).

1.1.2. Is live feed necessary?

Fish biologists categorise larvae into two types: precocial and altricial (Bengtson, 2003). Precocial larvae are those that, when the yolk sac is exhausted, appear as mini-adults, exhibiting fully developed fins and a mature digestive system including a functional stomach. Such fish can ingest and digest formulated diets as a first food. Altricial larvae are those that, when the yolk sac is exhausted, remain in a relatively undeveloped state. The digestive system is still rudimentary, lacking a stomach, and much of the protein digestion takes place in the epithelial cells of the hindgut (Govoni et al., 1986). Such a digestive system seems to be incapable of processing formulated diets in a manner that allows survival or growth of the larvae comparable to those fed live feed (Lubzens et al., 1989; Watanabe and Kiron, 1994). Live feeds are important for both larval categorises (precocial and altricial) as they are able to swim in the water column and are constantly available to the larvae. Furthermore, the movement of live feed in the water is likely to stimulate larval feeding responses (Kamler, 1992). Finally, live prey, with a thin exoskeleton and high water content, may be more palatable to the larvae once taken into the mouth, compared to the hard, dry formulated diets (Person-Le Ruyet, et al., 1993; Bengtson, 2003).

1.1.3. What is microalgae referring to?

In applied phycology the term microalgae refers to any microscopic algae, and even contains the oxygenic photosynthetic bacteria, i.e., the cyanobacteria. However, the microalgae that is used in this study is a "nano" microalgae (2-20μm).

1.2. How this study relates to live feed in aquaculture?

The relationship between a prey and its predator can be affected by many environmental factors. From the point of view of aquaculture, this relationship plays an important role, especially when stocks are cultured using live-food diets (Pillay, 1990). Aquaculturists are constantly seeking to improve food quality. One factor that might affect food quality is strain variation. Genetic variations between strains of microalgae have been shown to alter their relative quality as a food (Lopez Alonso et al., 1994). Factors such as temperature, which can be easily controlled in aquaculture, can also affect food quality (Bosque et al., 2001). Thus, studies into the influence of these factors will help keep feed cost low (Dhert, 1996). The rearing of the majority of marine fish larvae requires the use of the rotifer *Brachionus plicatilis* as a live feed. However, the quantity and quality of rotifers depend upon their diet (Ben-Amotz, 1984). Marine microalgae are one of the most nutritious diets for rotifers (Watanabe et al., 1983). But there are a variety of species and strains of these microalgae and they may be grown at different temperatures; these factors could affect their quality as food for rotifers.

1.3. Temperature affects on live food

Like many organisms, microalgae are affected by factors such as light (Molina Grima et al., 1996), salinity (Fan et al., 1998) and temperature (Thompson et al., 1992a; b). These factors can be easily controlled in many aquaculture practices. Temperature is one of the most important physical factors affecting any microalgae in aquaculture (Pillay, 1990). Temperature can strongly influence the growth potential of microalgae in culture (Goldman and Ryther, 1976). Temperature affects biological activity directly by altering chemical reactions, and indirectly by altering viscosity and diffusion rates (Cossins and Bowler, 1987). In addition, it influences the growth and biochemical composition of photoautotrophs primarily through its control of enzyme kinetics (Davison, 1991). Furthermore, temperature is known to affect membrane lipid composition and content (Hu, 2004). Growth temperature has also been shown to influence the pattern of cellular carbon and nitrogen quotas as well as cell volume (Montagnes and Franklin, 2001; Atkinson et al., 2003). Therefore, temperature is likely to influence the nutritional value of microalgae as a food for *B. plicatilis*.

The effects of temperature on the biochemical composition (e.g., the ratio of carbon and nitrogen, protein per cell, and chlorophyll *a*) of *Nannochloropsis* sp. (Volkman et al., 1993), *I. galbana* and many species of phytoplankton (Thompson, et al., 1992) have been investigated. The fatty acid composition of *Nannochloropsis* sp. (James et al., 1989) and *I. galbana* (Thompson et al., 1992) is also affected by temperature. Other research has related temperature to growth phase; Zhu et al. (1997) harvested *I. galbana* at 15 and 30°C in the exponential and early stationary growth phases and found variation in the carbohydrate, protein, lipid, and fatty acids

content.

Temperature also effects microalgae development (Thompson et al., 1992; Zhu et al., 1997; Montagnes and Franklin, 2001). Kain and Fogg (1958) showed that *I. galbana* grows best between 20 and 25°C, and growth was inhibited above 30°C. Lopez-Munoz et al. (1992) reported that at constant light intensity, the growth rate of *I. galbana* rises as temperature increase. Furthermore, cell size of *I. galbana* decreases when temperature increases (Montagnes and Franklin, 2001).

All of the above factors may alter the suitability of prey for rotifers. This work will bring together the above ideas to investigate the quality of *I. galbana* and *Nannochloropsis* sp. by measuring the following parameters: lipids, carbohydrate, protein, growth rate, cell size, production, and dry weight. This was done over the temperature range 10 to 30°C, at which aquaculturists normally grow their phytoplankton. Flagellates were then fed to the rotifer *B. plicatilis*, to test how temperature-induced differences on the prey affected the biochemical composition, growth rate, volume, production, fecundity, life history, survival and egg survival of the rotifer.

1.4. Strain importance in quality test

Even when *I. galbana* has been grown at the same temperature, in different studies, wide discrepancies appear in its growth and biochemistry (Ben-Amotz et al., 1985; Fernandez-Reiriz et al., 1989). Differences in culture conditions, the analytical methods used, and the growth phases in which cultures were sampled could explain some of these discrepancies. However, genetic variation between the samples could

be the primary cause of these discrepancies. Lopez Alonso et al. (1992; 1994) found a wide genetic variation in samples of *I. galbana*, and they suggested that 'geographical' genetic variation between strains of this species is likely to be high. Wikfors and Patterson (1994) discuss the origins of some *I. galbana* strains, describe differences between these strains, and recommend that researchers be cognizant of the cultural history of their *Isochrysis* strains because differences between them will affect their use as mariculture feeds.

Much of the previous research on *I. galbana* and *Nannochloropsis* sp. has studied a single strain, such as *I. galbana* Parke (8701) (Zhou et al., 1994) or *Nannochloropsis* sp. strain MFD-2 (James and Al-Khars, 1990). However, Lopez Alonso et al. (1992; 1994) reported that fatty acid profiles and total lipid content vary between strains of *I. galbana*. Helm and Laing (1987) compared the food value of *I. galbana* Parke strain to that of *I. galbana* (clone T-ISO) for bivalve larvae; they demonstrated dissimilarities in the polyunsaturated fatty acids (PUFA) content. As yet, no work has examined the potential differences in food quality of these two strains and other food strains for the rotifer *B. plicatilis*.

In fact, few studies have considered "strain' as a potentially important factor, even through strain selection of the microalgae is very important in terms of food quality (Lopez Alonso et al., 1992) and the content of PUFA among various strains (Lopez Alonso et al., 1994). Furthermore, studies have examined how variation in biochemical composition of *I. galbana* strains may affect growth parameters of the rotifer *B. plicatilis* (e.g., production time, egg survival, fatty acids).

The work proposed here does not aim to optimise the culture of *I. galbana*, but to determine if different prey strains exist, and if so, if they alter rotifer development, production, survival, carbohydrate content, protein content, total lipids

content, and dry weight. This study will test the prey quality of four strains of *I. galbana* collected from: Isle of Man (M), Tahiti (T), Greece (G), and Denmark (D) and one strain of *Nannochloropsis* sp. (N); for more information on these strains see Table 1, Chapter 2.

1.5. Aims

This study was designed to assess if microalgal strain and growth-temperature need to be considered by aquaculturists if they want to optimise their production. The study was divided into four main sections, each being presented as a chapter (written as potentially publishable manuscripts, see preface); each of these deals with a specific problem in detail. Chapter 2 examines if microalgal strain differences occur and if they have an impact on the predator *B. plicatilis*, at a single temperature. This study was designed to initially assess if strain differences were a potentially important factor to consider; which they were. Thus, the next steps were to assess if there were interactive effects of temperature and strain on microalgal growth and biochemical composition (Chapter 3), rotifer growth and development (Chapter 4), and rotifer biochemical composition and its relation to prey composition (Chapter 5). Finally, the general applied aspects from these chapters are synthesised in Chapter 6.

1.6. References

Atkinson, D., Ciotti, B.J., Montagnes, D.J.S., 2003. Protists decrease in size linearly with tempeature:*ca.*2.5%°C^{-1}. Proc. R. Soc. Lond. B. Biol. Sci. 270, 2605-2611.

Ben-Amotz, A., 1984. Production of nutritional microphytoplankton for use as food in marine fish hatcheries. In: Rosenthal, H., Sarig, S. (Eds.), The second seminar of the german-israel cooperation in aquaculture research. The german-israeli cooperation in science and technology, biologische anstalt, helgoland, Hamburg, pp. 195-200.

Ben-Amotz, A., Tornabene, T.G., Thomas, W.H., 1985. Chemical profile of selected species of microalgae with emphasis on lipids. J. Phycol. 21, 72-81.

Bengtson, D.A., 2003. Status of Marine Aquaculture in Relation to Live Prey: Past, Present and Future. In: Støttrup, J.G., McEvoy, L.A. (Eds.), Live Feeds in Marine Aquaculture. Blackwell, Cornwall, pp. 1-13.

Bold, H.C., 1950. Problems in the cultivation of algae. In: Brunel, J., Prescott, G.W., Tiffany, L.H. (Eds.), The culturing of algae. Charles Kettering F. Foundation, New York, pp. 11-17.

Bosque, T., Hernandez, R., Todoli, R., Oltra., 2001. Effect of salinity, temperature and food level on the demographic characteristics of the seawater rotifer *Synchaeta littoralis* Rousselet. J. Exp. Mar. Biol. Ecol. 258, 55-64.

Cossins, A.R., Bowler, K., 1987. Temperature Biology of Animals. Chapman and Hall, London, 339 pp.

Davison, I.R., 1991. Environmental effects on algae photosynthesis: temperature. J. Phycol 22, 77-87.

Dhert, P., 1996. Rotifers. In: Lavens, P., Sorgeloos, P. (Eds.), Manual on the production and use of live food for aquaculture. F. A.O. Technical paper. Food and Agriculture Organization of the United Nations, Rome, pp. 61-98.

Fan, Y., Liu, F., Li, H., 1998. Studies on polyunsaturated fatty acids of marine microalgae, II. Effects of culture condition on the contents of fatty acids of *Isochrysis galbana* 3011. Stud Mar Sin, 155-160.

Fernandez-Reiriz, M.J., Perez-Camacho, A., Ferreiro, M.J., Blanco, J., Planas, M., Campos, M.J., Labarta, U., 1989. Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. Aquaculture 83, 17-37.

Fukusho, K., 1985. Status of marine larval culture in Japan. In: Lee, C.-S., Liao, I.C. (Eds.), Reproduction and Culture of Milkfish, Tungkang Marine Laboratory, Tungkang (Taiwan), pp. 127-139.

Goldman, J.C., Ryther, J.H., 1976. Temperature influenced species competition in mass cultures of marine phytoplankton. Biotechnol. Bioeng. 18, 1125-1144.

Govoni, J.J., Boehlert, G.W., Watanabe, Y., 1986. The physiology of digestion in fish larvae. Environ. Biol. Fish. 16, 59-77.

Gross, F., 1937. Notes on the culture of some marine plankton organisms. J. Mar. Biol. Assoc. U.K. 21, 753-768.

Guillard, R.R.L., Ryther, J.H., 1962. Studies on marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacae* (Cleve) Gran. Can. J. Microbiol. 8, 229-239.

Helm, M.M., Laing, I., 1987. Preliminary Observations on the Nutritional Value of 'Tahiti *Isochrysis*' to Bivalve Larvae. Aquaculture 62, 281-288.

Hirata, H., 1974. An attempt to apply an experimental microcosm for the mass culture of marine rotifer, *Brachionus plicatilis* Mueller. Mem. Fac. Fish. Kagoshima Univ. 23, 163-172.

Hu, Q., 2004. Environmental Effects on Cell Composition. In: Richmond, A. (Ed.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Science Ltd, Cornwall, pp. 83-93.

James, C.M., Al-Khars, A.M., 1990. An intensive continuous culture system using tubular photobioreactors for producing microalgae. Aquaculture 87, 381-393.

James, C.M., Al-Hinty, S., Slman, A.E., 1989. Growth and ω3 fatty acid composition of marine microalgae under different temperature regimes. Aquaculture 77, 337-351.

Kain, J.M., Fogg, G.E., 1958. Studies on the growth of marine phytoplankton *Isochrysis galbana*. J. Mar. Biol. Assoc. U.K. 37, 781-788.

Kamler, E., 1992. Early Life History of Fish, an energetic approach. Chapman & Hall., London, 71-76 pp.

Lopez Alonso, D., Molina Grima, E., Sanchez Perez, J.A., Garcia Sanchez, J.L., Garcia Camacho, F., 1992. Isolation of clones of *Isochrysis galbana* rich in eicospapentaenoic acid. Aquaculture 102, 363-371.

Lopez Alonso, D., Segura del Castilo, C.I., Garcia Sanchez, J.L., Sanchez Perez, J.A., Garcia Camacho, F., 1994. Quantitative genetics of fatty acid variation in *Isochrysis galbana* (Prymnesiophyceae) and *Phaeodactylum tricornutum* (Bacillariophyceae). J. Phycol. 30, 553-558.

Lopez-Munoz, I., Abalde, J., Herrero, C., 1992. Growth and pigment content of four marine microalgae cultured under different temperature and light intensity conditions. Nova Acta Cient. Compostelana Biol 3, 59-65.

Lubzens, E., Zmora, O., 2003. Production and Nutritional Value of Rotifers. In: Støttrup, J.G., McEvoy, L.A. (Eds.), Live feeds in marine aquaculture. Blackwell Science Ltd, Cornwall, pp. 17-52.

Lubzens, E., Tandler, A., Minkoff, G., 1989. Rotifers as food in aquaculture. Hydrobiologia 186/187, 387-400.

Molina Grima, E., Fernandez Sevilla, J.M., Sanchez Perez, J.A., Garcia Camacho, F., 1996. A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances. J. Biotechnol. 45, 59-69.

Montagnes, D.J.S., Franklin, D.J., 2001. Effect of temperature on diatom volume, growth rate, and carbon and nitrogen content: Reconsidering some paradigms. Limnol. Oceanogr. 46, 2008-2018.

Person-Le Ruyet, J., Alexandre, J.C., Thebaud, L., Mugnier, C., 1993. Marine fish larvae feeding: Formulated diets or live prey? J. World Maricult. Soc. 24, 211-224.

Pillay, T.V.R., 1990. Aquaculture: principles and practices. Fishing News Book, London, 591 pp.

Provasoli, L., McLaughlin, J.J.A., Droop, M.R., 1957. The development of artificial media for marine algae. Arch. Mikrobiol. 25, 392-428.

Rognerud, C., 1887. Hatching of cod in Norway. Bull. US Fish. Commission. 7, 113-119.

Su, H.M., Su, M.S., Liao, I.C., 1994. Selection of super small-sized strain of the rotifer (*Brachionus plicatilis*) and its rearing conditions. J. Taiwan Fish. Res. 2, 19-29.

Thompson, P.A., Guo, M.X., Harrison, P.J., 1992. Effects of variation in temperature. 1. On the biochemical composition of eight species of marine phytoplankton. J. Phycol. 28, 481-488.

Thompson, P.A., Guo, M.X., Harrison, P.J., Whyte, J.N.C., 1992. Effects of variation in temperature. 2. On the fatty acid composition of eight species of marine phytoplankton. J. Phycol. 28, 488-497.

Volkman, J.K., Brown, M.R., Dunstan, G.A., Jeffrey, S.W., 1993. The biochemical composition of marine microalgae from the class eustigmatophyte. J. Phycol. 14, 451-454.

Watanabe, T., Kiron, V., 1994. Prospect in larval fish dietetics. Aquaculture 124, 223-251.

Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 89, 263-272.

Wikfors, G.H., Patterson, G.W., 1994. Differences in strains of *Isochrysis* of importance to mariculture. Aquaculture 123, 127-135.

Zhou, R., Sun, A., Yang, Z., 1994. Studies on the ecological factors of *Isochrysis galbana* Parke 8701. J. Ocean Univ. Qingdao/Qingdao Haiyang Daxue Xuebao 24, 181-186.

Zhu, C.J., Lee, Y.K., Chao, T.M., 1997. Effects of temperature and growth phase on lipid and biochemical composition of *Isochrysis galbana* TK1. J. Appl. Phycol. 9, 451-457.

Chapter 2.

Do strain differences in microalgae alter their relative quality as a food for the rotifer *Brachionus plicatilis*?

2.1. Introduction

Over the past thirty years, rotifers have become a valuable live food for the aquaculture of larval fish and crustaceans (Ito, 1960; Sulkin and Epifanio, 1975; Rothbard, 1977; Lubzens, 1981; Lubzens et al., 1989). *Brachionus plicatilis* is a rotifer routinely used in marine aquaculture, being fed to more than 60 marine finfish and 18 species of crustaceans (James et al., 1983; Pillay, 1990; Lubzens et al., 1997; Abu-Rezq et al., 2002). However, *B. plicatilis* is useful only if it is nutritious (Watanabe et al., 1978; Lubzens, 1987; Fernandez Reiriz et al., 1993), is of an adequate size (Yufera, 1982), grows rapidly (Lubzens et al., 1989; Walz, 1995), and has a low production cost. All of these factors may be affected by the quality of the prey fed to the rotifer (Lubzens et al., 2001). In fact, studies indicate that rotifer production and quality are affected by different prey species (Theilacker and McMaster, 1971; Scott and Baynes, 1978). There has recently been a growing recognition that strains of protists can be as different as different species (Fu et al., 1990; 1991a; b; Bouaicha et al., 2001). However, there remains a lack of information on how prey-strains within a species affect rotifers.

The flagellate *Isochrysis galbana* has been widely used in commercial mariculture for rearing invertebrates and has been suggested to be a good food source for *B. plicatilis* (Scott and Baynes, 1978; Hoff and Snell, 1987; Planas and Estevez, 1989). It is also well known that there are many strains of *I. galbana*, and these can differ in their qualities (Wikfors and Patterson, 1994). For instance, strains may differ in their biochemical composition (Whyte, 1987; Brown et al., 1997; Valenzuela-Espinoza et al., 2002), temperature tolerance (Kain and Fogg, 1958; Thompson et al., 1992), and nutritional value to invertebrates (Brown, et al., 1997;

Okauchi, 1990). These qualities might affect rotifers. Moreover, results from similar studies on rotifers that were fed *I. galbana* of an unspecified strain often differ (Scott and Middleton, 1979; Lubzens et al., 1985); it may be that these differences are due to prey-strain differences, rather than unaccounted for experimental error.

This study investigates the growth and composition of different strains of *I galbana*, and compares them to one strain of another species, *Nannochloropsis* sp. Then, it assesses the effect of these differences on the growth and compositional parameters of *B. plicatilis*. The main aim was to assess if prey-strain differences exist and if these differences should be considered an important factor, affecting aquaculture practices. Thus, the focus of this work is practical in nature, rather than being physiological.

2.2. Material and Methods

2.2.1. Algal and rotifer stock cultures

Strains of *Isochrysis galbana* from: the Isle of Man (M), Tahiti (T), Greece (G), Denmark (D), and one strain of *Nannochloropsis* sp. (N) were obtained from the plankton culture collection at Port Erin Marine Laboratory, Isle of Man (Table 1). The flagellates were grown in 200 ml flat bottom flasks with f/2 medium (Guillard, 1975). All strains and species were maintained in semi-continuous culture, by transfers every two weeks, at 20 °C, on a 12:12 light: dark cycle, at an irradiance of 100 $\mu E\ m^{-2}s^{-1}$.

Table 1

Information of *Isochrysis galbana* strains and *Nannochloropsis* sp. used in this study. f/2 medium: (Guillard, 1975), Chlorella medium (Culture Centre of algae and protozoa, 1972) (CCAP).

	Nannochloropsis sp. (N)	*I. galbana* (D)	*I. galbana* (T)	*I. galbana* (M)	*I. galbana* (G)
CCAP strain number	CCAP 211/78		CCAP 927/14	CCAP 927/1	CCMP1323
Class	Eustigmatophyceae	Prymnesiophyceae	Prymnesiophyceae	Prymnesiophyceae	Prymnesiophyceae
Isolated by	Unknown	Unknown	Haines, K	Parke, M.	Parke, M.
Isolation date	Unknown	Unknown	1977	1/1938	1/1938
Collection site	Japan	Local Waters Denmark	Society Island, Tahiti	Port Erin, Isle of Man, UK	Port Erin, Isle of Man, UK
Ocean	Pacific	North Atlantic	South Pacific	Irish Sea	Irish Sea
Nearest continent	Asia	Europe	Asia	Europe	Europe
Obtained from	CCAP	Maine biological Laboratory of Helsingr	CCAP	CCAP	Greek aquaculture farm
Collection culture medium	f/2	f/2	f/2	f/2	Chlorella
Cell length, width (μm)	~3, 2.5	~4, 3	~3, 2.5	~5, 4	~5, 4
Strain synonyms	Japanese *Chlorella*	*I. galbana*	TISO	ISO, *I. galbana*	ISO, *I. galbana*

The rotifer *Brachionus plicatilis* was obtained from the Larval-Rearing Centre at Port Erin Marine Laboratory, where it was maintained at 25 °C. Rotifers were collected onto a 45 μm mesh and rinsed with filtered seawater (Whatman GF/C), to remove contaminants, prey from the original cultures, and to concentrate rotifers. Then the rotifers were resuspended in filtered seawater (32 ppt) and fed on a diet of *Nannochloropsis* sp. To ensure uniform food distribution and to prevent sedimentation, gentle aeration was provided. Light conditions were the same as those provided for the flagellates.

2.2.2. Algae growth characteristics experiments

The four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) were obtained from stock cultures. Three replicates of each algal strain/species were grown in 500 ml round bottom flasks in modified f/2 medium. All replicates were maintained in exponential growth phase by semi-continuous culturing on a continuous light cycle. An irradiance of 100 $\mu E\ m^{-2}s^{-1}$ was provided, which is considered optimal for flagellate growth rate (Brown et al., 1993; Molina Grima et al., 1994; Molina Grima et al., 1996). All cultures were maintained for ~10 generations; flasks were suspended in a controlled water bath at 25 ± 1°C and were gently agitated at least once a day.

Growth rate was measured by determining abundance daily, using a Model II Coulter Multisizer, and associated software V.4.1 equipped with a population accessory (Coulter Electronics Ltd., England). Growth rate was calculated as the slope of *ln* numbers of cells vs time in days.

Cell volume (V) was estimated from cell length (L) and width (W) measurements, assuming a prolate spheroid shape (see Fig 1 for illustration). The volume was estimated using the following equation:

$$V=4/3\ \pi\ (W/2)^2\ (L/2)$$

Measurements were made on ≥ 40 randomly chosen, active cells obtained in log phase from each flask. Cell were placed in a 10 ml settling chamber and measured using an inverted microscope (Zeiss Axiovert 135tv) equipped with a video camera (JVC model 3-CCD, 750 lines horizontal resolution), attached to a Pentium IIPC with image analysis software (Scion Image for Windows, Scion Corp., MD, USA).

Flagellate production ($\mu m^3\ d^{-1}$) was calculated by multiplying mean cell volume by growth rates (Montagnes and Weisse, 2000). For dry weight and biochemical analyses, samples were filtered onto Whatman GF/F glass fibre filters. For dry weight three replicates, one from each replicate culture, were filtered and washed with a solution of 0.5 M ammonium formate (Babarro et al., 2001); filters were dried at 60 °C for 12h before weighting. For biochemical analyses replicate filters were immediately frozen in liquid nitrogen and stored for < 1 year; biochemical methods are described below (see 2.4).

2.2.3. Rotifer growth characteristics experiments

2.2.3.1. Rotifer rearing culture

Rotifers were collected from the stock culture onto a 45 μm mesh and rinsed with filtered seawater. Five hundred rotifers were placed in 100 ml of algae culture at the concentration of ~ 6 x 10^4 cell ml^{-1}, this food concentration is sufficiently high to maintain maximum growth rate for rotifer following recommendations of Hansen

et al. (1997). To maintain rotifers in exponential growth, to maintain algae concentration, and to increase the volume of the cultures, 100 ml of the flagellate culture at (~ 6 x 10^4 cell ml^{-1}) were added every day. Rotifers were cultured on this diet for ~7 generations at 25 °C; generation time was calculated following the equation of Hirayama and Kusano (1972). Rotifer growth rate was measured by determining abundance daily, and calculated as the slope of *ln* numbers vs. time in days.

Forty active rotifers were randomly selected and examined to measure body volume and egg volume, using the image analysis system described above. The volume was calculated following the method of Yufera (1982). Rotifer body volume and egg volume assumed to be a prolate spheroid shape. For each rotifer and egg, two linear dimensions were measured (length (L) and width (W)) (see Fig 1 for illustration), and the volumes were estimated using the following equation:

$$V=4/3\ \pi\ (W/2)^2\ (L/2)$$

The remaining culture was rinsed with filtered seawater (Whatman GF/C) and collected onto a 45 μm mesh to remove prey, and to concentrate the rotifers. The concentrated rotifers were used to estimate dry weight and biochemical composition (see 2.4 below). For dry weight, the concentrated rotifers were collected in filters and gently washed with a solution of 0.5 M ammonium formate to eliminate salt; filters were dried at 60°C for 12h before weighting. Three data points of the dry weight data (T, M, G) were estimated from the average of these points at 20 and 30°C (determined in chapter 4).

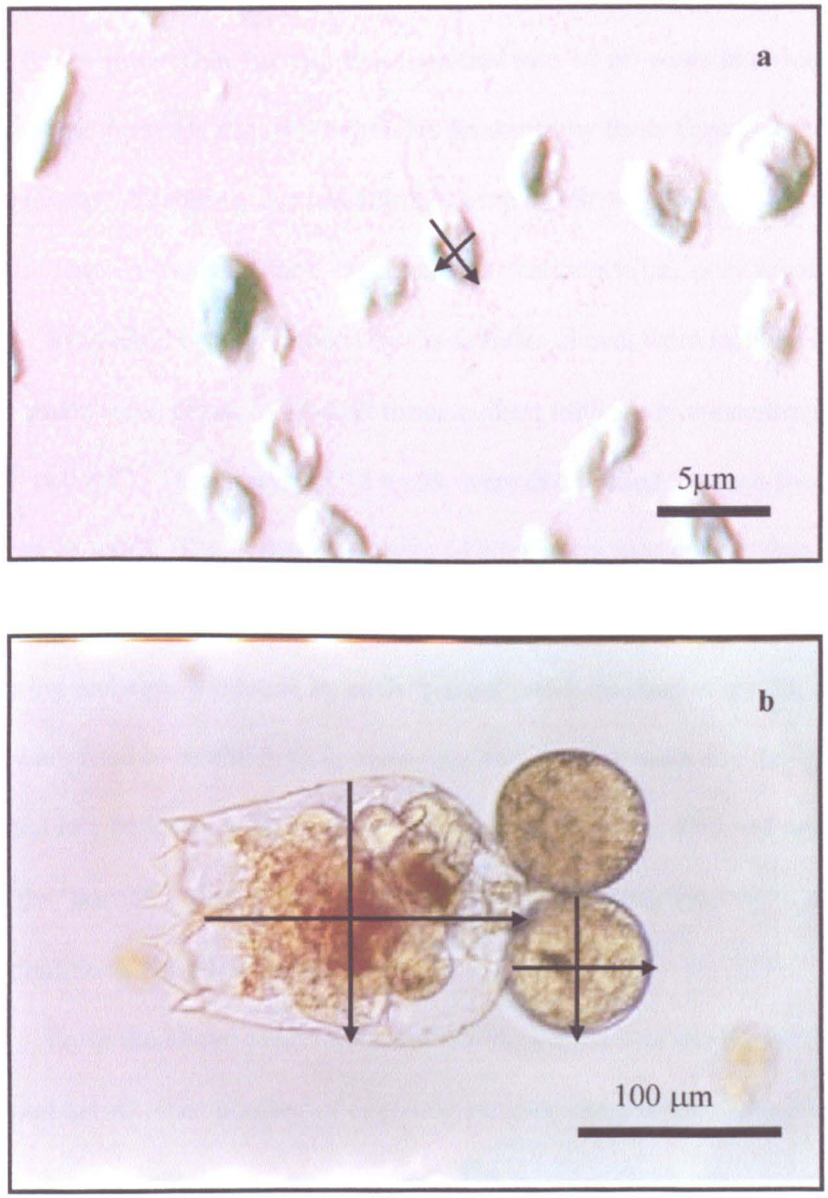

Fig1. **a.** Microalgae cell volume (μm^3) estimated assuming a prolate spheroid shape, from cell length (L) and width (W) measurements. **b.** For rotifer body volume (μm^3) and egg volume (μm^3) two linear dimensions were measured (length (L) and width (W)), assumed to be a prolate spheroid shape.

2.2.3.2. Rotifer individual culture

Parthenogenetic eggs, produced by amictic females, from the culture above (i.e., the different prey treatments), were collected in petri dishes. Offspring hatching from these eggs within one day were pipetted into 10 ml wells in tissue culture plates. The first offspring from females produced by these eggs were used for experiments. Therefore, the test animals were the first-laid eggs from offspring of amictic females, isolated from experimental treatments (i.e., prey strains/ species).

Individual eggs, produced by the females above, were isolated in 1 ml of the appropriate food-strain, in 12-well tissue culture trays; prey concentrations were ~ $6x10^4$ cell ml^{-1}. Three trays, of 12 wells, were established for each food-strain (i.e., 36 eggs in total). The average response of a tray was determined; thus n = 3.

Eggs hatched the following day, producing a "parent". The number of offspring and eggs produced by each "parent" were counted every 2h, for 12 h; these data were used to establish daily rates (see below). The next day the "parent" was isolated in a new tray with fresh culture medium and prey; attached eggs were left with the "parent". This process of evaluating the reproductive output of the "parent" was continued until the "parent" died.

From the above experiment, several measurements were made; these are outlined below. The number of eggs laid per day, per animal (fecundity), at different ages (i.e., different days) was estimated. For all cases there was an initial increase in fecundity followed by a decreased. The day of peak fecundity was recorded, and the rate of decline in fecundity (d^{-1}) was calculated as the slope of *ln* daily fecundity vs. time. Mortality rate of "parents" (d^{-1}) was also estimated as the slope of *ln* number of "parents" vs. time (starting when the first "parent" died); then the day to 50% survival was determined. Not all eggs produced by a "parent" hatched: the

percentage of egg hatching (hatched eggs %) was determined as the number of eggs in a tray, on a given day, that hatched divided by the total number of eggs produced on that day, multiplied by 100.

From the above analysis several parameters were also calculated. The different development stages were determined as outlined by Schmid-Araya and See (1991): duration of embryonic development (De), duration of post-embryonic development (Dj), duration of the interval between eggs (Di), duration of the reproductive period (Dr), duration of the post reproductive period (Dp), and duration of the life-span (Dl) (see Fig. 2 for further explanation on the determination of duration of different development stages). Furthermore, the average number of eggs laid by a female in her entire lifetime (net reproduction rate, R_0) was determined (Bosque et al., 2001; Bradford, 1976).

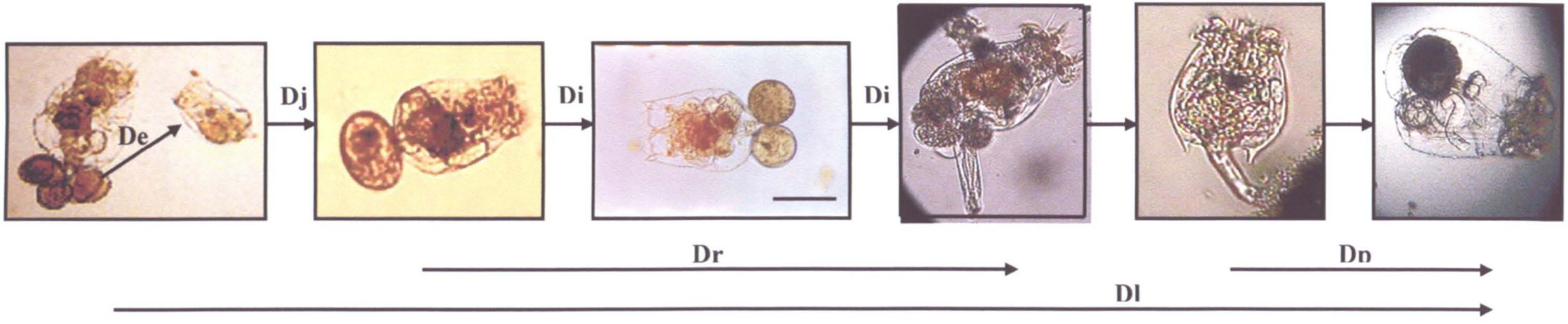

Fig. 2. The duration of different life phases of *Brachionus plicatilis*. De, duration of embryonic development (h); Dj, duration of post-embryonic development (h); Di, duration of the interval between egg-laying (h). Dr, duration of reproductive period (d); Dp, duration of post reproductive period (d); Dl, duration of life-span (d).

2.2.4. *Protein, carbohydrate, and lipid analysis for phytoplankton and rotifers*

Three replicates, one from each algal flask (see section 2.2 above), were collected for protein, carbohydrate, and lipid determination: 30 ml for protein, 50 ml for carbohydrate, and 100 ml for lipid. Cell concentrations were determined by Coulter counter and were typically ~10^6 cells ml^{-1}. As for the rotifer analysis, three replicate volumes of water, from separate flasks, each with ~$1x10^5$ organisms ml^{-1}, were sampled for chemical analysis. The same analytical methods were performed on both rotifers and algae.

Protein was quantified using the Bradford method (Bradford, 1976), after hydrolysis in NaOH 1N for 1h at 90 °C. Total carbohydrates were quantified as glucose by the phenol-sulfuric acid method (Kochert, 1978). Lipid was extracted following the method of Bligh and Dyer (1959), and quantified by the method of Pande et al. (1963). Calibration curves were made using: bovine albumin as the standard for proteins, D-glucose for carbohydrates, and tripalmitin for lipids.

2.2.5. *Statistical analyses*

Statistical analysis used STATISTICA version 5.0. Homogeneity of variance was tested by Cochran's test (Underwood, 1997). Data always passed tests for normality. One-way analyses of variance (ANOVA) were used to examine the differences between algal strains/species. Where necessary, data were transformed to meet the assumption of the parametric statistics. Further analysis by Tukey HSD test indicated where significant differences occurred. When transformation were not sufficient, non-parametric data were analysed by Kruskal-Wallis ANOVA, and the

Dunn non-parametric multiple comparison test was used to make pair-wise comparisons between average ranks (Zar, 1999). Regression was performed to determine biochemical composition relationships between prey-diets and rotifers and tested using ANOVA. In all analyses, the statistical significance was at $P<0.05$.

2.3. Results

2.3.1. Algal growth characteristics

Growth rate, cell volumes, production, and dry weight. Significant differences occurred in growth rates, cell volume, production, and dry weight between strains of *I. galbana* and *Nannochloropsis* sp. (Fig. 3, Table 2). For cell volume and production there were significant differences between all paired comparisons (Table 2).

Protein, carbohydrate, and lipid. Flagellate protein and carbohydrate content varied significantly not only on a per cell basis but also μm^{-3} between *I. galbana* strains and *Nannochloropsis* sp. (Fig. 4a, b, c, d, Table 2). Total lipid content also differed between *I. galbana* strains and *Nannochloropsis* sp. per cell, but there was no variation in lipid content μm^{-3} (Fig. 4e, f, Table 2). The variation between the strains was some times larger than differences between species, and in general, *I. galbana* strain M had the highest protein and lipid content (Fig. 2).

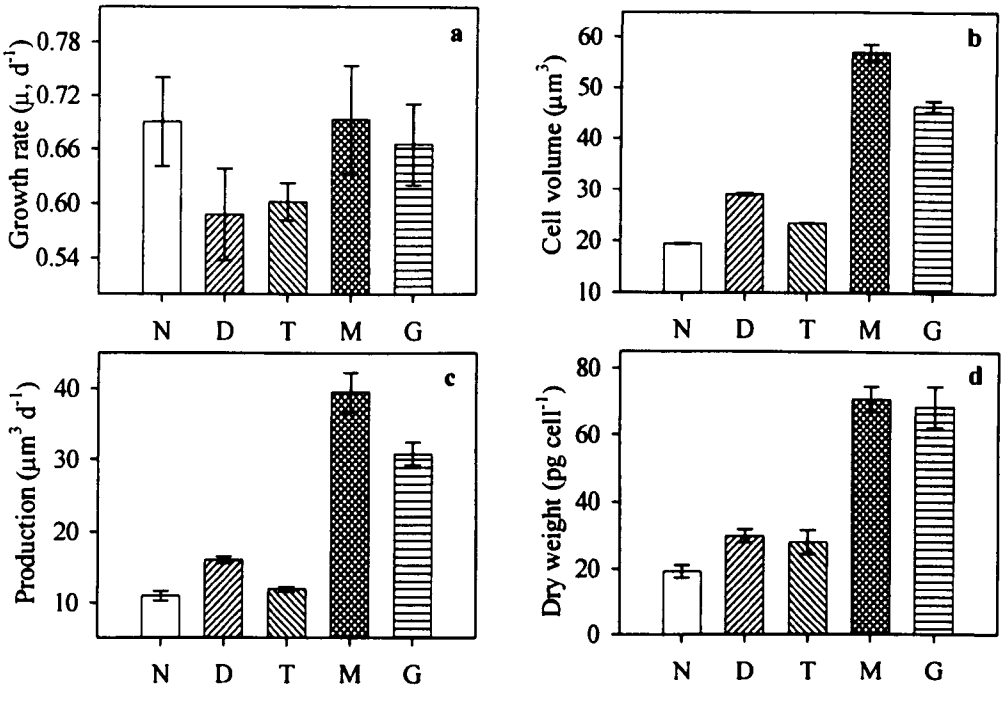

Fig. 3. Growth and production parameters of algal strains, **a.** growth rate (μ, d^{-1}), **b.** cell volume (μm^3), **c.** production ($\mu m^3 d^{-1}$), and **d.** dry weight (pg $cell^{-1}$) of four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 for strain designations). Bars are one standard error ±.

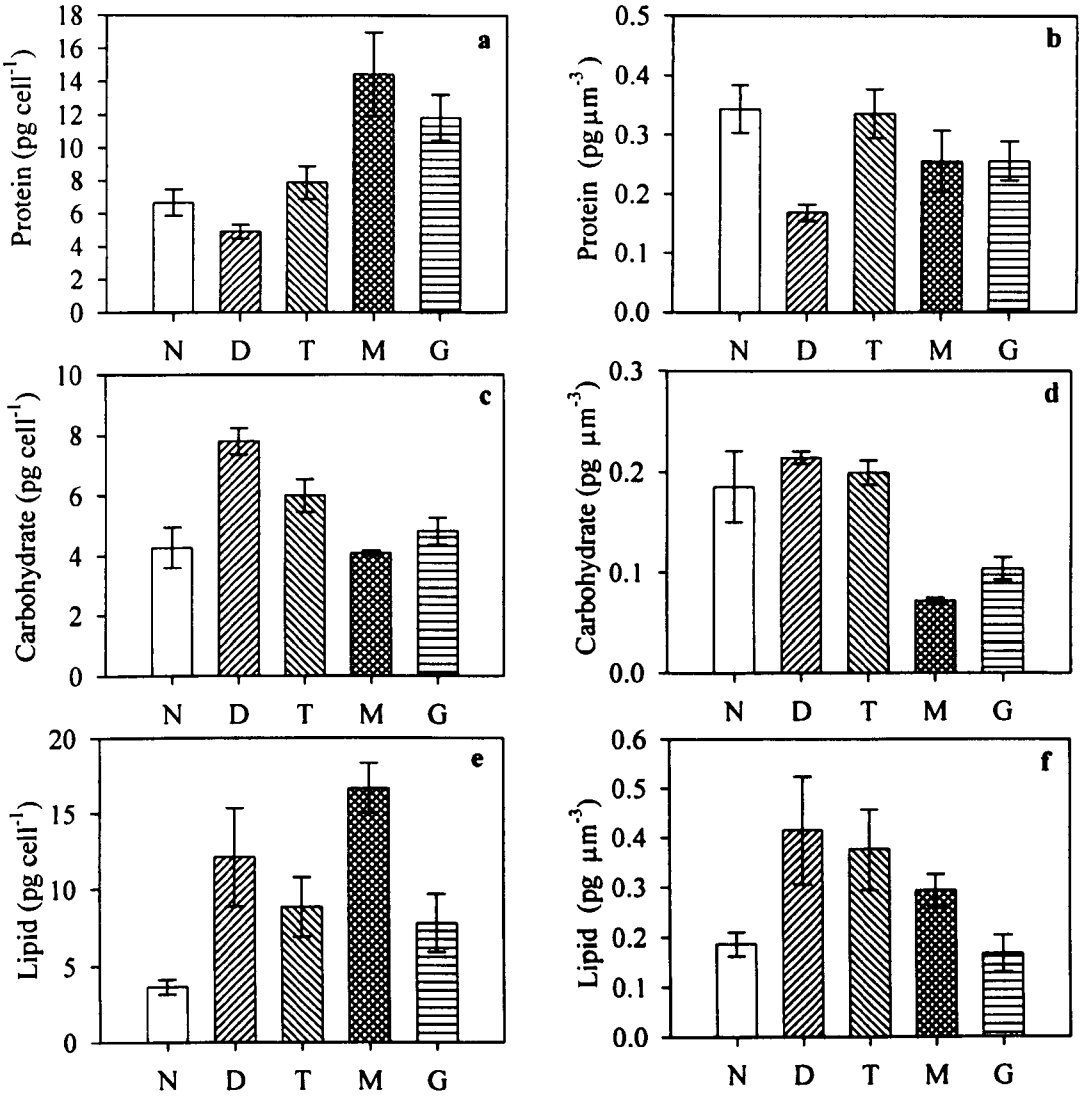

Fig. 4. Biochemical content of algal strains, **a.** protein (pg cell^{-1}), **b.** protein (pg µm^{-3}), **c.** carbohydrate (pg cell^{-1}), **d.** carbohydrate (pg µm^{-3}), **e.** total lipid (pg cell^{-1}), and **f.** total lipid (pg µm^{-3}), of four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 for strain designations). Bars are one standard error ±.

Table 2.

One-way ANOVA and Tukey HSD test for the effect of strain on algal parameters. Strains designation (N, D, T, M, and G) are described in Table 1. Tukey's test data are ordered from lowest (left) to highest (right); underscoring indicates where there were no significant differences at $p = 0.05$. For all tests, degrees of freedom were 4 for the parameter of interest, 10 for the residuals, and 14 for the total. Factors of cell volume and carbohydrate (pg μm^{-3}) were Log transformed.

Factors	ANOVA			Tukey's test
	MS	*F*	*P-level*	
Growth rate (μ, d^{-1})	0.029	8.794	0.003	T D N G M
Residual	0.002			
Cell volume (μm^3)	0.116	718.408	$3X10^{-12}$	N T D G M
Residual	0.000			
Production ($\mu m^3 d^{-1}$)	453.337	43.271	$2.8X10^{-6}$	N T D G M
Residual	10.477			
Dry weight (pg $cell^{-1}$)	1799.382	41.546	$3.3X10^{-6}$	N T D G M
Residual	43.310			
Protein (pg $cell^{-1}$)	45.731	7.5.536	0.005	D N T G M
Residual	6.093			
Carbohydrate (pg $cell^{-1}$)	7.114	10.263	0.002	N M G T D
Residual	0.693			
Lipid (pg $cell^{-1}$)	71.587	5.739	0.012	N G T D M
Residual	12.474			
Protein (pg μm^{-3})	0.015	3.513	0.049	D M G T N
Residual	0.004			
Carbohydrate (pg μm^{-3})	0.127	19.009	0.0001	M G N T D
Residual	0.007			
Lipid (pg μm^{-3})	0.037	2.836	0.083	D G M T N
Residual	0.013			

2.3.2. *Rotifer growth characteristics*

Net reproduction rate (R_o), hatched eggs %, and egg volume. Females that were reared on different algal strains yielded different net reproduction rates (R_o) (Fig. 5a, Table 3). Hatched eggs % differed between *I. galbana* T strain-fed rotifers and other tested rotifers (Fig. 5b, Table 3). However, no differences occurred between other reared rotifers, which were ~98 % successful (Fig. 5b). Diet strain did not affect rotifers egg volume (Table 3); averaged volume was ~5 x $10^5 \mu m^3$ (Fig. 5c).

Rotifer volume, dry weight, and growth rate. Significant differences occurred between rotifer body volumes between treatments (Fig. 5c, Table 3). Prey treatment altered rotifer dry weight (Fig. 5e, Table 3). Growth rate differed significantly only between rotifers fed on *I. galbana* D strain and *Nannochloropsis* sp. (Fig. 5f, Table 3).

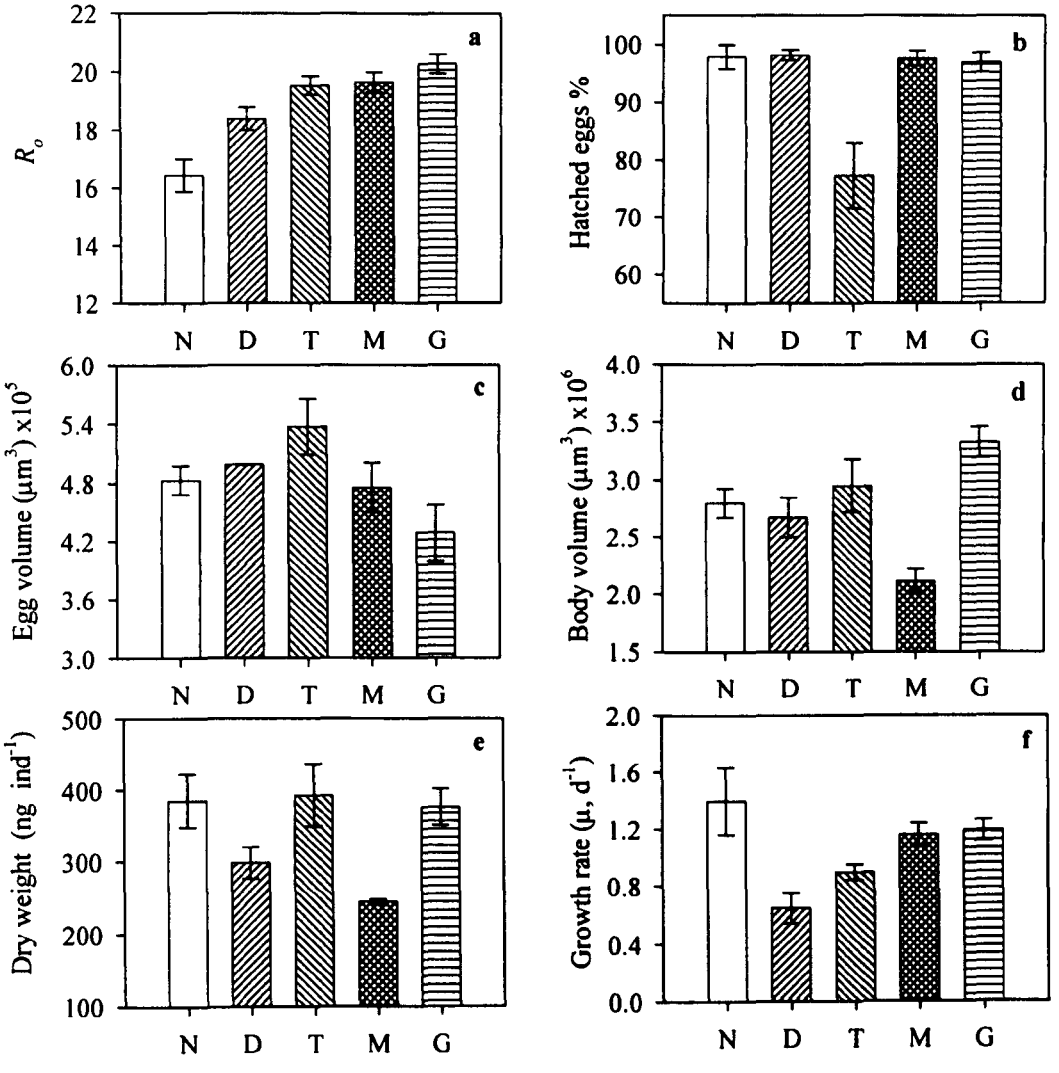

Fig. 5. Rotifer growth parameters **a.** number of eggs per life time (R_o), **b.** hatched eggs %, **c.** egg volume (µm^3), **d.** body volume (µm^3), **e.** dry weight (ng ind^{-1}), and **f.** growth rate (µ, d^{-1}), of the rotifer *Brachionus plicatilis* cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 for strain designations). Bars are one standard error ±.

Life history. In general, there were differences in the duration of life phases between the rotifers fed each of the four *I. galbana* strains and *Nannochloropsis* sp. (Fig. 6). Differences occurred in all phases of the life histories: duration of embryonic development (De), duration of post-embryonic development (Dj), duration of the interval between eggs (Di), duration of reproductive period (Dr), duration of post reproductive period (Dp), and duration of life-span (Dl) (Fig. 6 a, b, Table 3, 4).

From the observed life histories, there were several important points to note. Overall, the duration of De, Dj, and Di were short (hours) compared to those of Dr, Dp, and Dl (days) (Fig. 6 a, b). Furthermore, the duration of Dr, Dp, and Dl were sufficiently long to have a marked affect on rotifer population growth, although the differences in De, Dj, and Di would still have an effect. In general, rotifers offered *I. galbana* strain G had the longest reproductive period (Dr), senile time (Dp), and life-span (Dl) (Fig. 6, Table 3).

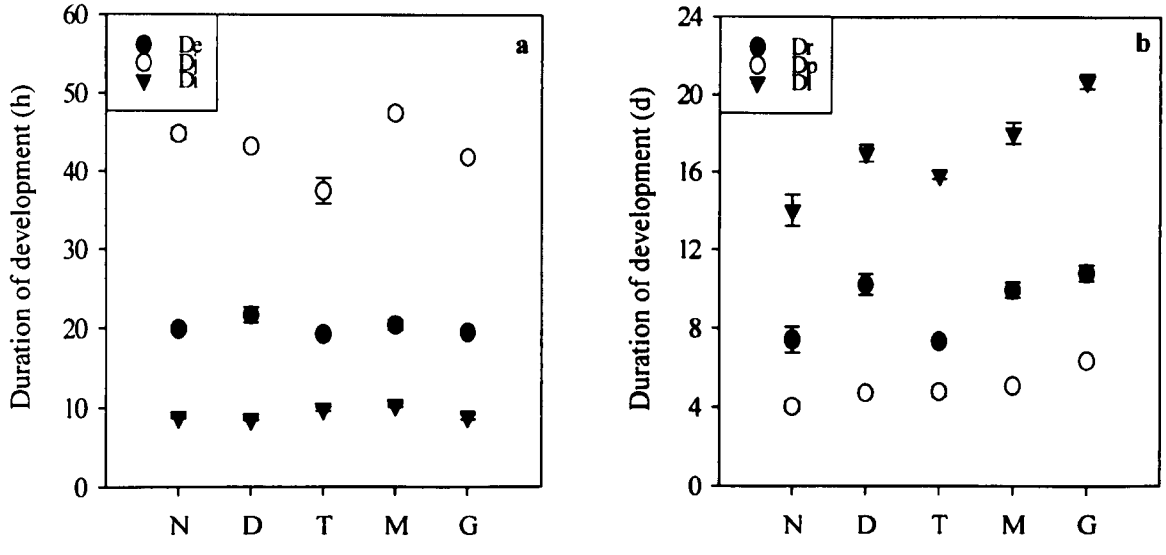

Fig. 6. The duration of different life phases of *Brachionus plicatilis* cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 for strain designations). **a.** Duration of development (h); De (•), duration of embryonic development; Dj (○), duration of post- embryonic development; Di (▼), duration of the interval between egg-laying. **b.** Duration of development (d); Dr (•), duration of reproductive period; Dp (○), duration of post reproductive period; Dl (▼), duration of life-span. Bars are one standard error ±.

Survivorship and fecundity. In general, the rotifers had similar survival (Fig. 5) and fecundity patterns (Fig. 6). No significant differences were found in the following: day to 50% survival (Fig. 5a), mortality rate (d^{-1}) (Fig. 5b), fecundity decline rate (d^{-1}) (Fig. 6a), and the day to peak fecundity (Fig. 6c, Table 3). However, rotifers fed *I. galbana* strain (T) had a higher number of eggs at the fecundity peak (Fig. 6b, Table 3).

Rotifer biochemical composition. Rotifers fed different algal strains had different biochemical compositions (Fig. 9, Table 3). Protein and total lipid content differed between rotifers fed different strains on both a per-individual and μm^{-3} basis. In contrast, carbohydrate content differed on a per-individual basis but not μm^{-3} (Table 3). Generally, rotifers fed *I. galbana* (M) had lower biochemical content while rotifers fed *I. galbana* (T) strain had higher carbohydrate and lipid content.

No relationship existed between flagellate and rotifer biochemical composition, i.e., for protein, carbohydrate, or total lipids in terms of dry weight and μm^{-3} ($P>0.05$ in all cases) (Fig. 10). The biochemical composition ratios of algal to rotifer were 2:1 for protein, 10:1 for carbohydrate and lipid μm^{-3} (Fig. 10a, c, e); and 1:2 for protein, 2:1 carbohydrate and lipid %DW (Fig. 10b, d, f). This study acknowledged the shortfall in rotifer compositions in terms of %DW, which might be due to ash content.

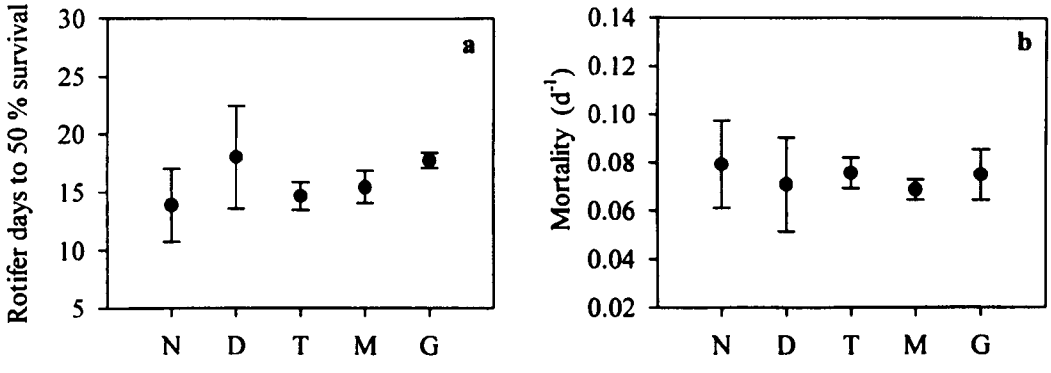

Fig. 7. The survival of rotifer *Brachionus plicatilis* cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 for strain designations). **a.** Time intervals to 50% survival and **b.** mortality rate (d^{-1}). Bars are one standard error ±.

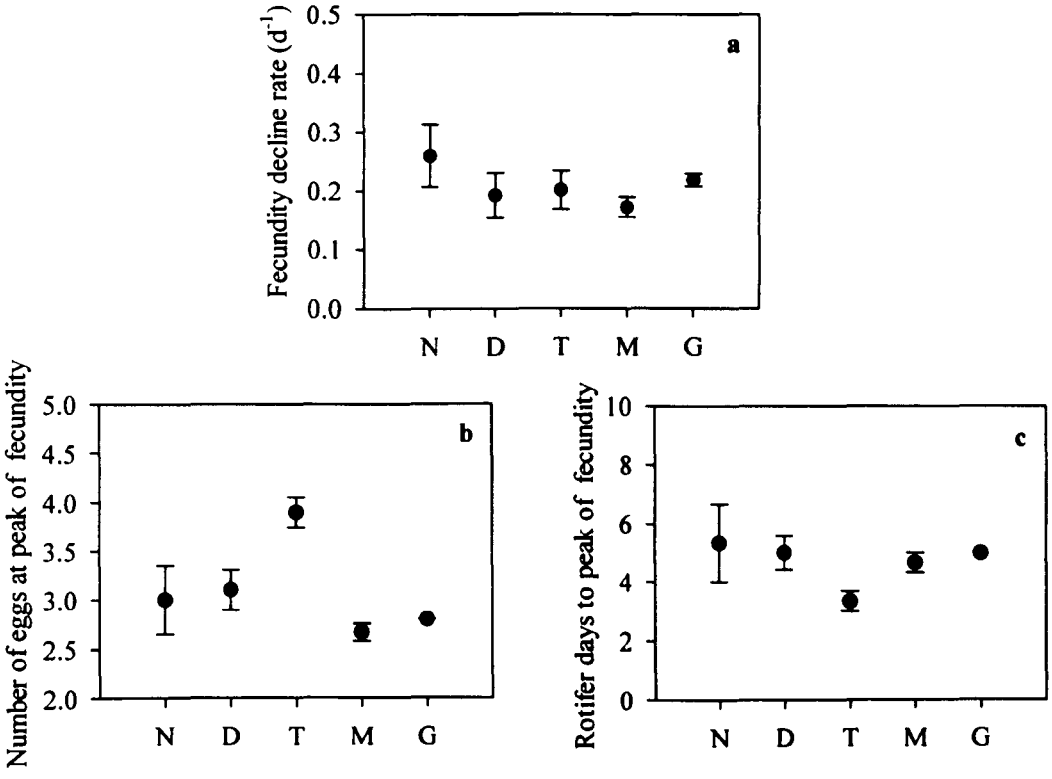

Fig. 8. Fecundity of the rotifer *Brachionus plicatilis* cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N)). **a.** Fecundity decline rate (d^{-1}), **b.** number of eggs at peak of fecundity, and **c.** days to peak of fecundity. Bars are one standard error ±.

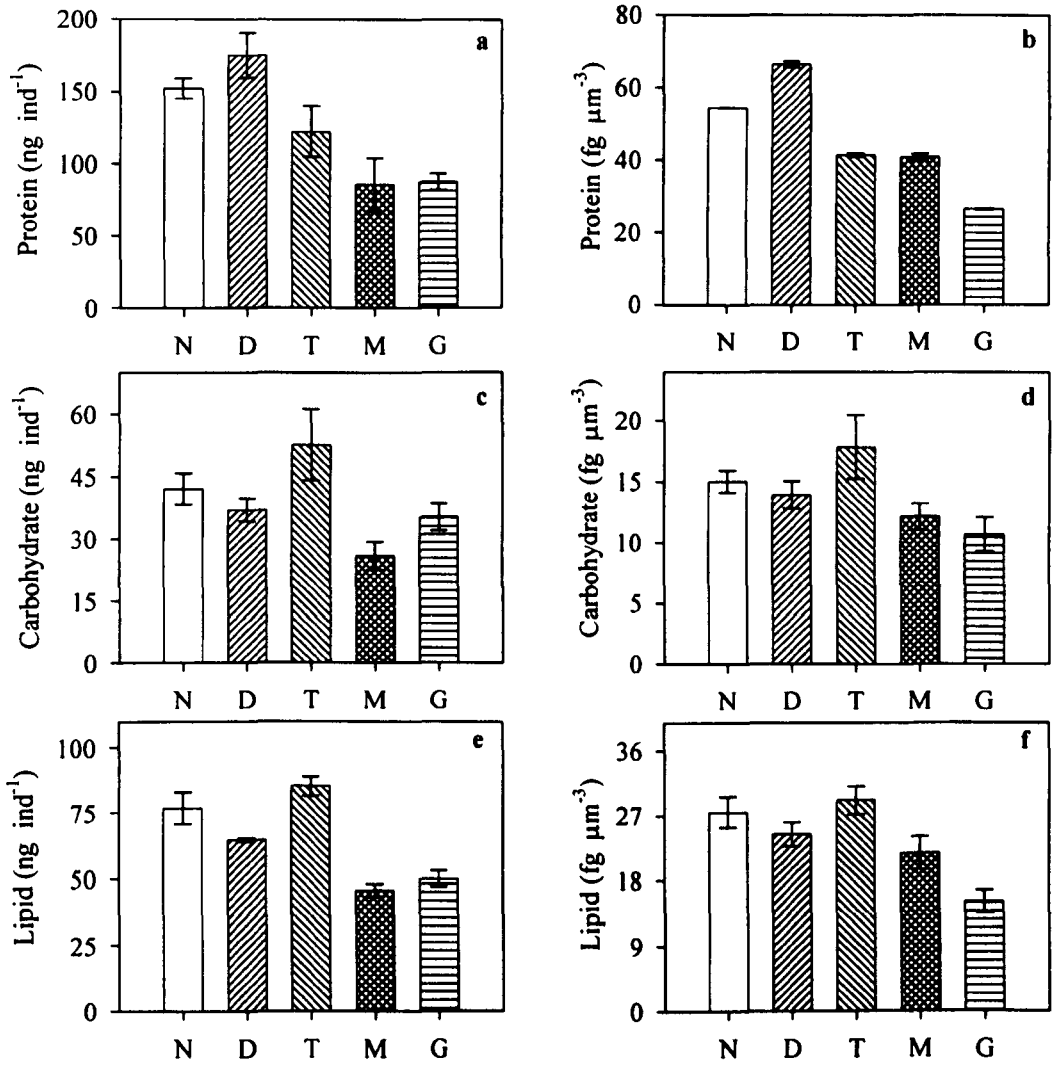

Fig. 9. Biochemical content of *Brachionus plicatilis*, **a.** protein (ng ind^{-1}), **b.** protein (fg μm^{-3}), **c.** carbohydrate (ng ind^{-1}), **d.** carbohydrate (fg μm^{-3}), **e.** total lipid (ng ind^{-1}), and **f.** total lipid (fg μm^{-3}) cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 for strain designations). Bars are one standard error ±.

Table 3

ANOVA and Tukey test for the indicated strain factor diet on rotifer growth parameters. Strains designation (N, D, T, M, and G) are described in Table 1. Tukey's test data are ordered from lowest (left) to highest (right); underscoring indicates where there were no significant differences at $p = 0.05$. For all tests degrees of freedom were 4 for the parameter of interest, 10 for the residuals, and 14 for the total. Factors of egg volume and growth rate have been Log transformed.

Factors	ANOVA			Tukey's test
	MS	*F*	*P-level*	
Net reproduction rate (R_o)	6.840	14.272	0.0003	N D T M G
Residual	0.481			
Hatched eggs %	249.44	9.885	0.002	T G M D N
Residual	25.234			
Egg volume	0.2	1.7	0.2	D G M T N
Residual	0.1			
Body volume	5.9×10^{11}	7.75	0.004	M D N T G
Residual	76.7			
Dry weight	12812.17	4.856	0.019	M D G N T
Residual	2638.667			
Growth rate (μ, d^{-1})	0.254	5.161	0.016	D T M G N
Residual	0.050			
Duration of interval between eggs (Di)	1.785	14.554	0.0003	N D G T M
Residual	0.123			
Duration of reproductive period (Dr)	8.005	12.776	0.0006	N T D M G
Residual	0.627			
Duration of post reproductive period (Dp)	2.192	21.562	6×10^{-6}	N D T M G
Residual	0.102			
Duration of life-span (Dl)	18.545	23.253	4×10^{-5}	N T D M G
Residual	0.798			
Days to 50%survival	26	1.7	0.2	N D T M G
Residual	15.5			
Total				
Mortality (d^{-1})	5.2×10^{-5}	0.099	0.98	N D T M G
Residual	0.001			

Fecundity decline rate (d^{-1})	0.2	2.8	0.1	N D T M G
Residual	0.1			
Day of peak of fecundity	0.446	2.62	0.09	N D T M G
Residual	0.17			
Number of eggs at peak of fecundity	0.696	5.866	0.011	M G N D T
Residual	0.119			
Protein (pg ind^{-1})	4657.695	7.997	0.003	M G T N D
Residual	582.519			
Carbohydrate (pg ind^{-1})	289.449	4.13	0.031	M G D N T
Residual	70.086			
Lipid (pg ind^{-1})	869.064	21.656	0.0001	G M D N T
Residual	40.13			
Carbohydrate (pg μm^{-3})	$2.3 X10^{-11}$	3.124	0.06	G M D N T
Residual	$7.2 X10^{-12}$			
Lipid (pg μm^{-3})	$9.2 X10^{-11}$	8.182	$6.5 X10^{-5}$	G M D N T
Residual	$1.1 X10^{-11}$			

Table 4

Non parametric Kruskal-Wallis ANOVA and Dunn test for the indicated strain factor diet on rotifer growth parameters. Strains designation (N, D, T, M, and G) are described in Table 1. Dunn test data are ordered from lowest (left) to highest (right); underscoring indicates where there were no significant differences at $p = 0.05$.

Factors	ANOVA			Dunn test
	H	*df*	*p-level*	
Protein (pg μm^{-3})	11.70	4	0.015	G M T N D
Duration of embryonic development (De)	10.117	4	0.05	T M D G N
Duration of post-embryonic development (Dj)	13.5	4	0.009	T G D N M

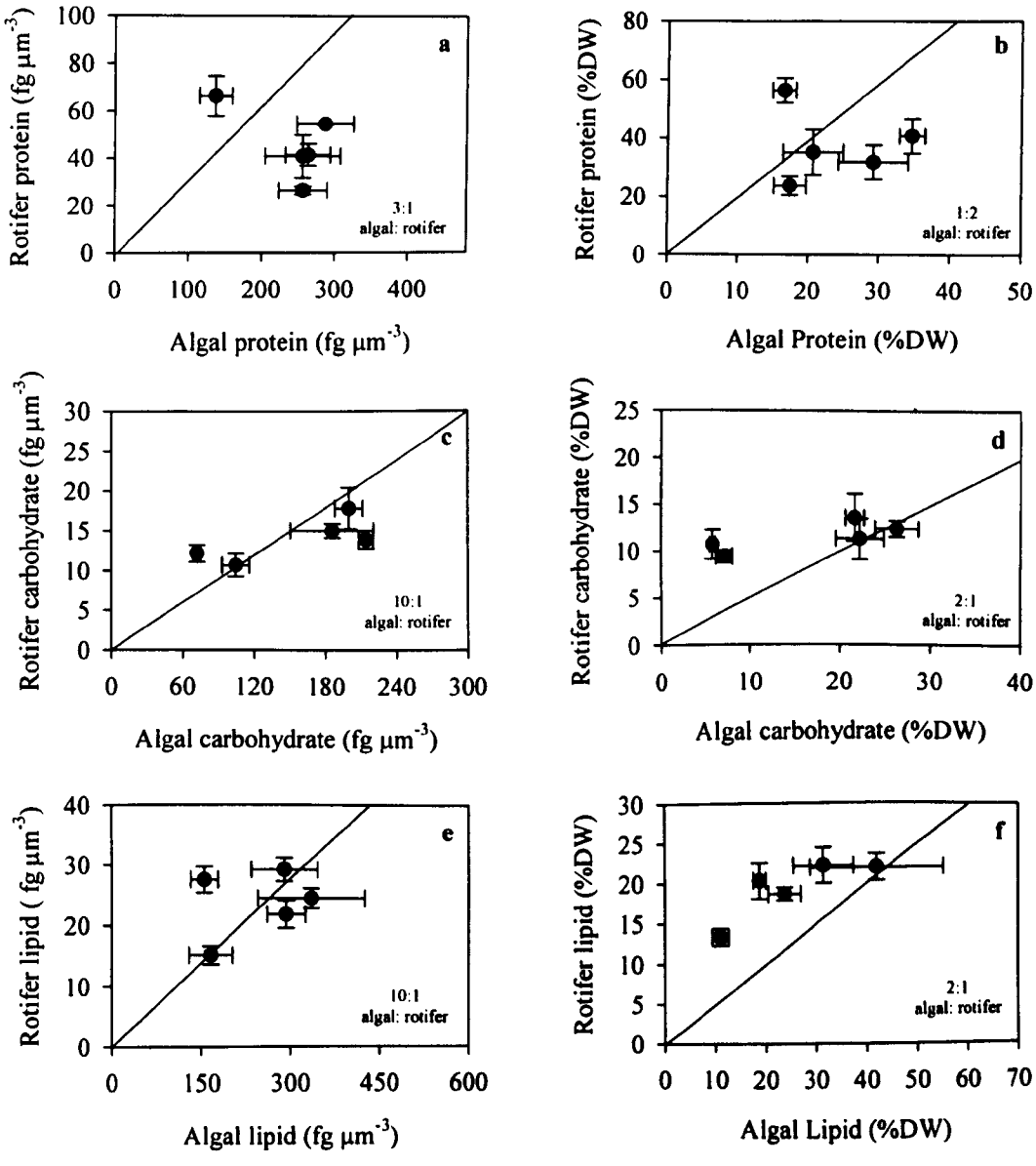

Fig. 10. The **a.** protein (pg μm^{-3}), **b.** protein (%DW), **c.** carbohydrate (fg μm^{-3}), **d.** carbohydrate (%DW), **e.** total lipid (fg μm^{-3}), and **f.** total lipid (%DW) relationship between rotifer *Brachionus plicatilis* and their algal prey guide lines show the ratio of biochemical compositions (algal: rotifer) indicated. Bars are one standard error ±.

Table 5

Some *Isochrysis galbana* strains and *Nannochloropsis* sp. used to determine growth and biochemical compositions parameters. Biochemical compositions, expressed as % of organic weight (%OW) and (pg cell^{-1}). Results by other authors are given for comparison. ID: illumination density; L/D: light/dark in *x*:*y* h; M: medium; T: temperature in °C; -: not stated in the original paper. T-ISO: *Isochrysis galbana* Tahiti strain.

ID	Author	Observations: ID, L/D, M, T	Growth rate (μ,d^{-1})	Cell size (μm^3)	Cell dry weight (pg)	Proteins		Carbohydrates		Lipids	
						(%OW)	(pg cell^{-1})	(%OW)	(pg cell^{-1})	(%OW)	(pg cell^{-1})
I. galbana	(Kain and Fogg, 1958)	~9000 lux, 24:0, -, 20	-	31	-	-	-	-	-	-	-
	(Dortch and Conway, 1984)	~350 µE m^{-2}s^{-1}, 24:0, f/2, 20	1.73	-	-	-	-	-	-	-	-
	(Ben-Amotz et al., 1985)	18 Wm^{-2}, 24:0, -, 25	1.6	-	-	37	-	11.2	7.1	-	-
	(Fabregas et al., 1986)	-,12:12, -, 15	-	-	-	-	5.34	-	2.29	-	-
	(Whyte, 1987)	1000 lux, 24:0, f/2, 20	-	-	-	28.06	-	-	-	21.16	-
	(Fernandez-Reiriz et al., 1989)	60 µE m^{-1} s^{-1}, 24:0,- , 20	-	-	23.4	39.97	9.35	15.21	3.45	28.60	5.28
	(Sukenik and Wahnon, 1991)	~100 µmol^{-2}s^{-1}, 24:0, f/2, 25	0.8	-	-	-	7.5	-	3.5	-	6
	(Nelson et al., 1992)	-, 14:10, f/2, 30	-	94.3	-	-	7.20	-	3.42	-	3.10
	(Zhu et al., 1997)	200 µmol^{-2}s^{-1}, 24:0, -, 15	-	-	-	32	-	28	-	-	-
	(Fidalgo et al., 1998)	115 µmol^{-2}s^{-1}, 12:12, -, 18	-	-	47.5	40.19	-	7.65	-	21.87	-
	(Renaud et al., 1999)	80 µmol^{-2}s^{-1}, 24:0, f/2, 25	0.47	-	32.1	29.5	-	15.5	-	23.4	-
	(Montagnes and Franklin, 2001)	50µmol^{-2}s^{-1}, 14:10, f/2, 25	0.68	70	-	-	-	-	-	-	-
	(Satoh et al., 2002)	100 µmol^{-1} s^{-1}, 12:12, f/2, 16		69.1	-	-	-	-	-	-	-
	This study (M)	100 µmol^{-2}s^{-1}, 24:0, f/2, 25	0.69	57	70.9	20	14.4	5.8	4.1	23.8	16.7

Table 5 continued

T-ISO	(Pillsbury, 1985)	-, 14:10, -, 26	0.87	-	-	41	-	18	-	23	-
	(Whyte, 1987)	1000 lux, 24:0, f/2, 20	-	-	-	33.44	-	-	-	25.06	-
	(Volkman et al., 1989)	80 μE $m^{-2}s^{-1}$, 12:12, f/2, 20	-	91	-	-	-	-	-	-	-
	(Laing et al., 1990)	Method not stated in the original paper	-	-	24.47	-	-	20.10	-	20.6	-
	(Thompson et al., 1991)	225 μmol $^{-2}s^{-1}$, 24:0, -, 17C°	1.39	50.2	-	-	-	-	-	-	-
	(Nelson et al., 1992)	-, 14:10 L:D, f/2, 30	1.45	56.3	-	-	5.97	-	2.81	-	4.51
	(Thompson et al., 1992	220 μmol $^{-2}s^{-1}$, 24:0, -, 10	0.52	38	-	-	6	-	1.8	-	1.8
	(Brown et al., 1993)	100 μE $m^{-2}s^{-1}$, 12:12, f/2, 25	-	-	45	27	12	8.5	3.7	17.5	7.8
	(Reitan et al., 1994)	~90 μmol $^{-2}s^{-1}$, 24:0, f/2, 25	0.54	-	-	-	-	-	-	-	-
	(Wikfors et al., 1996)	Method not stated in the original paper	-	51.3	17.1	-	11.4	-	0.042	-	3.69
	(Babarro et al., 2001)	100 μmol $^{-2}s^{-1}$, 24:0, f/2, 22	-	-	40.17	-	13.60	-	6.15	-	9.65
	(Renaud et al., 2002)	100 μmol $^{-2}s^{-1}$, 12:12, f/2, 30	0.89	-	23.1	45.3	-	12.4	-	21.2	-
	(Valenzuela-Espinoza et al., 2002)	72 μmol $^{-2}s^{-1}$, 24:0, f/2, 20	-	-	-	-	6	-	8	-	6
	This study (T)	100 μmol $^{-2}s^{-1}$, 24:0, f/2, 25	0.57	30	28	29.2	7.9	21.7	6	31.6	8.8
Nannochloropsis	(James et al., 1989)	140 μE m^{-1} s^{-1}, 24:0, -, 25	0.11	52	-	-	-	-	-	-	-
sp.	(Sukenik and Carmeli, 1989)	550 μmol $^{-2}s^{-1}$, 24:0, f/2, 25	0.64	10.5	-	-	1.40	-	1.40	-	3.04
	(Sukenik et al., 1990)	290 μmol $^{-2}s^{-1}$, 12:12, f/2, 25	-	10	-	-	2	-	2	-	2.2
	(Sukenik et al., 1993)	35 μmol $^{-2}s^{-1}$, 24:0, f/2, 25	-	-	-	-	4.20	-	4.20	-	2.62
	(Volkman et al., 1993)	~80 μE m^{-1} s^{-1}, 12:12, f/2, 27	-	-	5.3	-	1.1	-	1.1	-	0.7
	This study (N)	100 μmol $^{-2}s^{-1}$, 24:0, f/2, 25	0.68	23.6	19.2	34.7	6.7	22.3	4.3	18	3.6

2.4. Discussion

Isochrysis galbana is among the most widely-used phytoplankton species in mariculture (Wikfors and Ohno, 2001). Aquaculturists might consider all strains of a species to be identical. However, a literature review of *I. galbana* strains indicated differences in their growth and biochemical composition (Wikfors and Patterson, 1994), and these differences may affect their energetic and nutritional value as food for rotifers (Reitan et al., 1997). However, from earlier work it was unclear if strain differences are real or artefacts resulting from different experimental conditions. This study indicated that there are distinct *I. galbana* strain-differences, in terms of growth and biochemical parameters. The study then assessed some subsequent effects of these strains on rotifer growth, development, and biochemical composition. These differences are discussed below.

2.4.1. Algal strains

There is a growing awareness of differences between isolated strains of phytoplankton (Bouaicha et al., 2001). The recognition of such differences, in fact, is not new to aquaculturists, as several taxa have been recognised to have strain-specific responses, e.g., rotifers (Snell and Carrillo, 1984) and phytoplankton (Lopez Alonso et al., 1992). Differences in a variety of parameters occur between studies of *I. galbana* (Table 5). For example, *I. galbana* cell volume may differ by 25%, and growth rate may vary by 27% (Table 5). Strain-differences also occur for dry weight, and biochemical composition (Table 5). Even fatty acid composition exhibits marked differences (e.g., Scott and

Middleton, 1979; Lubzens, 1985). These observations raise the question: are differences due to experimental study conditions or inherent strain differences?

Microalgae are easily affected by different culturing conditions (Wikfors and Ohno, 2001). Conditions such as culture medium composition, temperature, and light intensity affect both flagellate growth and biochemical composition, and these could induce the differences indicated in Table 5. Alternatively, maintenance of long-term cultures may leave a genotypic/phenotypic mark (Falconer, 1982). Therefore, to assess if strains differ phenotypically under identical conditions this study examined several strains of *I. galbana* and compared them to one strain of *Nannochloropsis* sp. Furthermore, two strains were used that have the same history of isolation (*I. galbana* M and G strains), but were grown in different culture media and at different locations for almost a decade before the present experiment (Table 1).

The comparison between M and G strains indicated that although they did not differ in several growth and biochemical composition parameters, they did differ in cell volume and production estimates. However, those differences were not as large as those between other isolated strains (Fig. 3, 4, Table 2).

In fact, the variations between different isolated strains observed here, under identical conditions, were as large as between other studies (Table 5). Therefore, I suggest that the difference observed between published studies could be attributed to different isolates of *I. galbana*, rather than simply to experimental conditions. Such differences could be caused by the genetic variation that occurs between isolates of *I. galbana* (Lopez Alonso et al., 1994).

In this study *Nannochloropsis* sp. was used as an "out-group" to assess if strain differences could be as large as those between species. The differences between *I. galbana* strains were often as high as between the two species (Fig. 3, 4, Table 2). For example,

similar growth rates were observed for *Nannochloropsis* sp. and *I. galbana* strains T, and D, but there was a significant difference between them and *I. galbana* strain M (Fig. 3).

As *Isochrysis galbana* is one of the most commonly used species in culture (Wikfors and Ohno, 2001), differences between *I. galbana* strains could potentially affect aquaculture applications. Industry is interested in producing large quantities of a high-quality product at a minimum cost in short periods (Abu-Rezq et al., 1997; Abu-Rezq et al., 1999; Cheng-Wu et al., 2001); such inexpensive production could be achieved by selecting optimal strains. Furthermore, strains that vary in cell size and biochemical composition may also differ in their benefits to zooplankton and marine larvae (Vadstein et al., 1993). Thus, high nutritional quality is a requirement of many live-feed culturists (Abu-Rezeq and James, 1985; Sukenik et al., 1993; Albentosa et al., 1996). The data obtained in this study indicate that strain differences in the size, growth rates, and biochemical composition may affect their suitability for aquaculture.

The aim of this work was not to scan all *I. galbana* isolates or strains, but to assess if key differences exist between several commonly used strains in mariculture. However, from this limited survey, *I. galbana* strain M had a higher growth rate, cell volumes, production, dry weight, and in general, higher protein and lipids content than other strains (Fig. 3, 4, Table 2). Therefore, of the strains used, this one might be the preferred strain in terms of speed and high quality production. Having established differences between strains, the second step of this study was to assess the impacts of these prey-differences on rotifer quality.

2.4.2. Rotifer growth characteristics

Evaluation of rotifer growth parameters is important in many larval hatcheries, as rotifers need to have predictable productivity to ensure a reliable daily supply to the larvae (Navarro and Yufera, 1998). The present work indicates that feeding rotifers different algal strains has no or little affect on most growth parameters, and where significant differences occurred, they would have a minor impact on rotifer bulk production.

It was found that rotifer net reproduction rate, percentage of eggs hatched, body volume, dry weight, and different stages of rotifer life history were all affected by the food strain (Fig. 5, 6, Table 3, 4). These differences may be caused by the variation between food strains. However, such differences were generally minor, compared with differences that can be caused by several major factors such as hormones (Gallardo et al., 1997; 1999; 2000), ambient temperature (Hirayama and Kusano, 1972; Bosque et al., 2001), salinity (Bosque et al., 2001), food concentration (Schmid-Araya and See, 1991), rotifer population density (Carmona et al., 1994), and different algal prey species (Hirayama et al., 1979; Snell et al., 1983; Planas and Estevez, 1989). Thus, strain differences may not require such careful monitoring as other factors.

Nevertheless, some differences between rotifers fed different strains may still have an impact on their culture and could improve their ultimate productivity. Productive culture may be achieved by rearing rotifers with a long reproductive period sufficient to produce high quality eggs that hatch in large quantities. Cultures of rotifers fed on *I. galbana* strain G had all these beneficial attributes (Table 3). Therefore, it appears that this strain is the most suitable for rotifer culture to improve productivity.

Not only could prey affect rotifer productivity, it may also indirectly influence the ability of larvae to feed on rotifers. For instance, rotifers fed *I. galbana* strain M had a

smaller body size. This method of culturing rotifers might be beneficial, as small rotifers are required for the diet of many larvae during the first few weeks (Kamler, 1992). In contrast, larger, slower moving rotifers are needed for other larvae, as they are easy to capture (Luciani et al., 1983; Stemberger and Gilbert, 1987). If larger rotifers were desired, the data from this study suggest that the rotifers should be fed *I. galbana* strain G.

Although several parameters were affected by prey strain/species, many others were not: egg volume, mean time to 50% survival, mortality rate, fecundity decline rate, and time to peak of fecundity (Fig. 6, 7, Table 3). These results support the observation of Theilacker and McMaster (1971), and Scott and Baynes (1978) that, within the limits of suitable prey, the type of alga (species) has little influence on the yield of rotifers. The present study extends this observation to strains within a species, i.e., in general, although *I. galbana* strains differed in a number of attributes as food (see 4.1, above), they did not have a strong affect on rotifer productivity.

2.4.3. Rotifer biochemical composition

In many aquaculture practices rotifers ultimately supply all the nutrients for larvae development (Lubzens et al., 2001), and the nutritional quality of rotifers is altered by their diet (Watanabe et al., 1983). This study found differences in the biochemical composition of the rotifer's diet (Table 2). Furthermore, there were some differences in the composition of rotifers fed on different diets (flagellate strains), e.g., differences in protein, carbohydrate, and lipid content (Fig. 9). This supports the finding that the proportions of these nutrients change when rotifers are fed different species of algae or artificially prepared enrichment-diets (Nagata and Whyte, 1992; Øie and Olsen, 1997).

The differences recorded here between rotifers fed different strains, suggest that some strains are better than others. For instance, the highest protein content occurred in rotifers fed on *I. galbana* strain D. Protein synthesis is reduced at low rotifer growth rates (Caric et al., 1993), and *I. galbana* strain D had the lowest growth rate (Fig. 5); this may explain the low protein content. In contrast, rotifers fed on *I. galbana* strain T had the highest lipid content and the highest carbohydrate content (Fig. 9). Therefore, a high level of one of these nutrients does not necessarily suggest that all nutrients will be high within a single strain.

One might expect that rotifer composition reflects that of its prey. However, a clear relationship between rotifers and their food in terms of protein, carbohydrate, or total lipids expressed as either dry weight or μm^{-3}, was absent in this study (Fig. 10). Similar studies have compared prey and rotifer composition and expressed findings in terms of dry weight (Frolov et al., 1991; Fernandez Reiriz et al., 1993; Reitan et al., 1997). However the findings of these previous studies are contradictory; for example, Frolov et al. (1991) obtained a positive correlation between protein and lipid content of rotifer and their prey, but no correlations were established for total carbohydrates. In contrast, Fernandez Reiriz et al. (1993) found a rotifer-prey relation in carbohydrate and lipid content but no relation between protein content. However, all these researches found a positive relationship between rotifers and their diet in terms of lipid content per dry weight, but this was not the case in this study (Fig. 10f). Thus, rotifer composition does not necessarily reflect that of its prey.

A second observation from this study is that the composition of all three biochemical attributes is substantially higher μm^{-3} in the prey, compared to the rotifer (Fig. 10). Why might this be so? Based on volume estimates of the rotifer pseudocoelom (from illustrations in the literature and personal observations, assuming this space is devoid of

material), I was able to account for a two-fold difference between the rotifer and the prey (calculations not shown), but the differences are 3 to 10 fold (Fig. 10). Thus, my pseudocoelom estimates may be incorrect, or there is another unaccounted factor affecting these estimates. However, biochemical composition on a dry weight basis is relatively similar between prey and rotifer. In either case, this discrepancy should be noted if volume: biochemical relationships are applied to rotifers and their prey in the future.

The most important observation, however, is the influence of dietary strains on rotifer biochemical composition, which could be as large as 25- 50% (Fig. 9, Table 3). The results of this work show that algal strains affect rotifer body volume (Fig. 5d, Table 3) hence altering the content of biochemical compounds each individual can hold (Fig. 9a, c, e, Table 3), and the concentration of the component μm^{-3} of the volume. Therefore, some strains may be preferred to others to ensure high biochemical content particularly if used as rotifer enrichment. Thus, aquaculturists are recommended to rear and enrich their rotifers with *I. galbana* strain T before addition to larvae tanks if their culture method and strains matches the one used in this study.

2.5. Application of results to aquaculture

This study found that there was no single "best" strain of *I. galbana* for the rotifer. Rather there was a series of potential trade-offs; one stain providing some benefits, while another provides others. Furthermore, different stages of the rotifer rearing procedure sometimes required different strains for optimal results.

However, some general recommendations can be made. From this study it can be recommend that *I. galbana* strain M be used to provide the highest growth rate and *I.*

galbana strain G to provide the highest production; both high growth and production are attributes sought after by aquaculturists (Yufera et al., 1983; James and Dias, 1984; Abu-Rezeq, James, 1985; Bou-Abbas et al., 1985; Rafiuddin and Neelakantan, 1990; Abu-Rezq et al., 1997; Suantika et al., 2000). However, aquaculturists are also interested in the lipid content of their food (Ben-Amotz et al., 1985; Kanazawa, 1985; Brown et al., 1997; Fernandez-Reiriz and Labarta, 1996; Evjemo and Olsen, 1997; Babarro et al., 2001); if *I. galbana* is used as lipid enrichment for rotifers before feeding to larvae, then strain T should be used. These are some examples of recommendations from this study; Table 3 outlines all the differences and might be used as a guideline to choose the best strain for a specific purpose.

It must be noted that the purpose of this study was not to identify optimal strains but to highlight that algal strains differ in their quality and may affect rotifer grow and biochemical composition. There thus appears to be a need to continue to identify strain differences. Possibly, however, the most important message from this study is that aquaculturists should identify the strains that they use, either by the geographical regions where strains were isolated (e.g., Table 1) or ideally by molecular methods (Lopez Alonso et al., 1994).

2.6. References

Abu-Rezeq, T., James, C.M., 1985. Production and nutritional quality of the rotifer *Brachionus plicatilis* in relation to different *Chlorella* cell densities. Annu. Res. Rep. Kuwait Inst. Sci. Res. 10, 30-34.

Abu-Rezq, T., Al-Abdul-elah, K., Duremdez, R., Al-Marzouk, A., James, C.M., Al-Gharabally, H., Al-Shimmari, J., 2002. Studies on the effect of using the rotifer, *Brachionus plicatilis* , treated with different nutritional enrichment media and antibiotics on the growth and survival of blue-fin sea bream, *Sparidentex hasta* (Valenciennes), larvae. Aquacult. Res. 33, 117-128.

Abu-Rezq, T.S., Al-Shimmari, J., Dias, P., 1997. Live food production using batch culture and chemostat systems in Kuwait. Hydrobiologia 358, 1-3.

Abu-Rezq, T.S., Al-Musallam, L., Al-Shimmari, J., Dias, P., 1999. Optimum production conditions for different high-quality marine algae. Hydrobiologia 403, 97-107.

Albentosa, M., Perez-Camacho, A., Labarta, U., Fernandez-Reiriz, M.J., 1996. Evaluation of live microalgal diets for the seed culture of *Ruditapes decussatus* using physiological and biochemical parameters. Aquaculture 148, 11-23.

Babarro, J.F., Reiriz, M.J.F., Labarta, U., 2001. Influence of preservation techniques and freezing storage time on biochemical composition and spectrum of fatty acids of *Isochrysis galbana* clone T-ISO. Aquacult. Res. 32, 565-572.

Ben-Amotz, A., Tornabene, T.G., Thomas, W.H., 1985. Chemical profile of selected species of microalgae with emphasis on lipids. J. Phycol. 21, 72-81.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Bosque, T., Hernandez, R., Todoli, R., Oltra., 2001. Effect of salinity, temperature and food level on the demographic characteristics of the seawater rotifer *Synchaeta littoralis* Rousselet. J. Exp. Mar. Biol. Ecol. 258, 55-64.

Bou-Abbas, M., James, C.M., Al-Khars, A.M., Al-Hinty, S., Salman, A.E., 1985. Production of the rotifer *Brachionus plicatilis* for aquaculture in Kuwait. Kuwait Bull. Mar. Sci., 198.

Bouaicha, N., Chezeau, A., Turquet, J., Quod, J.P., Puiseux Dao, S., 2001. Morphological and toxicological variability of *Prorocentrum lima* clones isolated from four locations in the south-west Indian Ocean. Toxicon 39, 1195-1202.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal. Biochem. 72, 248-254.

Brown, M., Dunstan, G., Jeffrey, S., J-M, L., 1993. The influence of irradiance on the boichemical composition on the Prymnesiophyta *Isochrysis* sp. (CLONE T-ISO). J. Phycol 29, 601-612.

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151, 315-331.

Brown, M.R., Dunstan, G.A., Jeffrey, S.W., Volkman, J.K., Barrett, S.M., LeRoi, J.M., 1993. The influence of irradiance on the biochemical composition of the prymnesiophyte *Isochrysis sp.* (clone T-ISO). J. Phycol. 29, 601-612.

Caric, M., Sanko-Njire, J., Skaramuca, B., 1993. Dietary effects of different feeds on the biochemical composition of the rotifer (*Brachionus plicatilis* Mueller). Aquaculture 110, 141-150.

Carmona, M.J., Serra, M., Miracle, M.R., 1994. Effect of population density and genotype on life-history traits in the rotifer *Brachionus plicatilis* O.F. Mueller. J. Exp. Mar. Biol. Ecol. 182, 223-235.

Cheng-Wu, Z., Zmora, O., Kopel, R., Richmond, A., 2001. An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). Aquaculture 195, 1-2.

Culture Center of algae and protozoa, 1972. Amendment to the 1971 list of strains, Cambridge, UK, 4 pp.

Dortch, Q., Conway, H.L., 1984. Interactions between nitrate and ammonium uptake: variation with growth rate, nitrogen source and species. Mar. Biol. 79, 151-164.

Evjemo, J.O., Olsen, Y., 1997. Lipid and fatty acid content in cultivated live feed organisms compared to marine copepods. Hydrobiologia 358, 159-162.

Fabregas, J., Herrero, C., Cabezas, B., Abalde, J., 1986. Biomass production and biochemical composition in mass cultures of the marine microalga *Isochrysis galbana* Parke at varying nutrient concentrations. Aquaculture 53, 101-113.

Falconer, D.S., 1982. Introduction to Quantitative Genetics. Longman Inc., New York, 77-133 pp.

Fernandez Reiriz, M.J., Labarta, U., Ferreiro, M.J., 1993. Effects of commercial enrichment diets on the nutritional value of the rotifer (*Brachionus plicatilis*). Source Aquaculture 112, 2-3.

Fernandez-Reiriz, M.J., Labarta, U., 1996. Lipid classes and fatty acid composition of rotifers (*Brachionus plicatilis*) fed two algal diets. Hydrobiologia 330, 73-79.

Fernandez-Reiriz, M.J., Perez-Camacho, A., Ferreiro, M.J., Blanco, J., Planas, M., Campos, M.J., Labarta, U., 1989. Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. Aquaculture 83, 17-37.

Fidalgo, J.P., Cid, A., Torres, E., Sukenik, A., Herrero, C., 1998. Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga *Isochrysis galbana*. Aquaculture 166, 1-2.

Frolov, A.V., Pankov, S.L., Geradze, K.N., Pankov, S.A., Spektorova, L.V., 1991. Influence of the biochemical composition of food on the biochemical composition of the rotifer *Brachionus plicatilis*. Aquaculture 97, 181-202.

Fu, Y., Hirayama, K., Natsukari, Y., 1990. Strains of the rotifer *Brachionus plicatilis* having particular patterns of isozymes. In: Hanyu, I. (Ed.), Second Asian Fisheries Forum, Tokyo (Japan), pp. 37-40.

Fu, Y., Hirayama, K., Natsukari, Y., 1991a. Morphological differences between two types of the rotifer *Brachionus plicatilis* O.F. Mueller. J. Exp. Mar. Biol. Ecol. 151, 29-41.

Fu, Y., Hirayama, K., Natsukari, Y., 1991b. Genetic divergence between S and L type strains of the rotifer *Brachionus plicatilis* O.F. Mueller. J. Exp. Mar. Biol. Ecol. 151, 43-56.

Gallardo, W.G., Hagiwara, A., Snell, T.W., 2000. Effect of juvenile hormone and serotonin (5-HT) on mixis induction of the rotifer *Brachionus plicatilis* Muller. J. Exp. Mar. Biol. Ecol. 252, 97-107.

Gallardo, W.G., Hagiwara, A., Tomita, Y., Snell, T.W., 1999. Effect of growth hormone and gamma -aminobutyric acid on *Brachionus plicatilis* (Rotifera) reproduction at low food or high ammonia levels. J. Exp. Mar. Biol. Ecol. 240, 179-191.

Gallardo, W.G., Tomita, Y., Hagiwara, A., Soyano, K., Snell, T.W., 1997. Effect of dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH), acetone, and ethanol on the population growth, mictic female production, and body size of the rotifer *Brachionus plicatilis* Muller. Bulletin of the Faculty of Fisheries, Nagasaki University. Nagasaki, 15-22.

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Chanley, M.H. (Ed.), Culture of Marine Invertebrate Animals. Plenum Press, New York, pp. 29-60.

Hansen, B., Moller, T., Wittrup, L., 1997. Particle grazing efficiency and specific growth efficiency of the rotifer *Brachionus plicatilis* (Muller). J. Exp. Mar. Biol. Ecol. 215, 217-233.

Hirayama, K., Kusano, T., 1972. Fundamental studies on physiology of rotifer for its mass culture - II. Influence of water temperature on population growth of rotifer. Bull. Jap. Soc. Sci. Fish. 38, 1357-1363.

Hirayama, K., Takagi, K., Kimura, H., 1979. Nutritional effect of eight species of marine phytoplankton on population growth of the rotifer, *Brachionus plicatilis*. Bull. Japan. Soci. Sci. Fish. 45, 11-16.

Hoff, F.H., Snell, T.W., 1987. Plankton Culture Manual. Florida Aqua Farms, Inc., Dade. USA., 12-35 pp.

Ito, T., 1960. On the culture of the mixochaline rotifer *Brachionus plicatilis* O. F. Muller in the sea water. Rep. Fac. Fish. Prefect. Univ. Mie. 3, 708-740.

James, C., Ben-Abbas, M.B., Al-Khars, A.M., Al-Hinty, S., Salman, A.E., 1983. Production of the rotifers *Brachionus plicatilis* for aquaculture in Kuwait. Hydrobiologia 104, 77-84.

James, C.M., Dias, P., 1984. Mass culture and production of the rotifer *Brachionus plicatilis* using baker's yeast and marine yeast. Annu. Res. Rep. Kuwait Inst. Sci. Res., 49-51.

James, C.M., Al-Hinty, S., Slman, A.E., 1989. Growth and ω3 fatty acid composition of marine microalgae under different temperature regimes. Aquaculture 77, 337-351.

Kain, J.M., Fogg, G.E., 1958. Studies on the growth of marine phytoplankton *Isochrysis galbana*. J. Mar. Biol. Assoc. U.K. 37, 781-788.

Kamler, E., 1992. Early Life History of Fish, an energetic approach. Chapman & Hall., London, 71-76 pp.

Kanazawa, A., 1985. Essential fatty acid and lipid requirement of fish, 281-298 pp.

Kochert, G., 1978. Quantitation of the macromolecular components of microalgae. Cambridge University Press, Cambridge, 189-195 pp.

Laing, I., Child, A.R., Janke, A., 1990. Nutritional value of dried algae diets for larvae of Manila clam (*Tapes philippinarum*). J. Mar. Biol. Assoc. U.K. 70, 1-12.

Lopez Alonso, D., Molina Grima, E., Sanchez Perez, J.A., Garcia Sanchez, J.L., Garcia Camacho, F., 1992. Isolation of clones of *Isochrysis galbana* rich in eicospapentaenoic acid. Aquaculture 102, 363-371.

Lopez Alonso, D., Segura del Castilo, C.I., Garcia Sanchez, J.L., Sanchez Perez, J.A., Garcia Camacho, F., 1994. Quantitative genetics of fatty acid variation in *Isochrysis galbana* (Prymnesiophyceae) and *Phaeodactylum tricornutum* (Bacillariophyceae). J. Phycol. 30, 553-558.

Lubzens, E., 1981. Rotifer resting eggs and their application to marine aquaculture. Spec. Publ. European Maricult. Soc. 6, 193-180.

Lubzens, E., 1987. Raising rotifers for use in aquaculture. Hydrobiologia 147, 245-255.

Lubzens, E., Marko, A., Tietz, A., 1985. De novo synthesis of fatty acids in the rotifer, *Brachionus plicatilis*. Aquaculture 47, 27-37.

Lubzens, E., Tandler, A., Minkoff, G., 1989. Rotifers as food in aquaculture. Hydrobiologia 186/187, 387-400.

Lubzens, E., Zmora, O., Barr, Y., 2001. Biotechnology and aquaculture of rotifers. Hydrobiologia. 446, 337-353.

Lubzens, E., Minkoff, G., Barr, Y., Zmora, O., 1997. Mariculture in Israel - past achievements and future directions in raising rotifers as food for marine fish larvae. Hydrobiologia 358, 13-20.

Luciani, A., Chasse, J.L., Clement, P., 1983. Aging in *Brachionus plicatilis* : The evolution of swimming as a function of age at two different calcium concentrations. Hydrobiologia 104, 141-146.

Molina Grima, E., Garcia Camacho, F., Sanchez Perez, J.A., Garcia Sanchez, J.L., 1994. Biochemical productivity and fatty acid profiles of *Isochrysis galbana* Parke and *Tetraselmis* sp. as a function of incident light intensity. Process Biochem. 29, 119-126.

Molina Grima, E., Fernandez Sevilla, J.M., Sanchez Perez, J.A., Garcia Camacho, F., 1996. A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances. J. Biotechnol. 45, 59-69.

Montagnes, D.J.S., Weisse, T., 2000. Fluctuating temperatures affect growth and production rates of planktonic ciliates. Aquat. Microb. Ecol. 21, 97-102.

Montagnes, D.J.S., Franklin, D.J., 2001. Effect of temperature on diatom volume, growth rate, and carbon and nitrogen content: Reconsidering some paradigms. Limnol. Oceanogr. 46, 2008-2018.

Nagata, W.D., Whyte, J.N.C., 1992. Effects of yeast and algal diets on the growth and biochemical composition of the rotifer *Brachionus plicatilis* (Mueller) in culture. Aquacult. Fish. Manage. 23, 13-21.

Navarro, N., Yufera, M., 1998. Influence of the food ration and individual density on production efficiency of semicontinuous cultures of Brachionus-fed microalgae dry powder. Hydrobiologia 387, 1-3.

Nelson, J.R., Guarda, S., Cowell, L.E., Heffernan, P.B., 1992. Evaluation of microalgal clones for mass culture in a subtropical greenhouse bivalve hatchery: Growth rates and biochemical composition at 30 degree C. Aquaculture 106, 3-4.

Øie, G., Olsen, Y., 1997. Protein and lipid content of the rotifer *Brachionus plicatilis* during variable growth and feeding condition. Hydrobiologia 358, 1-3.

Okauchi, M., 1990. Food value of *Isochrysis aff. galbana* for the growth of pearl oyster spat. Nippon Suisan Gakkaishi/Bull. Jap. Soc. Sci. Fish. 56, 1343.

Pande, S.V., Parvin, R., Venkitasubramanian, 1963. Microdetermination of lipids and serum total fatty acids. Anal. Biochem 6, 415-425.

Pillay, T.V.R., 1990. Aquaculture: principles and practices. Fishing News Book, London, 591 pp.

Pillsbury, K.S., 1985. The relative food value and biochemical composition of five phytoplankton diets for queen conch, *Strombus gigas* (Linne) larvae. J. Exp. Mar. Biol. Ecol. 90, 221-231.

Planas, M., Estevez, A., 1989. Effects of diet on population development of the rotifer *Brachionus plicatilis* in culture. Helgolander Meeresuntersuchungen 43, 171-181.

Rafiuddin, A.S., Neelakantan, K., 1990. Production of rotifer, *Brachionus plicatilis* (Muller) fed with different cell densities of microalgae, *Chlorogibba trochisciaeformis* (Geiter). Proc. Indian Acad. Sci. (Anim. Sci.). 99, 519-523.

Reitan, K.I., Rainuzzo, J.R., Olsen, Y., 1994. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. J. Phycol. 30, 972-979.

Reitan, K.I., Rainuzzo, J.R., Oeie, G., Olsen, Y., 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155, 207-221.

Renaud, S.M., Thinh, L.V., Parry, D.L., 1999. The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. Aquaculture 170, 147-159.

Renaud, S.M., Thinh, L., Lambrinidis, G., Parry, D.L., 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 211, 195-214.

Rothbard, S., 1977. Observation on adult forms and experiments in growth of larvae of the fresh water shrimp *Macrobrachium nipponense* (De Haan). Bemidgeh 29, 115-125.

Satoh, M., Hirachi, Y., Yoshioka, A., Kobayashi, M., Oyama, Y., 2002. Determination of cellular levels of nonprotein thiols in phytoplankton and their correlations with susceptibility to mercury. J. Phycol. 38, 983-990.

Schmid-Araya, J.M., See, L., 1991. The effect of food concentration on the life histories of *Brachionus plicatilis* (O. F. M.) and *Encentrum linnhei* Sccot. Arch. Hydrobiol. 121, 87-102.

Scott, A.P., Baynes, S.M., 1978. Effect of algal diet and temperature on the biochemical composition of the rotifer, *Brachionus plicatilis*. Aquaculture 14, 247-260.

Scott, A.P., Middleton, C., 1979. Unicellualar algae as a food for turbot (*Scophthalmus maximus* L.) larvae - the importance of dietary long-chin polyunsatrurated fatty acids. Aquaculture. 14, 227-240.

Snell, T.S., Carrillo, K., 1984. Body size variation among strains of the rotifer *Brachionus plicatilis*. Aquaculture 37, 359-367.

Snell, T.W., Bieberich, C.J., Fuerst, R., 1983. The effects of green and blue-green algal diets on the reproductive rate of the rotifer *Brachionus plicatilis*. Aquaculture 31, 21-30.

Stemberger, R.S., Gilbert, J.J., 1987. Rotifer threshold food concentrations and the size-efficiency hypothesis. Ecology 68, 181-187.

Suantika, G., Dhert, P., Nurhudah, M., Sorgeloos, P., 2000. High-density production of the rotifer *Brachionus plicatilis* in a recirculation system: consideration of water quality, zootechnical and nutritional aspects. Aquacult. Eng. 21, 201-214.

Sukenik, A., Carmeli, Y., 1989. Regulation of eicosapentaenoic acid content by light in the marine eustigmatophyte *Nannochloropsis sp*, Marine Biotechnology, Tokyo (Japan), pp. 18.

Sukenik, A., Wahnon, R., 1991. Biochemical quality of marine unicellular algae with special emphasis on lipid composition. 1. *Isochrysis galbana*. Aquaculture 97, 61-72.

Sukenik, A., Zmora, O., Carmeli, Y., 1993. Biochemical quality of marine unicellular algae with special emphasis on lipid composition. 2. *Nannochloropsis* sp. Aquaculture 117, 313-326.

Sulkin, S.D., Epifanio, C.E., 1975. Comparison of rotifers and other diets for rearing early larvae of the blue crab *Callinectes sapidus* Rathbun. Estuarine Coastal Mar. Sci. 3, 109-113.

Theilacker, G.H., McMaster, M.F., 1971. Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as a food for larval anchovies. Mar. Biol. 10, 183-188.

Thompson, P.A., Harrison, P.J., Parslow, J.S., 1991. Influence of irradiance on cell volume and carbon quota for ten species of marine phytoplankton. J. Phycol. 27, 351-360.

Thompson, P.A., Guo, M.X., Harrison, P.J., 1992. Effects of variation in temperature. 1. On the biochemical composition of eight species of marine phytoplankton. J. Phycol. 28, 481-488.

Underwood, A.J., 1997. Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge, 504 pp.

Vadstein, O., Oeie, G., Olsen, Y., 1993. Particle size dependent feeding by the rotifer *Brachionus plicatilis*. In: Miracle, M.R. (Ed.), 6. Int. Rotifer Symp. Hydrobiologia, Banyoles (Spain), pp. 261-267.

Valenzuela-Espinoza, E., Millan-Nunez, R., Nunez-Cebrero, F., 2002. Protein, carbohydrate, lipid and chlorophyll a content in *Isochrysis aff. galbana* (clone T-Iso) cultured with a low cost alternative to the f/2 medium. Aquacult. Eng. 25, 207-216.

Volkman, J.K., Brown, M.R., Dunstan, G.A., Jeffrey, S.W., 1993. The biochemical composition of marine microalgae from the Class Eustigmatophyte. J. Phycol. 14, 451-454.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 128, 219-240.

Walz, N., 1995. Rotifer populations in plankton communities: Energetic and life history strategies. Experientia 51, 437-453.

Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 89, 263-272.

Watanabe, T., Kitajima, C., Arakawa, T., Fukusho, K., Fujita, S., 1978. Nutritional quality of rotifer *Brachionus plicatilis* as a living feed from the viewpoint of essential fatty acids for fish. Bull. Jap. Soc. Sci. Fish. 44, 1109-1114.

Whyte, J.C., 1987. Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves. Aquaculture 60, 231-241.

Wikfors, G.H., Patterson, G.W., 1994. Differences in strains of *Isochrysis* of importance to mariculture. Aquaculture 123, 127-135.

Wikfors, G.H., Ohno, M., 2001. Impact of algal research in aquaculture. J. Phycol. 37, 968-974.

Wikfors, G.H., Patterson, G.W., Ghosh, P., Lewin, R.A., Smith, B.C., Alix, J.H., 1996. Growth of post-set oysters, *Crassostrea virginica*, on high-lipid strains of algal flagellates *Tetraselmis spp.* Aquaculture 143, 3-4.

Yufera, M., 1982. Morphometric characterization of a small-sized strain of *Brachionus plicatilis* in culture. Aquaculture 27, 55-61.

Yufera, M., Lubian, L.M., Pacual, E., 1983. Effect of four species of marine algae on population growth of two strains of *Brachionus plicatilis* in culture. Invest. Pesq. (Barc.). 47, 325-337.

Zar, J.H., 1999. Biostatistical Analysis. Prentice Hall International, Inc, New Jersey, USA, 663 pp.

Zhu, C.J., Lee, Y.K., Chao, T.M., 1997. Effects of temperature and growth phase on lipid and biochemical composition of *Isochrysis galbana* TK1. J. Appl. Phycol. 9, 451-457.

Chapter 3.

The combined effect of strain and temperature on the quality of microalgal growth, production, and biochemical composition

3.1. Introduction

Live algae are the best and the preferred food source for many aquatic animals (Borowitzka, 1997). Marine microalgae are widely used for feeding bivalve larvae and spat (Knauer and Southgate, 1999), early larvae of both crustaceans (Kanazawa et al., 1985) and marine fish (Reitan et al., 1997), rotifers (Watanabe et al., 1983), and brine shrimp (Lavens and Sorgeloos, 1996). Thus, it is not surprising that microalgae culture remains an integral part of rearing such animals (Volkman et al., 1989). Many microalgae species have been investigated, and the most nutritionally efficient ones have been retained. Among the numerous species tested is *Isochrysis galbana*, and the strains of this species are among the most widely used (Anon, 1975; Baynes and Scott, 1979; Bourne, 1988; Boussiba et al., 1988; Brown, 1991; Sukenik and Wahnon, 1991; Cho et al., 1999; Dai and Wu, 2000; Babarro et al., 2001a; Babarro et al., 2001b). This study continues the work of investigating the quality of *Isochrysis galbana* strains.

Isochrysis galbana strains have received increasing interest because they have distinctive qualities, which separate them from other flagellate species. *I. galbana* is easy to grow (Watanabe et al., 1983) and is in the size range preferred by many live prey (Brown et al., 1997; Hansen et al., 1997). Compared with other species of algae it elicits higher growth rates of not only live prey for aquaculture species, but also of small larval invertebrates which are reared directly for aquaculture (Lavens and Sorgeloos, 1996). The lack of a cell wall and their small size allows microalgae such as *I. galbana* to be digested by small larval invertebrates (Babinchak and Ukeles, 1979). Furthermore, *I. galbana* produces essential fatty acids (Yongmanitchai and Ward, 1991), which are required for the proper

development and survival of many marine larvae (Enright et al., 1986; Brown et al., 1997; Reitan et al., 1997; Sargent et al., 1999; Sargent et al., 1997).

Isochrysis galbana can grow under a wide range of environmental conditions, such as temperatures between 10 - 25°C (Thompson et al., 1992), a range of photoperiods (Price et al., 1998), light intensities between 15 to 218 Wm^{-2} (Molina Grima et al., 1994), and in different types of culture medium (Dortch and Conway, 1984). However, the optimum growth under these conditions may vary between strains.

Comparative studies on *I. galbana* have described differences between strains isolated at different times and from different places. These include differences in such characteristics as amino acids and sugars (Brown, 1991), fatty acid profiles (Grima et al., 1992), nutritional value to invertebrates (Brown et al., 1997; Mourente et al., 1995), and other growth and biochemical variables (Chapter 2, Table 5). These data suggest that strains may differ in their tolerance to different environmental conditions and have different optimal conditions (Muller-Feuga et al., 2003). Achieving the best harvest for an aquaculturist, at a minimum cost, requires knowledge of how the growth of these strains may vary in various conditions that can be easily controlled.

Temperature is one of the conditions that can be easily controlled by the aquaculturist. It is an important physical factor that can increase microalgal growth up to an optimum point after which growth rate often rapidly decreases (Muller-Feuga et al., 2003). Temperature also influences microalgal cell volume (Montagnes and Franklin, 2001; Muller-Feuga et al., 2003), and this may affect their use as "live-particles" required by different larval stages and species (Muller-Feuga et al., 2003; Thompson et al., 1992). Temperature also affects the nutritional value (Thompson et

al., 1992 a; b), and biochemical composition of microalgae, which is important for mariculture (Watanabe et al., 1983).

As the biochemical composition of microalgae can be manipulated by changing the temperature (Thompson et al., 1992a; b; Renaud et al., 1995; Renaud et al., 2002), and the effects may vary between strains, knowledge of how strains respond to different environments is of practical use to mariculturists. Temperature and strain choice may then be used with an optimal level of specific nutrients to fulfil the requirements of the feeding animal. The aim of this study was to assess the independent and interactive responses of different strains at various temperatures, employing both strains and temperatures commonly used in aquaculture practices.

3.2. Material and Methods

3.2.1. Stock cultures

Strains of *Isochrysis galbana* from: the Isle of Man (M), Tahiti (T), Greece (G), Denmark (D), and one strain of *Nannochloropsis* sp. (N) were obtained from the plankton culture collection at Port Erin Marine Laboratory, Isle of Man (Chapter 2, Table 1). The flagellates were grown in 200 ml flat bottom flasks in f/2 medium (Guillard, 1975). All strains and species were maintained in semi-continuous culture, by transfers every two weeks, at 20°C, on a 12:12 light: dark cycle, at an irradiance of 100 $\mu E\ m^{-2}s^{-1}$.

2.2.2. *Growth characteristics*

The four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) were obtained from stock cultures. Three replicates of each algal strain/species were grown in 500 ml round bottom flasks in modified f/2 medium (Guillard, 1975) at different temperature (15, 20, 25, 30 ± 1°C). All replicates were maintained in the exponential growth phase by semi-continuous culturing on a 24:0 light: dark cycle. An irradiance of 100 $\mu E\ m^{-2}s^{-1}$ was provided, which is considered optimal for flagellate growth rate (Brown et al., 1993; Molina Grima et al., 1994; Molina Grima et al., 1996). All cultures were maintained for ~10 generations; flasks were suspended in a controlled water bath at the four temperatures and were gently agitated at least once a day.

Growth rate was measured by determining abundance, daily, using a Model II Coulter Multisizer, and associated software V.4.1 equipped with a population accessory (Coulter Electronics Ltd., England). Growth rate was calculated as the slope of *ln* numbers of cells vs time in days.

Cell volume (V) was estimated from cell length (L) and width (W) measurements, assuming a prolate spheroid shape (see Fig 1, Chapter 2 for illustration). The volume was estimated using the following equation:

$$V=4/3\ \pi\ (W/2)^2\ (L/2)$$

Measurements were made on ≥ 40 randomly chosen, active cells obtained in exponentional phase from each flask. Cell were placed in a 10 ml settling chamber and were measured using an inverted microscope (Zeiss Axiovert 135tv) equipped with a video camera (JVC model 3-CCD, 750 lines horizontal resolution), attached to

a Pentium IIPC with image analysis software (Scion Image for Windows, Scion Corp., MD, USA).

Flagellate production ($\mu m^3 d^{-1}$) was calculated by multiplying mean cell volume by growth rate (Montagnes and Weisse, 2000). For dry weight and biochemical analyses, samples were filtered onto Whatman GF/F glass fibre filters. For dry weight, three replicates one from each replicate culture, were filtered and washed with a solution of 0.5 M ammonium formate (Babarro et al., 2001); filters were dried at 60°C for 12h before weighting. For biochemical analyses replicate filters were immediately frozen in liquid nitrogen and stored for < 1 year; biochemical methods are described below (see 2.3).

3.2.3 Protein, carbohydrate, and lipid analysis

Three replicates, one from each algal flask (see section 2.2 above), were collected for protein, carbohydrate, and lipid determination: 30 ml for protein, 50 ml for carbohydrate, and 100 ml for lipid analysis. Cell number was determined by Coulter Counter and was always ~10^6 cells ml^{-1}.

Protein was quantified using the Bradford method (Bradford, 1976), after hydrolysis in NaOH 1N for 1h at 90°C. Total carbohydrates were quantified as glucose by phenol-sulfuric acid method (Kochert, 1978). Lipids were extracted following the method of Bligh and Dyer (1959) and quantified by the method of Pande et al. (1963). Calibration curves were made using: bovine albumin as the standard for proteins, D-glucose for carbohydrates, and tripalmitin for lipids.

3.2.5. *Statistical analyses*

The relationships between temperature and the algal growth parameters assumed a linear response and were fitted using simple linear regression (see Discussion for justification). The linear responses were then compared by analysis of covariance (ANCOVA), followed by Tukey's tests. Tukey's test was first used to compare the slopes of the regressions (Zar, 1999). Then, if the slopes were not significantly different, the means elevations were compared, also using the Tukey test. Production was the one growth parameter to not show a significant response to temperature (see Results); for production all data (at all temperatures) were considered replicates for a species/strain, and one-way ANOVA followed by the Tukey HSD test were applied to the data.

Biochemical data did not follow clear trends with temperature, so the above ANCOVA-based analysis could not be applied to these data. Instead, data were assessed by two-way analyses of variance (ANOVA), with temperature and strain as the source of variance, and protein, carbohydrate, and lipid $cell^{-1}$ or μm^{-3} as the dependent variables.

For all tests, data always passed tests for normality, and homogeneity of variance was tested by Cochran's test (Underwood, 1997). Where necessary, data were transformed to meet the assumption of the parametric statistics (Zar, 1999). In some cases data remained heterogeneous even after transformation. In these cases ANOVA was still applied since this technique is robust to departure from homogenous variances (Underwood, 1997). All statistical analysis was performed using STATISTCA version 5.0, and the statistical significance level was at $P<0.05$.

3.3. Results

3.3.1. Algal growth characteristics

Linear relationships were fitted to all growth related data, except for those between *Nannochloropsis* sp. growth rate and temperature and for all cases of production vs. temperature, as in these cases there were no significant relationships with temperature (Fig. 1). When linear relationships occurred ANCOVA was used to compare the slopes; when individual slopes were not significantly different, the means elevations were compared.

For the response of growth rate to temperature, there were no significant differences between slopes or mean elevations (of *I. galbana* strains only), indicating no strain differences (Fig. 1, Table 1). Furthermore, all slopes of *I. galbana* strains were positive with temperature (mean slope: 0.025 ± 0.006 d^{-1} $°C^{-1}$).

For the response of both cell volume and dry weight to temperature (Fig. 1), there were significant differences between slopes for both parameters (Table 1). For cell volume, there were two distinct groups: *Nannochloropsis* sp., *I. galbana* strain D, and T had similar slopes (mean slope: 1.1 ± 0.2 μm^3 $°C^{-1}$), and *I. galbana* strain M and G had similar slopes (mean slope: 5.2 ± 0.2 μm^3 $°C^{-1}$). Whereas for dry weight, *Nannochloropsis* sp. and *I. galbana* strain D had similar slopes (mean slope: 2.19 ± 0.68 pg $°C^{-1}$), and *I. galbana* strain M and G had similar slopes (mean slope: 6.5 ± 1.15 pg $°C^{-1}$). For both of these parameters the data suggest that not only were there strain differences in these responses but also one or two *I. galbana* strains were more similar to *Nannochloropsis* sp. than to other *I. galbana* strains.

There was no significant linear response of production to temperature (i.e., the slope of the line was not significantly different from zero), although there appeared to be an elevated rate for at 15 °C for *I. galbana* strains M and G. Thus, rather than evaluating strain differences by ANCOVA, ANOVA followed by a Tukey's test was applied to log-transformed data, treating all measurements as replicates with and without values at 15 °C included (i.e., n = 9 and 12). When this analysis was conducted there were significant differences between species and strains (Table 2).

One general trend that appeared to arise from the growth parameters (Fig. 1, Table 1, 2) was that *I. galbana* strains M and G were similar and distinctly different from the others. Furthermore, strains D and T were relatively similar to *Nannochloropsis* sp.

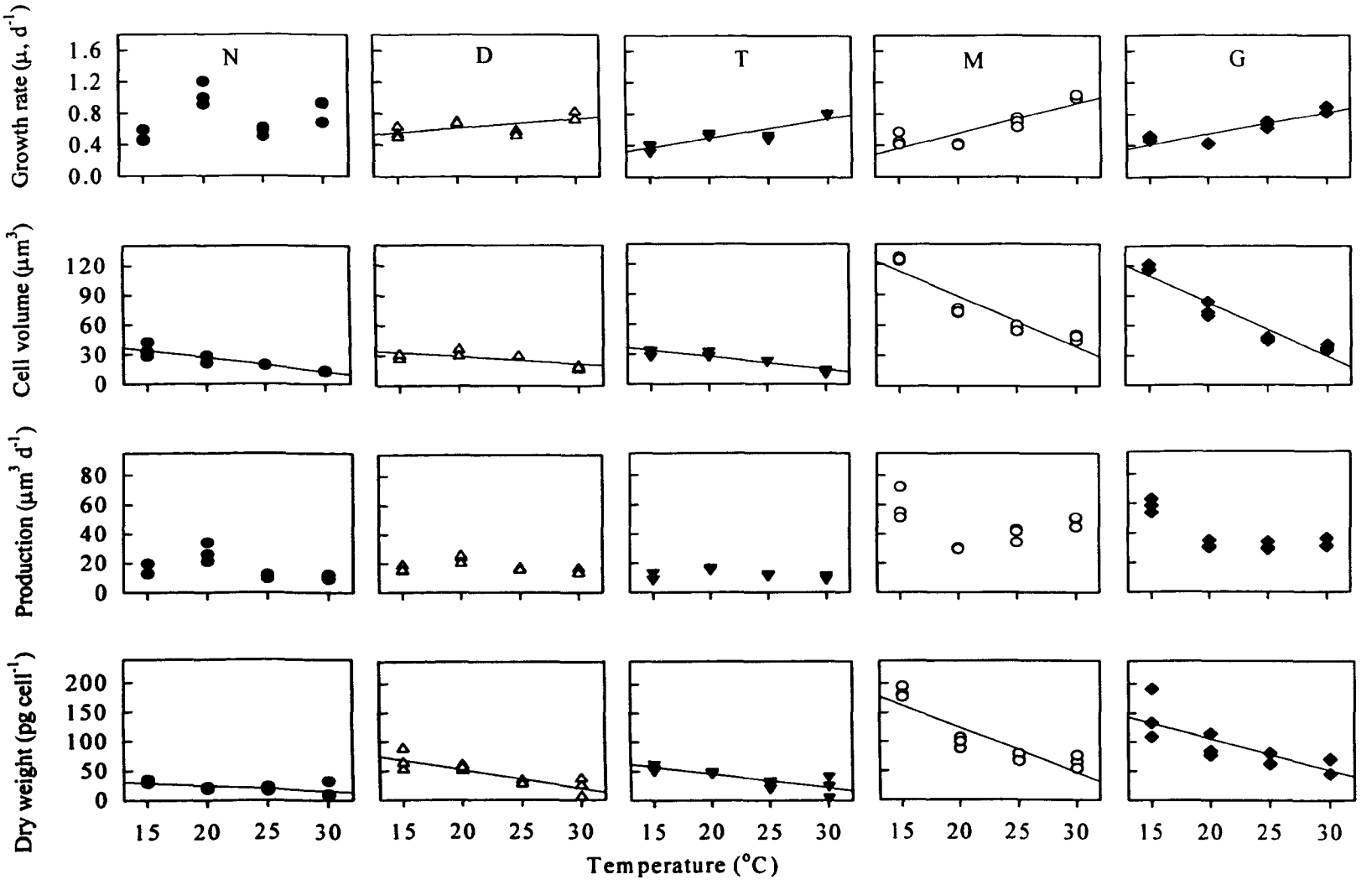

Fig. 1. Growth and production parameters of four strains of *Isochrysis galbana* (D, T, M, G), and one strain of *Nannochloropsis* sp. (N) (see Table 1 for strain designations): growth rate (μ, d^{-1}), cell volume (μm^3), production ($\mu m^3 d^{-1}$), and dry weight (pg $cell^{-1}$). Symbols of ●, □, ▼, ○, □ are for N, D, T, M, and G, respectively.

Table 1

Analysis of covariance (ANCOVA) for slopes of four *I. galbana* strains (D, T, M, and G) regression lines between algal growth factors and temperature. For algal growth rate and temperature ANCOVA was applied to examine: a) slopes, and b) intercepts. When significant, Tukey's test was used for post hoc comparison. Symbols are as described in Table 1, Chapter 2. Tukey's test data are ordered from lowest (left) to highest (right); underscoring indicates where there were no significant differences at $P = 0.05$. For growth rate, degrees of freedom were 1 for the intercepts, 1 for temperature, 3 for strain, and 43 for the error. For cell volume and dry weight, degrees of freedom were 1 for the intercepts, 1 for temperature, 4 for strain, and 54 for the error.

Factors	ANCOVA with (N)				Tukey's test
	SS	*MS*	*F*	*P-level*	
Growth rate (μ, d^{-1})					
Intercepts (elevations)	0.005	0.005	0.438	0.512	
Temperature	0.969	0.969	80.04	2.3×10^{-11}	
Strain (slopes)	0.064	0.021	1.765	0.168	D T M G
Error	0.521	0.012			
Cell volume (μm^3)					
Intercepts	39098.7	39098.7	201.72	0	
Temperature	14154.5	14154.5	73.026	1.3×10^{-11}	
Strain (slopes)	32970.4	8242.60	42.525	4.4×10^{-16}	N D T M G
Error	10466.7	193.828			
Dry weight (pg $cell^{-1}$)					
Intercepts	77095.5	77095.5	161.50	0	
Temperature	28928.9	28928.9	60.601	2.2×10^{-10}	
Strain (slopes)	61769.6	15442.4	32.349	9.2×10^{-14}	N T D M G
Error	25777.8	477.367			

Table 2.

One-way ANOVA and Tukey's test for the effect of strain on algal production. Strains designation (N, D, T, M, G) are described in Chapter 2, Table 1. Tukey's test data are ordered from lowest (left) to highest (right); underscoring indicates where there were no significant differences at $P = 0.05$. For all temperatures n=12, degrees of freedom were 4 for the parameter of interest, 55 for the residuals, and 59 for the total. For all temperatures except at 15 °C n=9, degrees of freedom were 4 for the parameter of interest 40 ,for the residuals, and 44 for the total. When n=12 production factor) $\mu m^3 d^{-1}$) were log transformed. When n=9 the data were heterogeneous even after transformation.

Factors	ANOVA			Tukey's test
	MS	*F*	*P-level*	
Production ($\mu m^3 d^{-1}$) (n=12)	0.7074	44.504	$1.3X10^{-16}$	N T D G M
Residual	0.0159			
Production ($\mu m^3 d^{-1}$) (n=9)	1176	32.15	$5.2X10^{-12}$	N T D G M
Residual	36.57			

3.3.2. Algal biochemical composition

Unlike the relationships between growth parameters and temperature, biochemical composition showed no clear linear trends with temperature. Consequently, two-way ANOVA was applied to these data (Table 3). For all biochemical composition parameters (both $cell^{-1}$ and μm^{-3}), except for lipid μm^{-3} for *I. galbana*, there were significant interactions between temperature and strain, indicating the complexity of these relationships (Table 3). Thus, further detailed statistical analysis was not performed; rather, the trends illustrated in Fig. 2 and Fig. 3 were visually interpreted.

In general, protein content for both strains and species (both μm^{-3} and $cell^{-1}$) increased with temperature to a maximum and then often decreased. However, the temperature where levels reached a maximum differed between strain/species (Fig. 2, 3). Carbohydrate levels (both μm^{-3} and $cell^{-1}$) exhibited no consistent pattern with temperature between species/strains: in some cases it increased, in others it decreased, and in others it increased to a maximum and then decreased. Lipid levels $cell^{-1}$ were relatively invariant with temperature, but lipid μm^{-3} increased with temperature for all strains/species.

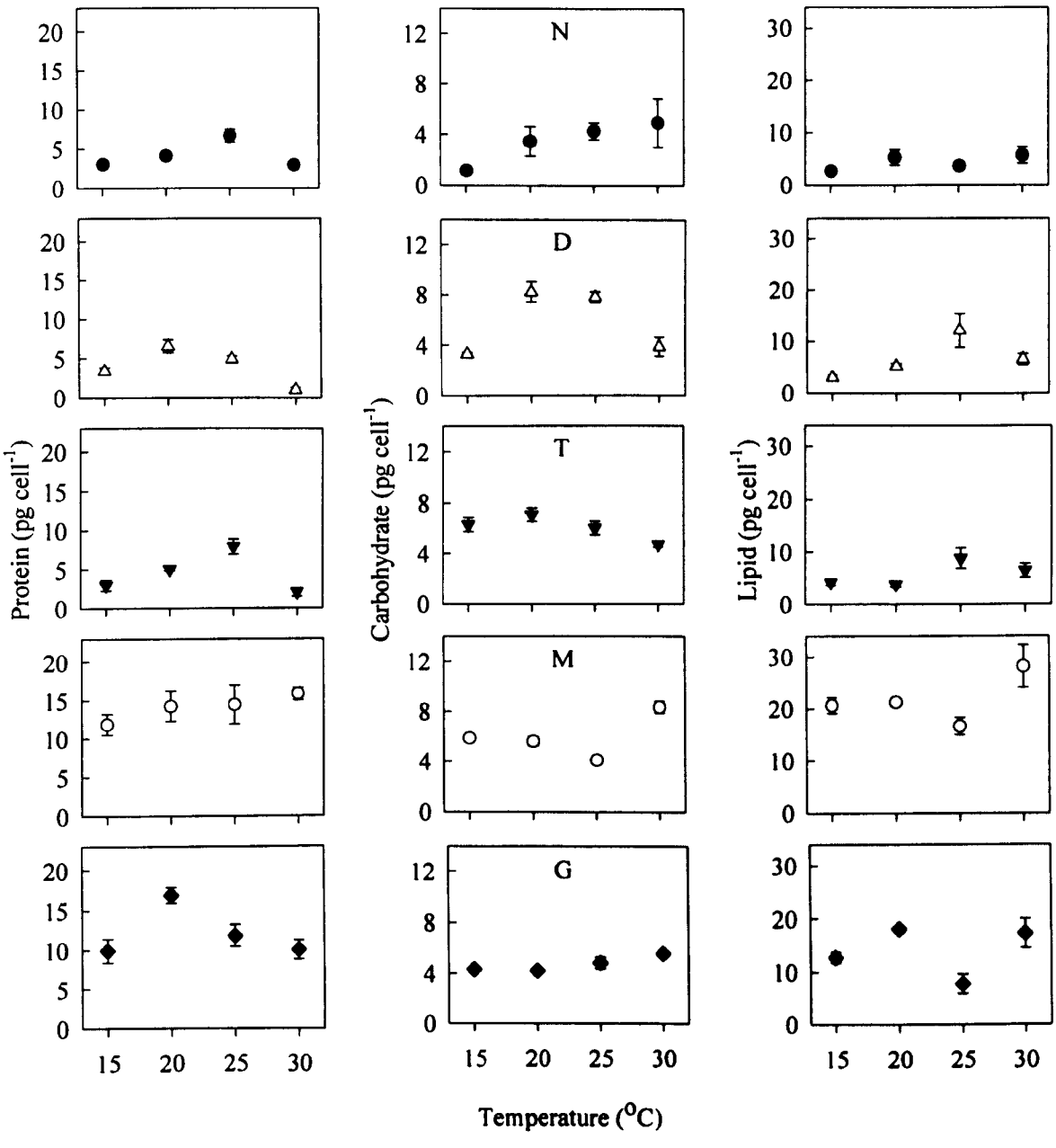

Fig. 2. Biochemical content of four strains of *Isochrysis galbana* (D, T, M, G), and one strain of *Nannochloropsis* sp. (N) (see Table 1 for strain designations). Presented per cell: protein (pg cell^{-1}) Carbohydrate (pg cell^{-1}), and total lipid (pg cell^{-1}). Symbols of ●, □, ▼, ○, □ are for N, D, T, M, and G, respectively.

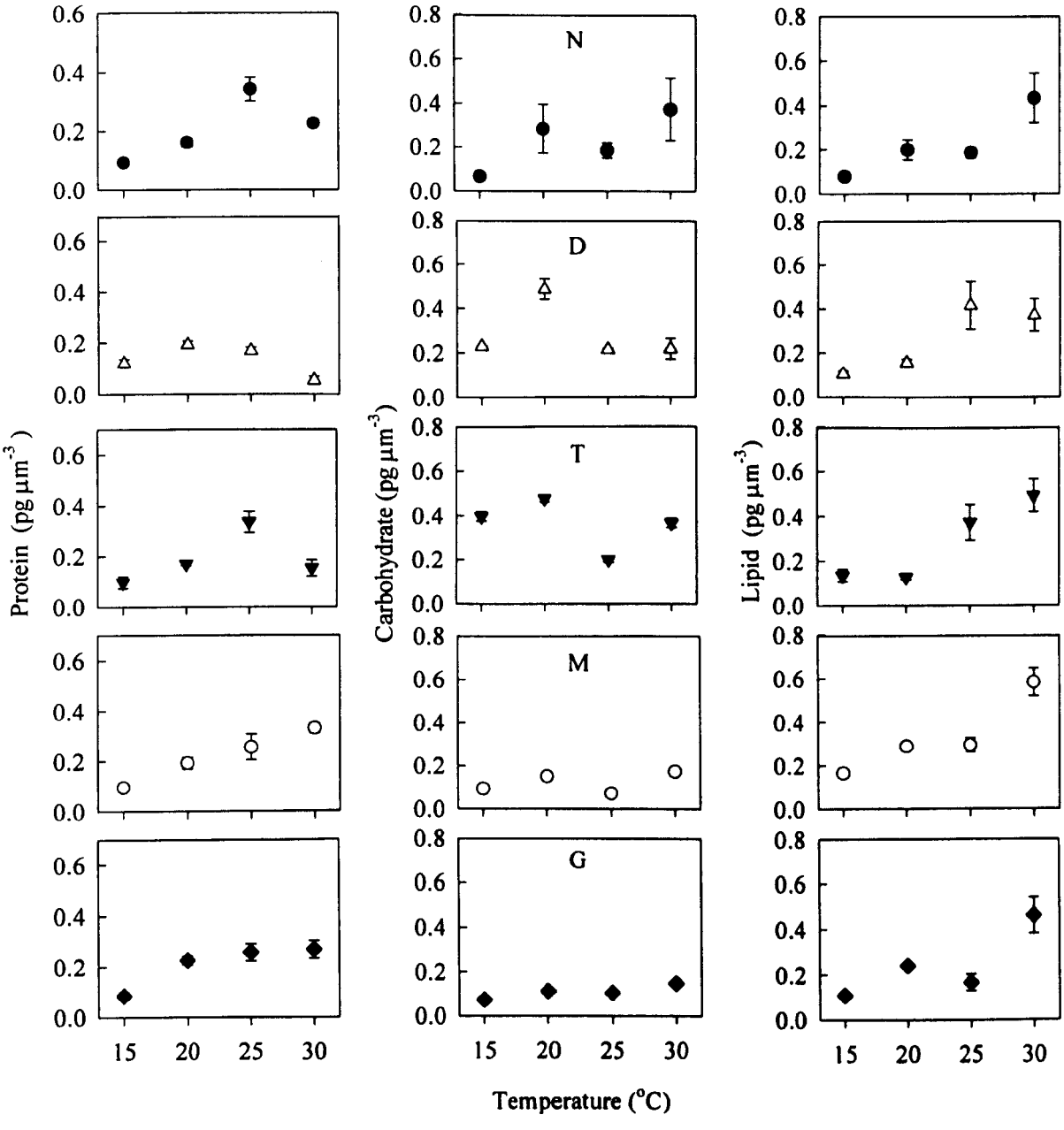

Fig. 3. Biochemical content of four strains of *Isochrysis galbana* (D, T, M, G), and one strain of *Nannochloropsis* sp. (N) (see Table 1 for strain designations). Presented μm^{-3}: protein (pg μm^{-3}), carbohydrate (pg μm^{-3}), and total lipid (pg μm^{-3}). Symbols of ●, □, ▼, ○, □ are for N, D, T, M, and G, respectively.

Table 3

Two -way ANOVA and Tukey's test for the effect of strain and temperature on algal biochemical compositions. Symbol (N) is as described in Table 1, Chapter 2. For ANOVA with (N) (test between species and strains) all tests degrees of freedom were 3 for temperature, 4 for strain, 12 for the interaction, and 40 for the residual. For ANOVA without (N) (test between strains only) all tests degrees of freedom were 3 for temperature, 3 for strain, 9 for the interaction, and 32 for the residual.

Factors		ANOVA with (N)			ANOVA without (N)		
		MS	*F*	*P-level*	*MS*	*F*	*P-level*
Protein (pg cell^{-1}) [b/-]							
	Temperature	13250.6	10.42	3.4×10^{-5}	39.01	9.314	0.0001
	Strain	180.74	0.142	0.965	326.5	77.96	8.4×10^{-15}
	Interaction	4024.1	3.164	0.003	12.44	2.971	0.011
	Residual	1272					
Carbohydrate (pg cell^{-1}) [a/-]							
	Temperature	6.982	5.427	0.003	3.515	6.149	0.002
	Strain	14.69	11.42	2.8×10^{-6}	4.498	7.869	0.0005
	Interaction	8.599	6.684	2.2×10^{-6}	9.897	17.31	6.1×10^{-10}
	Residual	1.287			0.572		
Lipid (pg cell^{-1}) [b/b]							
	Temperature	0.933	58.92	9.8×10^{-15}	0.08	5.625	0.003
	Strain	0.076	4.765	0.003	0.99	70.56	3.3×10^{-14}
	Interaction	0.046	2.897	0.006	0.1	6.812	2.1×10^{-5}
	Residual	0.016			0.014		
Protein (pg μm^{-3}) [-/-]							
	Temperature	0.077	40.63	3.2×10^{-12}	9×10^{-5}	0.03	0.993
	Strain	0.014	7.248	0.0002	0.023	7.387	0.0007
	Interaction	0.013	6.734	2×10^{-7}	0.02	6.421	3.6×10^{-5}
	Residual	0.002			0.003		
Carbohydrate (pg μm^{-3}) [a/a]							
	Temperature	0.073	12.16	8.5×10^{-6}	0.457	58.48	4.4×10^{-13}
	Strain	0.137	22.93	6.7×10^{-10}	2.592	331.7	3.6×10^{-24}
	Interaction	0.021	3.477	0.00146	0.147	18.77	2.2×10^{-10}
	Residual						

Lipid (pg μm^{-3}) [c/-]							
	Temperature	$1.7x10^{-9}$	42.06	$1.9x10^{-12}$	0.191	29.82	$2.2x10^{-9}$
	Strain	$9.9x10^{-10}$	25.15	$1.9 x10^{-10}$	0.076	11.8	$2.3x10^{-5}$
	Interaction	$1.2x10^{-9}$	30.81	$2x10^{-17}$	0.012	1.842	0.099
	Residual				0.006		

ANOVA with (N)/ ANOVA without (N)

[a] heterogeneous

[b] log transformed

[c] square root transformed

[-] no transformation

3.4. Discussion

Temperature influences microalgal physiology (Goldman, Ryther, 1976), growth (Thompson et al., 1992; Renaud et al., 1995), and nutritional value (Thompson et al., 1992; Renaud et al., 2002). These changes can then affect the efficiency of biomass transfer between trophic levels in aquaculture practices (Scott and Baynes, 1978; Brown et al., 1997). However, the response of microalgal growth and biochemical composition to temperature varies between species (Aaronson, 1973; Tomaselli et al., 1988; Thompson et al., 1992). Thus, aquaculturists must pay attention to which prey species they use. The present study takes this concept one step further and asks the question: do aquaculturists also need to consider which strains they use? In fact, there are significant differences between stains, in terms of growth and biochemical parameters, and these are influenced by temperature. Below is a discussion of the potential impact of these affects.

3.4.1. Growth and production of strains at various temperatures

Positive relationships between microalgal growth rate and temperature have been well documented (Thompson et al., 1992; Montagnes and Franklin, 2001). Recently, it has been suggested that the typical response of the growth rates of protists to increasing temperature is linear over a defined growth range (Eppley, 1972; Montagnes et al., 2003). Above a critical temperature growth rate typically declines rapidly, due to stressful conditions (Eppley, 1972; Montagnes et al., 2003). For most *I. galbana* strains that critical temperature is ~30°C (Nelson et al., 1992; Renaud et al., 1995; 2002), and it is ~20°C for *Nannochloropsis* sp.(James et al., 1989; Brown,

Jeffrey, 1992). The *I. galbana* growth data in this study, which did not extend the rate beyond 30°C, indicate no critical upper limit (Fig. 1) while, in contrast, a critical temperature of ~20°C was supported for *Nannochloropsis* sp. (Fig. 1).

The average slope of *I. galbana* strains growth rate lines was 0.025 ± 0.006 d^{-1} $°C^{-1}$; this is within the range found by studies that examined the response of growth to temperature for *I. galbana* (from 0.01 to 0.035 d^{-1} $°C^{-1}$: Montagnes and Franklin, 2000; Montagnes et al., 2003). It was, however, lower than the average slope for protists (mean slope: 0.065 ± 0.005 d^{-1} $°C^{-1}$: Montagnes et al. 2003). Thus, this study suggests that the response of *I. galbana* strains is not as great as the typical response for protists in general.

The implication of these results for aquaculture is clear. If a maximum growth rate is desired for *Nannochloropsis* sp. it need not be grown above 20°C. In contrast, most of *I. galbana* strains could easily be grown at 30°C, but above this temperature a drop in growth might be expected (Nelson et al., 1992; Renaud et al., 1995; Renaud et al., 2002). Furthermore, as in most *I. galbana* strains, growth rate followed a linear relationship over their defined growth temperature rage, we can predict the growth of these strains at various temperatures, assuming a slope of ~ 0.025 ± 0.006 d^{-1} $°C^{-1}$.

Parameters other than growth are also significantly affected by temperature changes, but these are also significantly influenced by different strains (Fig. 1, Table 1). In fact, since volume and dry weight decreased with increasing temperature while growth rate increased, production (the amount of volume made per day) was invariant with temperature for all strains and species, except at 15°C for two strains (Fig. 1, Table 1, 2). The linear decrease in cell volume with temperature is a general phenomenon for protists (Atkinson et al., 2003). Such a phenomenon was seen in

this study, although it differed significantly between strains (Fig. 1, Table 1). The response of volume decrease, scaled to 15°C (see Atkinson et al., 2003), varied from 3.6 for N, D, and T to 4.3 % of mean cell volume $°C^{-1}$ for M, and G. These scaled values agree favourably with the range given by Atkinson et al. (2003) but were above the mean typical response for protists (2.5% $°C^{-1}$; Atkinson et al., 2003). Thus although the growth data of *I. galbana* is less responsive to temperature than for other protists, its volume response is greater.

For aquaculture, the impact of decreased volume with temperature is twofold. Firstly, the size of the microaglae may affect their use as food, as predators may differ in their size selectivity (Vadstein et al., 1993; Hansen et al., 1997). Secondly, the variation in cell size between strains may affect their production. Since the decrease in cell volume resulted in an invariant relationship between temperature and production, aquaculturist interested in total yield (either in terms of cell volume or dry weight) may not need to consider temperature effects. In contrast, strain effects on production do need to be considered (Fig. 1, Table 2) (Wikfors and Patterson, 1994).

In conclusion, despite the growth rate data, the data presented here indicate that there are differences between flagellate strains at the various temperatures, and such differences were as large as between species (Fig. 1, Table 1, 2). Thus, the general concern that aquaculturists have shown for species differences may also be applicable to strains.

3.4.2. Biochemical composition of strains at various temperatures

Temperature can have a strong effect on the gross biochemical composition of flagellates (Goldman and Mann, 1980; Nelson et al., 1992; Renaud et al., 1995; Renaud et al., 2002). In general, both from this study and from most of the literature, lipid tends to increase with temperature, protein tends to reach a maximum level over a range of temperatures, and no consistent relationship is observed between temperature and carbohydrate.

Increasing lipid content with increasing temperature has been reported for many microalgae species (Aaronson, 1973; Tomaselli et al., 1988; Thompson et al., 1992; Renaud et al., 1995). At higher temperatures lipid content of *I. galbana* reached a maximum (Brown et al., 1993; Molina Grima et al., 1994; Zhu et al., 1997). Data from this study also indicate the same response per cell and μm^{-3}, but the maximum lipid level was strain and species dependent, and the lipid content in each strain at different temperatures was significantly different (Fig. 2, 3, Table 3).

The protein trend, however, always indicated a maximum level over the temperature range. Protein per cell, for instance, showed a U-shaped relationship between 10 and 25°C for *I. galbana* T strain and other species (Thompson et al., 1992). Furthermore, at higher temperatures (25 to 35 °C) protein content in *I. galbana* T strain and other species increases and then sharply decreases (Renaud et al., 2002). In addition, for *Nannochloropsis* sp. the total amino acid content (a proxy for protein) between 25 and 30°C increased with temperature (James et al., 1989). However, the previous work on microalgal protein content records variations between species at such temperatures, and maximum protein content was rather species dependent. In this study protein content increased and then decreased, which

supports the overall trend recorded in the literature for both *I. galbana* and *Nannochloropsis* sp. However, both strain and species have been shown here to differ in their maximum protein content, both $cell^{-1}$ and μm^{-3} (Fig. 1, 2).

In contrast to the lipid and protein trends, there is no clear effect of temperature on carbohydrate levels. Generally, in microalgal the biochemical composition of rapidly growing cells is characterised by a high protein and low carbohydrate content (Zhu et al., 1997). However, the literature on microalgal biochemical composition indicates fluctuations in carbohydrate content with temperature (Thompson et al., 1992; Renaud et al., 2002). This study supports that finding (Fig. 2, 3), but most importantly, strains showed significant interaction with temperature, indicating that both temperature and strain must be considered when examining microalgae carbohydrate content.

For aquaculture, *I. galbana* and *Nannochloropsis* sp. are photosynthetic producers of useful products such as lipid (e.g., isoprenoid derivatives, carotenoids, and fatty acids), and protein (e.g., amino acids). Therefore, the optimum lipid and protein content of these strains might need to be predicted. This study suggests higher temperatures are needed for *I. galbana* strains M and G to yield maximum lipid and protein levels. For *Nannochloropsis* sp. and *I. galbana* strain T maximum lipid and protein levels are produced between 25-30°C, whereas maximum lipid and protein production are produced at lower temperatures (20-25°C) for *I. galbana* strain D. Therefore, although most of strains here followed the general trend of microalgae lipid and protein quotas, they behaved differently.

3.5. Application of results to aquaculture

Despite the variations observed between the *I. galbana* strains, we recommend that most of them can grow successfully at 30°C, but higher temperature tolerances beyond that may vary between strains. Growth and biochemical analyses reported here were not designed to define the optimum algal diet, but rather to determine how algal growth and biochemical composition varies between strains under temperatures used by most aquaculturists. Such information may be useful for the best application of these strains as diets. The observed variation in biochemical composition content does, however, illustrate the range for manipulation of algal biochemical constituents. Thus, we suggest that the production and nutritional quality of the strains could be improved by cultivating them under defined conditions.

3.6. References

Aaronson, S., 1973. Effects of incubation temperature on the macromolecular and lipid content of the phytoflagellate *Ochromonas danica*. J. Phycol. 9, 111-113.

Anon, 1975. Algal culture; development of a working system. Fish Farming Int. 2, 28-29.

Atkinson, D., Ciotti, B.J., Montagnes, D.J.S., 2003. Protists decrease in size linearly with tempeature:*ca*.2.5%°C^{-1}. Proc. R. Soc. Lond. B. Biol. Sci. 270, 2605-2611.

Babarro, J.M.F., Reiriz, M.J.F., Labarta, U., 2001. Influence of preservation techniques and freezing storage time on biochemical composition and spectrum of fatty acids of *Isochrysis galbana* clone T-ISO. Aquacult. Res. 32, 565-572.

Babinchak, J.A., Ukeles, R., 1979. Epifluorescence microscopy, a technique for the study of feeding in *Crassostrea virginica* veliger larvae. Mar. Biol. 51, 69-76.

Baynes, S.M., Scott, A.P., 1979. Unicellular algae in the diet of turbot larvae. Br. Phycol. J. 14, 119.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Borowitzka, M.A., 1997. Microalgae for aquaculture: opportunities and constraints. J. Appl. Phycol. 9, 393-401.

Bourne, N., 1988. Scallop breeding studies. J. Shellfish Res. 7, 111-112.

Boussiba, S., Sandbank, E., Shelef, G., Cohen, Z., Vonshak, A., Ben-Amotz, A., Arad, S., Richmond, A., 1988. Outdoor cultivation of the marine microalga *Isochrysis galbana* in open reactors. Aquaculture 72, 247-253.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal. Biochem. 72, 248-254.

Brown, M.R., 1991. The amino-acid and sugar composition of 16 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 145, 79-99.

Brown, M.R., Jeffrey, S.W., 1992. Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 1. Amino acids, sugars and pigments. J. Exp. Mar. Biol. Ecol. 161, 91-113.

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151, 315-331.

Brown, M.R., Dunstan, G.A., Jeffrey, S.W., Volkman, J.K., Barrett, S.M., LeRoi, J.M., 1993. The influence of irradiance on the biochemical composition of the prymnesiophyte *Isochrysis sp.* (clone T-ISO). J. Phycol. 29, 601-612.

Cho, J.Y., Jin, H.J., Lim, H.J., Whyte, J.N.C., Hong, Y.K., 1999. Growth activation of the microalga *Isochrysis galbana* by the aqueous extract of the seaweed *Monostroma nitidum*. J. Appl. Phycol. 10, 561-567.

Dai, J.b., Wu, Q.y., 2000. The Growth and Biochemical Quality of Marine Unicellular Microalga *Isochrysis galbana* in Outdoor and Indoor Mass Culture. Mar. Sci. 24, 32-34.

Dortch, Q., Conway, H.L., 1984. Interactions between nitrate and ammonium uptake: variation with growth rate, nitrogen source and species. Mar. Biol. 79, 151-164.

Enright, C.T., Newkirk, G.F., Craigie, J.S., Castell, J.D., 1986. Evaluation of phytoplankton as diets for juvenile *Ostrea edulis*. J. Exp. Mar. Biol. Ecol. 96, 1-13.

Eppley, R.W., 1972. Temperature and Phytoplankton Growth in the Sea. Fishery bulletin 70, 1063-1085.

Goldman, J.C., Ryther, J.H., 1976. Temperature influenced species competition in mass cultures of marine phytoplankton. Biotechnol. Bioeng. 18, 1125-1144.

Goldman, J.C., Mann, R., 1980. Temperature influenced variations in speciations and chemical composition of marine phytoplankton in outdoor mass cultures. J. Exp. Mar. Biol. Ecol. 46, 29-39.

Grima, E.M., Perez, J.A.S., Sanchez, J.L.G., Camacho, F.G., Alonso, D.L., 1992. EPA from *Isochrysis galbana* . Growth conditions and productivity. Process Biochem 27, 299-305.

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), Culture of Marine Invertebrate Animals. Plenum Press, New York, pp. 29-60.

Hansen, B., Moller, T., Wittrup, L., 1997. Particle grazing efficiency and specific growth efficiency of the rotifer *Brachionus plicatilis* (Muller). J. Exp. Mar. Biol. Ecol. 215, 217-233.

James, C.M., Al-Hinty, S., Slman, A.E., 1989. Growth and ω3 fatty acid composition of marine microalgae under different temperature regimes. Aquaculture 77, 337-351.

Kanazawa, A., Taki, Y., Primavera, J.H., Llobrera, J.A., 1985. Nutrition of penaeid prawns and shrimps, Workshop on the Reproduction and Culture of Milkfish, Tungkang Marine Laboratory, Tungkang (Taiwan), pp. 123-130.

Knauer, J., Southgate, P.C., 1999. A review of the nutritional requirements of bivalves and the development of alternative and artificial diets for bivalve aquaculture. Rev. Fish. Sci. 7, 3-4.

Kochert, G., 1978. Quantitation of the macromolecular components of microalgae. Cambridge University Press, Cambridge, 189-195 pp.

Lavens, P., Sorgeloos, P., 1996. Manual on the production and use of live food for aquaculture. Food and Agriculture Organization of the United Nations, 83 pp.

Molina Grima, E., Garcia Camacho, F., Sanchez Perez, J.A., Garcia Sanchez, J.L., 1994. Biochemical productivity and fatty acid profiles of *Isochrysis galbana* Parke and *Tetraselmis* sp. as a function of incident light intensity. Process Biochem. 29, 119-126.

Molina Grima, E., Fernandez Sevilla, J.M., Sanchez Perez, J.A., Garcia Camacho, F., 1996. A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances. J. Biotechnol. 45, 59-69.

Montagnes, D.J.S., Weisse, T., 2000. Fluctuating temperatures affect growth and production rates of planktonic ciliates. Aquat. Microb. Ecol. 21, 97-102.

Montagnes, D.J.S., Franklin, D.J., 2001. Effect of temperature on diatom volume, growth rate, and carbon and nitrogen content: Reconsidering some paradigms. Limnol. Oceanogr. 46, 2008-2018.

Montagnes, D.J.S., Kimmance, S.A., Atkinson, D., 2003. Using Q_{10}: Can growth rates increase linearly with temperature? Aquat. Microb. Ecol. 32, 307-313.

Mourente, G., Medina, A., Gonzalez, S., Rodriguez, A., 1995. Variations in lipid content and nutritional status during larval development of the marine shrimp *Penaeus kerathurus*. Aquaculture 130, 187-199.

Muller-Feuga, A., Cahu, C.L., Robert, R., Robin, J., Divanach, P., 2003. Uses of Microalgae in Aquaculture. In: Støttrup, J.G., McEvoy, L.A. (Eds.), Live feeds in marine aquaculture. Blackwell Science Ltd, Cornwall, pp. 253-299.

Nelson, J.R., Guarda, S., Cowell, L.E., Heffernan, P.B., 1992. Evaluation of microalgal clones for mass culture in a subtropical greenhouse bivalve hatchery: Growth rates and biochemical composition at 30 degree C. Aquaculture 106, 3-4.

Pande, S.V., Parvin, R., Venkitasubramanian, 1963. Microdetermination of lipids and serum total fatty acids. Anal. Biochem 6, 415-425.

Price, L.L., Yin, K., Harrison, P.J., 1998. Influence of continuous light and L:D cycles on the growth and chemical composition of Prymnesiophyceae including coccolithophores. J. Exp. Mar. Biol. Ecol. 223, 223-234.

Reitan, K.I., Rainuzzo, J.R., Oeie, G., Olsen, Y., 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155, 207-221.

Renaud, S.M., Thinh, L., Lambrinidis, G., Parry, D.L., 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 211, 195-214.

Renaud, S.M., Zhou, H.C., Parry, D.L., Thinh, L.-V., Woo, K.C., 1995. Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, *Nitzschia paleacea*, and commercial species *Isochrysis* sp. (clone T.ISO). J. Appl. Phycol. 7, 595-602.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D., 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179, 217-229.

Sargent, J.R., McEvoy, L.A., Bell, J.G., 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture 155, 1-4.

Scott, A.P., Baynes, S.M., 1978. Effect of algal diet and temperature on the biochemical composition of the rotifer, *Brachionus plicatilis*. Aquaculture 14, 247-260.

Sukenik, A., Wahnon, R., 1991. Biochemical quality of marine unicellular algae with special emphasis on lipid composition. 1. *Isochrysis galbana*. Aquaculture 97, 61-72.

Thompson, P.A., Guo, M.X., Harrison, P.J., 1992. Effects of variation in temperature. 1. On the biochemical composition of eight species of marine phytoplankton. J. Phycol. 28, 481-488.

Thompson, P.A., Guo, M.X., Harrison, P.J., Whyte, J.N.C., 1992. Effects of variation in temperature. 2. On the fatty acid composition of eight species of marine phytoplankton. J. Phycol. 28, 488-497.

Tomaselli, L., Giovannetti, L., Sacchi, A., Bocci, F., 1988. Effects of temperature on growth and biochemical composition in *Spirulina platensis* strain M2. Elsevier Applied Science, London, 305-314 pp.

Underwood, A.J., 1997. Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge, 504 pp.

Vadstein, O., Oeie, G., Olsen, Y., 1993. Particle size dependent feeding by the rotifer *Brachionus plicatilis*. In: Gilbert, J.J., Lubzens, E., Miracle, M.R. (Eds.), 6. Int. Rotifer Symp. Hydrobiologia, Banyoles (Spain), pp. 261-267.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 128, 219-240.

Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 89, 263-272.

Wikfors, G.H., Patterson, G.W., 1994. Differences in strains of *Isochrysis* of importance to mariculture. Aquaculture 123, 127-135.

Yongmanitchai, W., Ward, O.P., 1991. Screening of algae for potential alternative sources of eicosapentaenoic acid. Phytochemistry 30, 2963-2967.

Zar, J.H., 1999. Biostatistical Analysis. Prentice Hall International, Inc, New Jersey, USA, 663 pp.

Zhu, C.J., Lee, Y.K., Chao, T.M., 1997. Effects of temperature and growth phase on lipid and biochemical composition of *Isochrysis galbana* TK1. J. Appl. Phycol. 9, 451-457.

Chapter 4.

Microalgae strain and temperature effects on *Brachionus plicatilis* development and survival

3.1. Introduction

The rearing of many marine fish larvae requires the use of the rotifer *Brachionus plicatilis* as a live food (Lubzens et al., 1989). Although *B. plicatilis* is not a natural live food for fish, it has been widely used due its ideal size, quick reproductive rate, and ability to feed on a variety of microalgae. Microalgae are frequently used as a diet for the rotifer *B. plicatilis* and can be used as the main food source (Tamaru et al., 1993; Øie et al., 1994). Rotifers are then used as live feed in the rearing of marine fish larvae.

Many reports indicated the positive nutritional effects of microalgae on rotifer cultivation (Hirayama et al., 1979; Okauchi and Fukusho, 1984; Tamaru and Murashige et al., 1993). However, most of these studies have focused on the effect of food concentration (Schmid-Araya and See, 1991), food species (Yufera et al., 1983; Yufera and Pascual, 1984), or feeding condition (Carmona et al., 1994; Yufera and Navarro, 1995). Few studies have examined the impact of microalgal strains or the temperature at which they are grown, two factors that affect microalgal quality (see Chapter 3).

Microalgae used in rotifer production can affect several rotifer growth parameters, which then may be used to assess the production of rotifers. For instance, microalgae species have been reported to affect rotifer growth rate (Snell et al., 1983; Korstad et al., 1989), rotifer length and individual biomass (Scott and Baynes, 1978; Okauchi and Fukusho, 1984), and the ability of rotifers to survive and remain viable during rinsing and transfer to larval tanks (Øie and Olsen, 1993). This study investigates the effect of food quality (influenced by strain/species and prey growth-temperature) on rotifer production by carefully examining a number of

parameters associated with their growth and survival. In this way, it was possible to assess more specifically the influence of prey quality.

Although growth rate, fecundity, and survival of rotifers grown on different algal species have been well documented, there is no information available on the effect of different algal strains on rotifer culture. Furthermore, there is no information available on the effect of these strains at various growth-temperatures. The importance of gaining knowledge about the effect of strains has become apparent in rearing fish larvae since more than one strain of the same species has been used worldwide under different temperature conditions.

4.2. Material and Methods

4.2.1. Algal and rotifer stock cultures

Strains of *Isochrysis galbana* from: the Isle of Man (M), Tahiti (T), Greece (G), Denmark (D), and one strain of *Nannochloropsis* sp. (N) were obtained from the plankton culture collection at Port Erin Marine Laboratory, Isle of Man (see Chapter 2, Table 1). The flagellates were all grown in 200 ml flat bottom flasks with f/2 medium (Guillard, 1975). All strains and species were maintained in semi-continuous culture, by transfers every two weeks, at 20 °C, on a 12:12 light: dark cycle, at an irradiance of 100 $\mu E\ m^{-2}s^{-1}$.

The rotifer *Brachionus plicatilis* was obtained from the Larval-Rearing Centre at Port Erin Marine Laboratory, where it was maintained at 25 °C. Rotifers were collected onto a 45 μm mesh and rinsed with filtered seawater (Whatman

GF/C), to remove contaminants, to remove prey from the original cultures, and to concentrate rotifers. Then the rotifers were resuspended in filtered seawater (32 ppt) and fed on a diet of *Nannochloropsis* sp. To ensure uniform food distribution and to prevent sedimentation, gentle aeration was provided. Light conditions were the same as those provided for the flagellates.

4.2.2. Algal culture diet treatment

The four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) were obtained from stock cultures. Each algal strain/species was grown in 3 L round bottom flasks in modified f/2 medium and grown at different temperatures. All flasks were maintained in exponential growth phase by semi-continuous culturing on a 24:0 light: dark cycle. An irradiance of 100 $\mu E\ m^{-2}s^{-1}$ was provided, which is considered optimal for flagellate growth rate (Brown et. al., 1993; Molina Grima et. al., 1994; Molina Grima et. al., 1996). All cultures were maintained for >10 generations; flasks were suspended in a controlled water bath at 15, 20, 25, and 30 ± 1°C and were gently agitated at least once a day. Each of these flasks was kept as a prey treatment for rotifer rearing cultures.

4.2.3. Rotifer rearing cultures

Rotifers were collected from the stock culture onto a 45 µm mesh and rinsed with filtered seawater. Five hundred rotifers were placed in each of the three replicate rotifer culture treatments; each group was fed on a different prey that was grown at a differed temperatures. These rotifers were fed 100 ml of the algal culture

treatment at the concentration of ~ 6 x 10^4 cell ml^{-1}, this food concentration is sufficiently high to maintain maximum growth rate for rotifer following recommendations of Hansen et. al. (1997). The concentration of the algal culture was monitored using a Model II Coulter Multisizer, and associated software V.4.1 equipped with a population accessory (Coulter Electronics Ltd., England). To maintain rotifers in exponential growth, to maintain algae concentration, and to increase the volume of the cultures, every day 100 ml of each of the flagellate culture treatment at (~ 6 x 10^4 cell ml^{-1}) was added to the rotifer culture. Rotifers were cultured on this diet for ~7 generations at 25 °C; generation time was calculated following the equation of Hirayama and Kusano (1972).

To measure rotifer body volume and egg volume, forty active rotifers were randomly selected and examined. These were from each of the rotifer replicate culture treatments (i.e., fed different algal prey that were grown at differed temperatures). Rotifers were placed in a 10 ml settling chamber and were measured using an inverted microscope (Zeiss Axiovert 135tv) equipped with a video camera (JVC model 3-CCD, 750 lines horizontal resolution), attached to a Pentium IIPC with image analysis software (Scion Image for Windows, Scion Corp., MD, USA). The volume was calculated following the method of Yufera (1982). Rotifer body volume and egg volume was assumed to be a prolate spheroid shape. For each rotifer and egg, two linear dimensions were measured (length (L) and width (W)), and the volumes were estimated from the following equation:

$$V=4/3\ \pi\ (W/2)^2\ (L/2)$$

The remainder of the rotifer culture was rinsed with filtered seawater (Whatman GF/C) and collected onto a 45 μm mesh to remove prey and to concentrate the rotifers. The concentrated rotifers were used to estimate dry weight.

For dry weight, the concentrated rotifers were collected on (Whatman GF/C) filters and gently washed with a solution of 0.5 M ammonium formate to remove salts; filters were dried at 60°C for 12h before weighting. Due to methodological problems data for three dry weight measurements (T, M, G) at 25°C were estimated from the average of the data at 20 and 30°C.

Rotifer growth rate was measured by determining abundance daily and was calculated as the slope of *ln* numbers vs. time in days. Rotifer mass production (ng d^{-1}) was calculated by multiplying growth rate and the estimate of individual dry weight, whereas volume production ($\mu m^3 d^{-1}$) was measured by multiplying growth rate and the measurement of individual rotifer body volume.

4.2.4. Rotifer individual cultures

Parthenogenetic eggs, produced by amictic females, from the cultures above (i.e., the different prey treatments), were collected in petri dishes. Offspring hatching from these eggs within one day were pipetted into 10 ml wells in tissue culture plates. The first offspring from females produced by these eggs were used for experiments. Therefore, the test animals were the first-laid eggs from offspring of amictic females, isolated from experimental treatments (i.e., prey strains/species, growth-temperature).

Individual eggs, produced by the females above, were isolated in 1 ml of the appropriate food-strain, in 12-well tissue culture trays; prey concentrations were ~ 6 x 10^4 cell ml^{-1}. Three trays, of 12 wells, were established for each food-strain (i.e., 36 eggs in total). The average response of a tray was determined; thus n=3.

Eggs hatched the following day, producing a "parent". The number of offspring and eggs produced by each parent were counted every 2h, for 12; these data were used to establish daily rates (see below). The next day the parent was isolated in a new tray with fresh culture medium and prey; attached eggs were left with the parent. This process of evaluating the reproductive output of the parent was continued until the parent died.

From the above experiment, several measurements were made; these are outlined below. The number of eggs laid per day, per animal (fecundity), at different ages (i.e., different days), was estimated. For all cases there was an initial increase in fecundity followed by a decrease. The day of peak fecundity was recorded, and the rate of decline in fecundity (d^{-1}) was calculated as the slope of *ln* daily fecundity vs time. Mortality rate of parents (d^{-1}) was also estimated as the slope of *ln* number of parents vs. time (starting when the first parent died); then the day to 50% survival was determined. Not all eggs produced by a parent hatched: the percentage of egg hatching (hatched eggs %) was determined as the number of eggs in a tray, on a given day, that hatched divided by the total number of eggs produced on that day, multiplied by 100.

From the above analysis several other parameters were also calculated. The different development stages were determined as outlined by Schmid-Araya and See (1991): duration of embryonic development (De), duration of post-embryonic development (Dj), duration of the interval between eggs (Di), duration of the reproductive period (Dr), duration of the post reproductive period (Dp), and duration of the life-span (Dl) (see Fig. 1, Chapter2 for further explanation on the determination of duration of different development stages). Furthermore, the

average number of eggs laid by a female in her entire lifetime (net reproduction rate; R_0) was determined (Bradford, 1976; Bosque et. al., 2001).

4.2.5. Statistical analyses

Two-way analyses of variance (ANOVA) were used to examine the differences in rotifer responses when they were fed different algal strains/species. Data always passed tests for normality. Where data were heteroscedastic, they were transformed (following recommendations of Zar (1999); see Results for details of transformations). However, in some cases data remained heteroscedastic, and ANOVA was still applied since this technique is robust to departure from homogenous variances (Underwood, 1997). Further analysis by Tukey's test indicated where significant differences occurred. In all analyses, the statistical significance was at $P<0.05$. Statistical analysis used STATISTCA version 5.0. Homogeneity of variance was tested by Cochran's test (Underwood, 1997).

4.3. Results

4.3.1. Affect on growth parameters

There were strong interactions between prey strains and prey growth-temperature on rotifer egg volume, body volume, net reproduction rate (R_o), hatched eggs %, dry weight, mass production, and volume production (Fig. 1, 2, Table 1). No obvious trends were observed in these parameters.

In general, the range of variation in egg volume, net reproduction rate (R_o), and hatched eggs % were from 3.4 to 5.4 x $10^5 \mu m^3$, 15 to 28 eggs, and from 73 to 100% hatched eggs, respectively (Fig. 1, 2). Rotifer body volume ranged from 2 to 3.3 x 10^6 μm^3. Whereas, mass production and volume production ranged from 87 to 292 (ng ind^{-1} d^{-1}), and from 71 to 178 (μm^3 d^{-1}), respectively (Fig. 2).

Prey strain and prey growth-temperature altered the growth rate of the rotifer, and no interaction occurred between these factors (Fig. 2, Table 1). The microalgal grown at low temperatures produced rotifers that had higher growth rates (Fig. 2). Overall, of the strains tested here, *I. galbana* T strain fed rotifers had the highest average growth rate (Table 1).

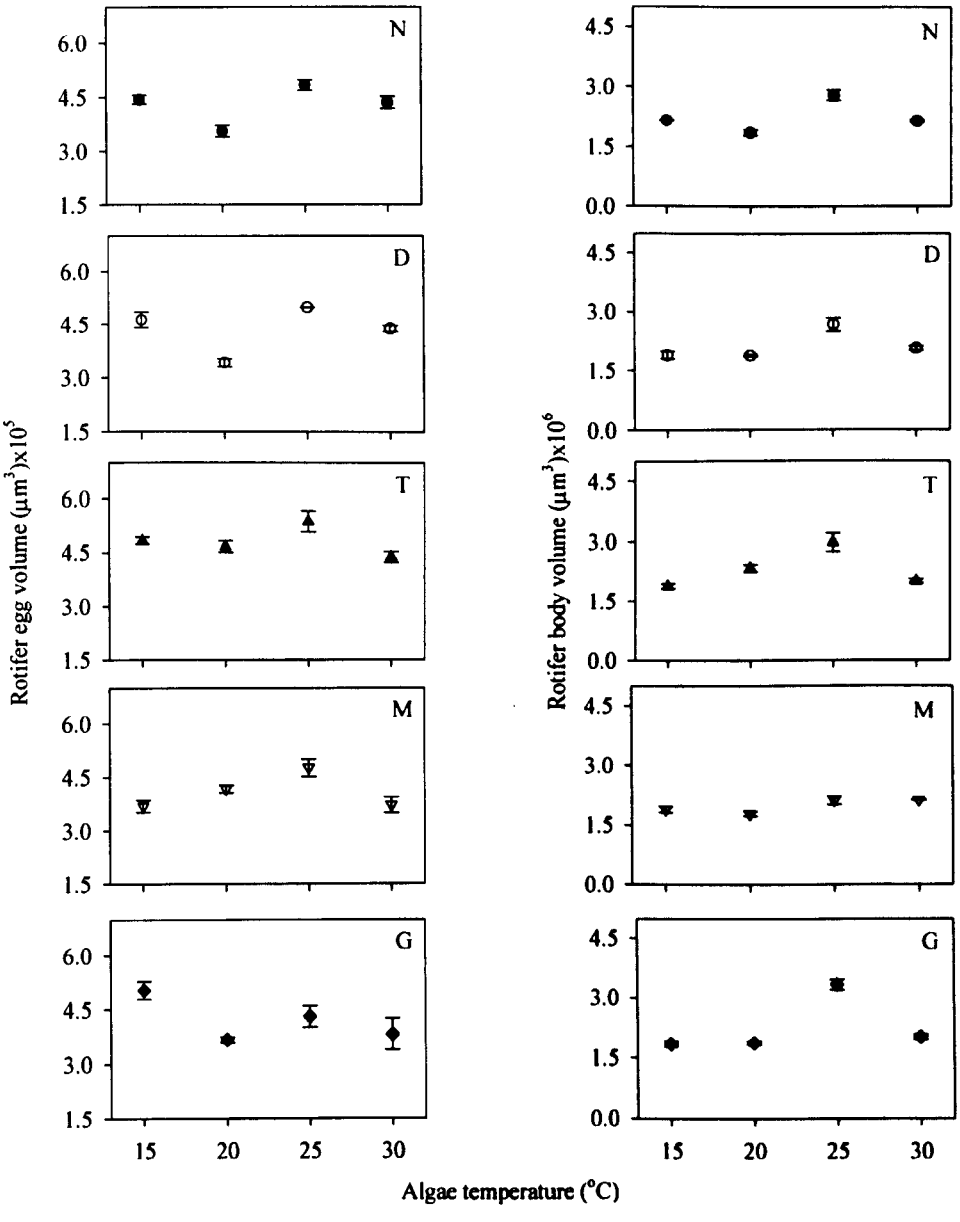

Fig. 1. Egg volume (μm^3) and body volume (μm^3) of the rotifer *Brachionus plicatilis* cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 Chapter 2 for strain designations). Bars are one standard error ±.

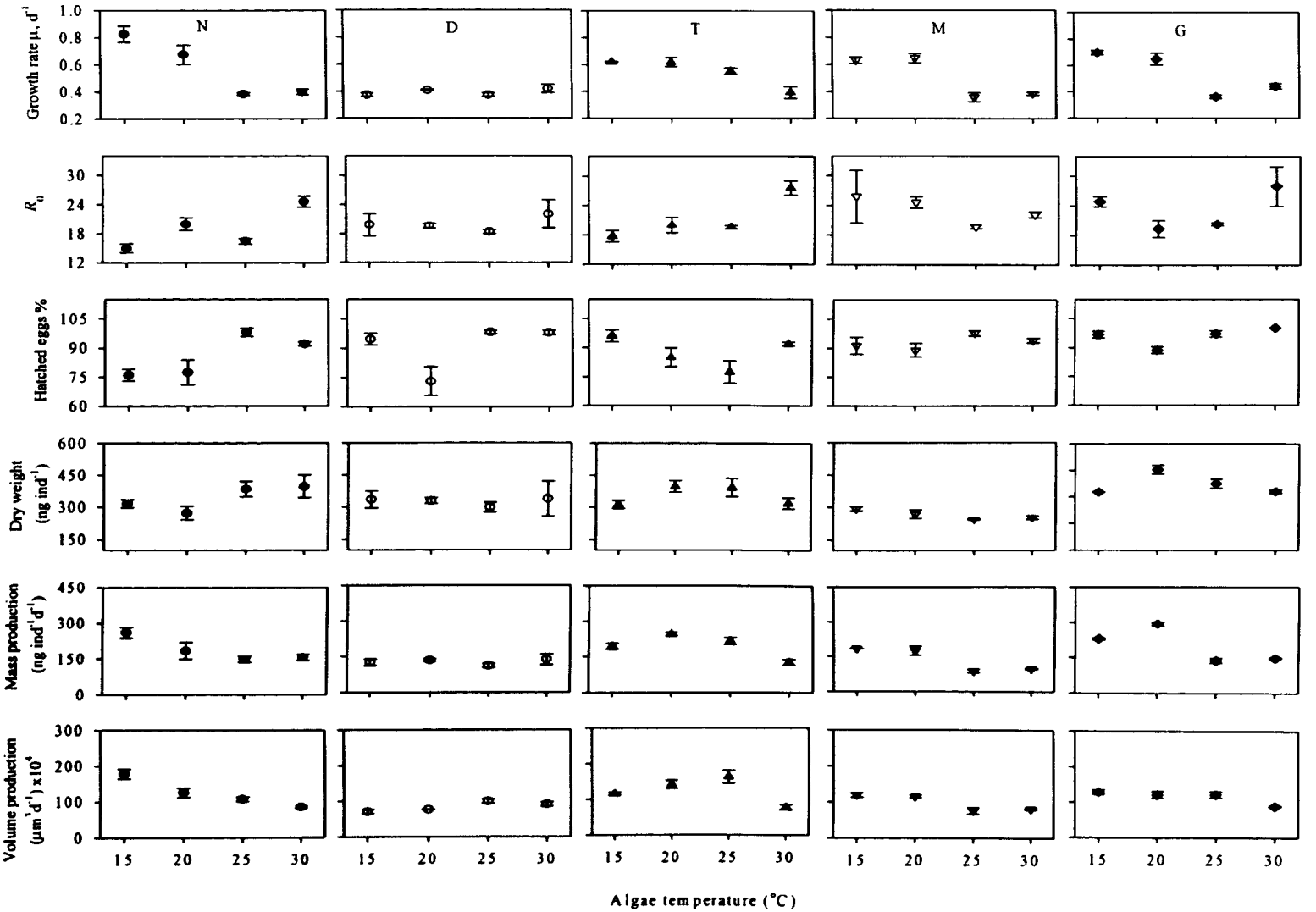

Fig. 2. The rotifer *Brachionus plicatilis* growth parameters: growth rate (μ, d^{-1}), number of eggs per life time (R_o), hatched eggs %, dry weight (ng ind^{-1}), mass production (ng ind^{-1} d^{-1}), and volume production ($\mu m^3 d^{-1}$) cultured on various algae strains (four strains of *Isochrysis galbana* (M, T, G, D)), and one strain of *Nannochloropsis* sp. (N) (see Table 1 Chapter 2 for strain designations). Bars are one standard error ±.

Table 1

Two-way ANOVA and Tukey's test for the indicated diet strain and growth-temperature factor on rotifer growth parameters. Symbols are as described in Table 1, Chapter 2. Tukey's test data are ordered from lowest (left) to highest (right); underscoring indicates where there were no significant differences at $p = 0.05$. For all tests degrees of freedom were 3 for temperature, 4 for strain, 12 for the interaction, and 40 for the residual. The body volume Factor was log transformed, and net reproduction rate (R_o) was 1/x transformed. Data of dry weight (ng ind^{-1}) and mass production (ng ind^{-1} d^{-1}) were heterogeneous even after transformation.

Factors		ANOVA			Tukey's test
		MS	*F*	*P-level*	
Egg volume (μm^3)					
	Algal temperature	$2.7x10^{10}$	18.443	$1.1x10^{-7}$	
	Algal strain	$9.4x10^{9}$	6.547	0.0004	
	Interaction	$5x10^{10}$	3.423	0.002	
	Residual	$1.4x10^{9}$			
Body volume (μm^3)					
	Algal temperature	0.078	91.264	$6.2x10^{-18}$	
	Algal strain	0.006	7.122	0.0002	
	Interaction	0.006	7.42	$6.2x10^{-7}$	
	Residual	0.001			
Growth rate (μ, d^{-1})					
	Algal temperature	0.008	43.51	$1.2x10^{-12}$	25 30 20 15
	Algal strain	0.002	7.728	0.0001	D M G N T
	Interaction	0.0002	1.528	0.155	
	Residual	0.0002			
Net reproduction rate (R_o)					
	Algal temperature	0.0004	11.12	$1.9x10^{-5}$	
	Algal strain	0.0002	5.917	0.0008	
	Interaction	0.0001	3.047	0.004	
	Residual	$3.8x10^{-5}$			

Hatched eggs %				
	Algal temperature	471.69	13.62	2.9×10^{-6}
	Algal strain	188.26	5.44	0.001
	Interaction	158.42	4.58	0.0001
	Residual	34.64		
Dry weight (ng ind^{-1})				
	Algal temperature	2225.91	0.736	0.54
	Algal strain	20816.8	6.881	0.0003
	Interaction	68333.9	2.26	0.027
	Residual	3024.91		
Mass production (ng ind^{-1} d^{-1})				
	Algal temperature	22267.6	36.13	1.8×10^{-11}
	Algal strain	14684.5	23.79	4×10^{-10}
	Interaction	4445.93	7.203	8.9×10^{-7}
	Residual	617.239		
Volume production (μm^3 d^{-1})				
	Algal temperature	4.1×10^{-11}	19.92	4.7×10^{-8}
	Algal strain	3.7×10^{-11}	17.77	1.9×10^{-8}
	Interaction	1.9×10^{-11}	9.169	4.1×10^{-8}
	Residual	2.1×10^{-10}		

4.3.2. Affect on fecundity and survival

In general, the treated rotifers had different fecundity patterns (Fig. 3), but a similar survival pattern (Fig. 4). No interaction occurred between strain and prey growth-temperature on rotifer days to peak of fecundity (Fig. 3, 5, Table 2). Tukey's test shows that there were no significant differences in days to peak of fecundity between prey temperature of 15, 20, and 25°C (~day 3.72 ± 0.14), but at 30°C the peak of reproduction was delayed until day ~7.07 ± 0.22 (Fig. 5).

An interaction occurred between strain and prey growth-temperature on the fecundity decline rate (d^{-1}) of the rotifers (Fig. 5, Table 2). The range of variation in rotifer fecundity decline rate (d^{-1}) was from 0.05 to 0.34 d^{-1} (Fig. 6). In contrast, no significant differences were found in either: day of 50% survival, or mortality rate (Fig. 6, Table 2). The average of day of 50% survival was 15.18 ± 0.65, and the average mortality rate was 0.077 d^{-1} ± 0.004 (Fig. 6).

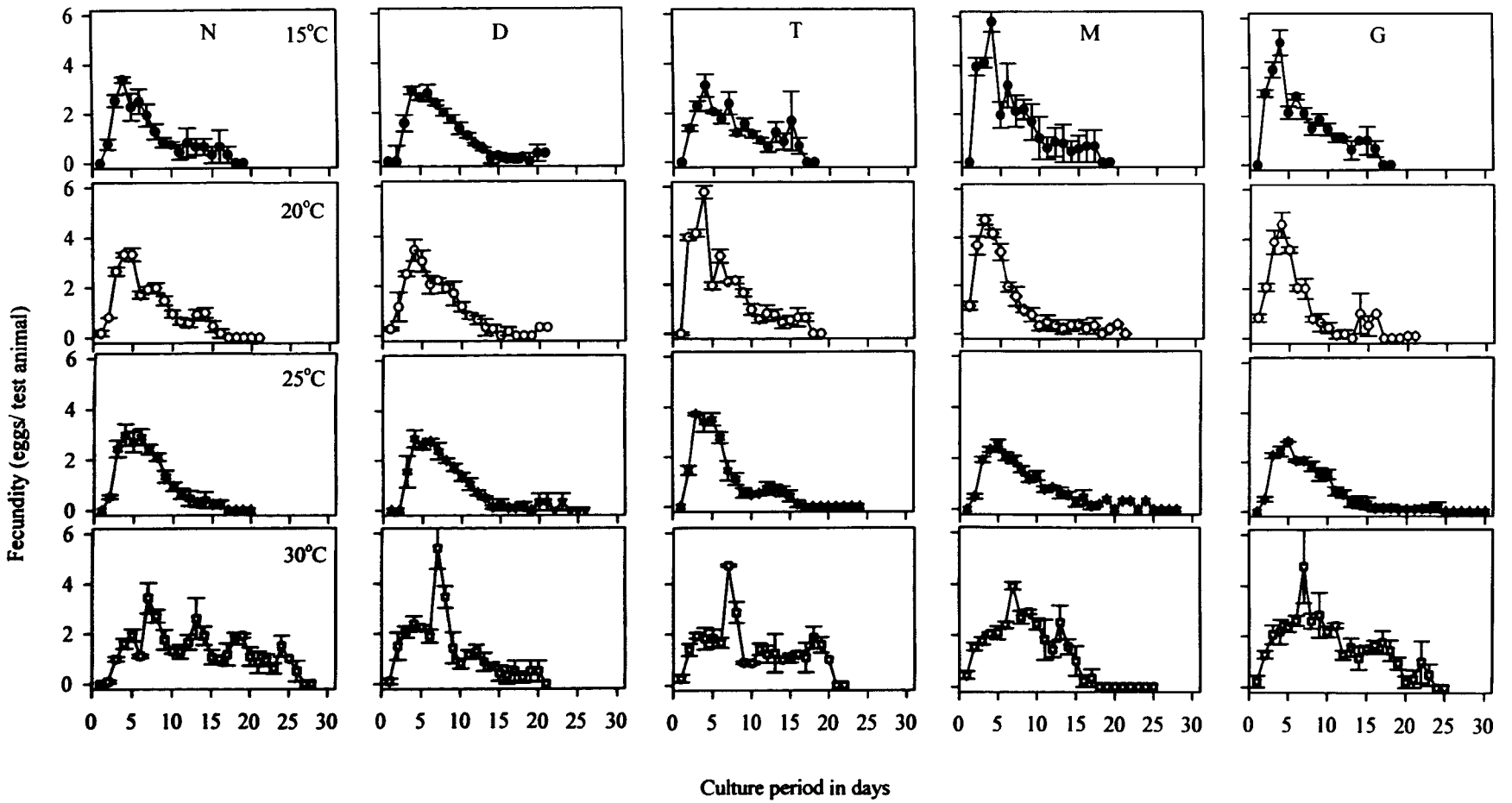

Fig. 3. Fecundity pattern of the rotifer *Brachionus plicatilis* cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 Chapter 2 for strain designations). Bars are one standard error ±.

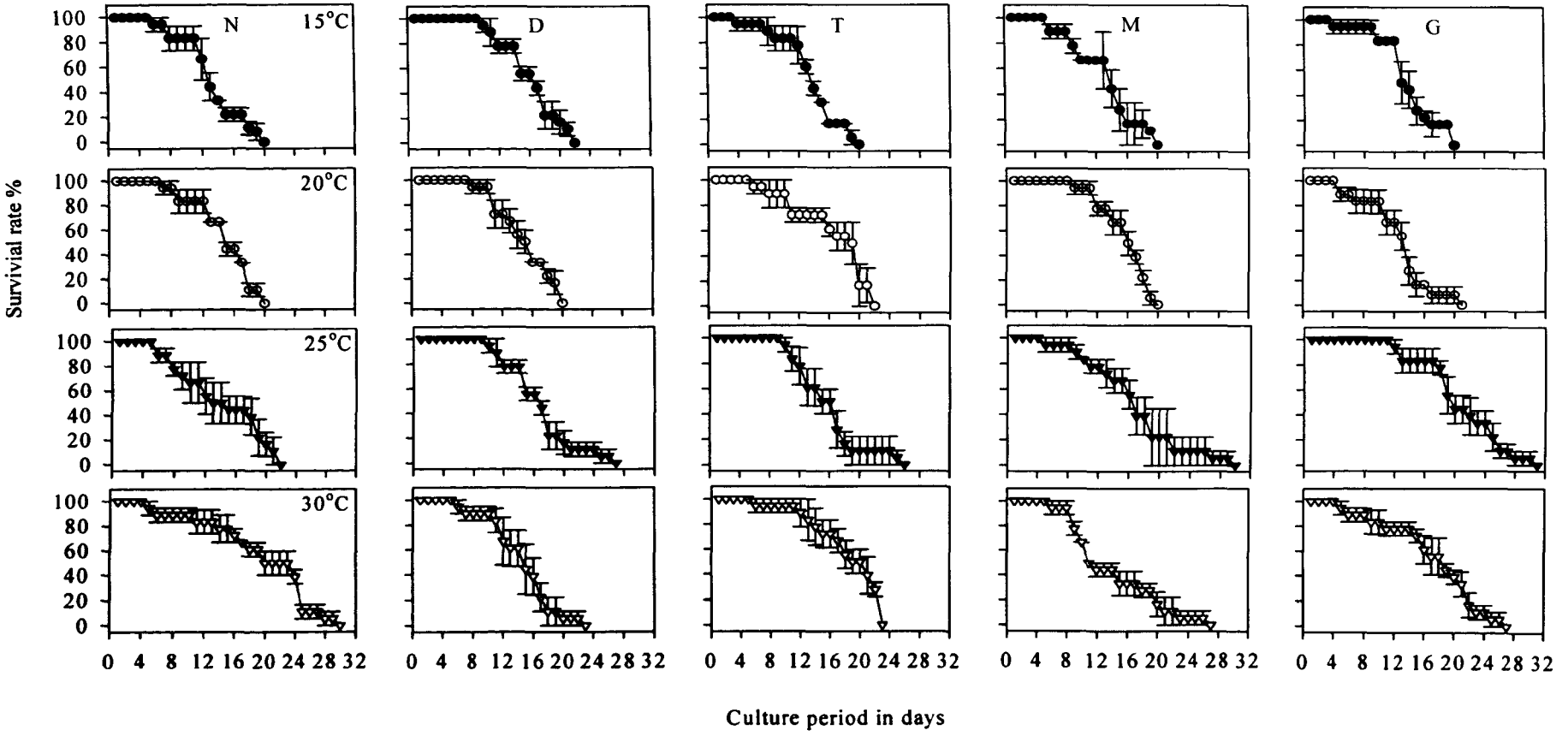

Fig. 4. The survival pattern of the rotifer *Brachionus plicatilis* cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 Chapter 2 for strain designations). Bars are one standard error ±.

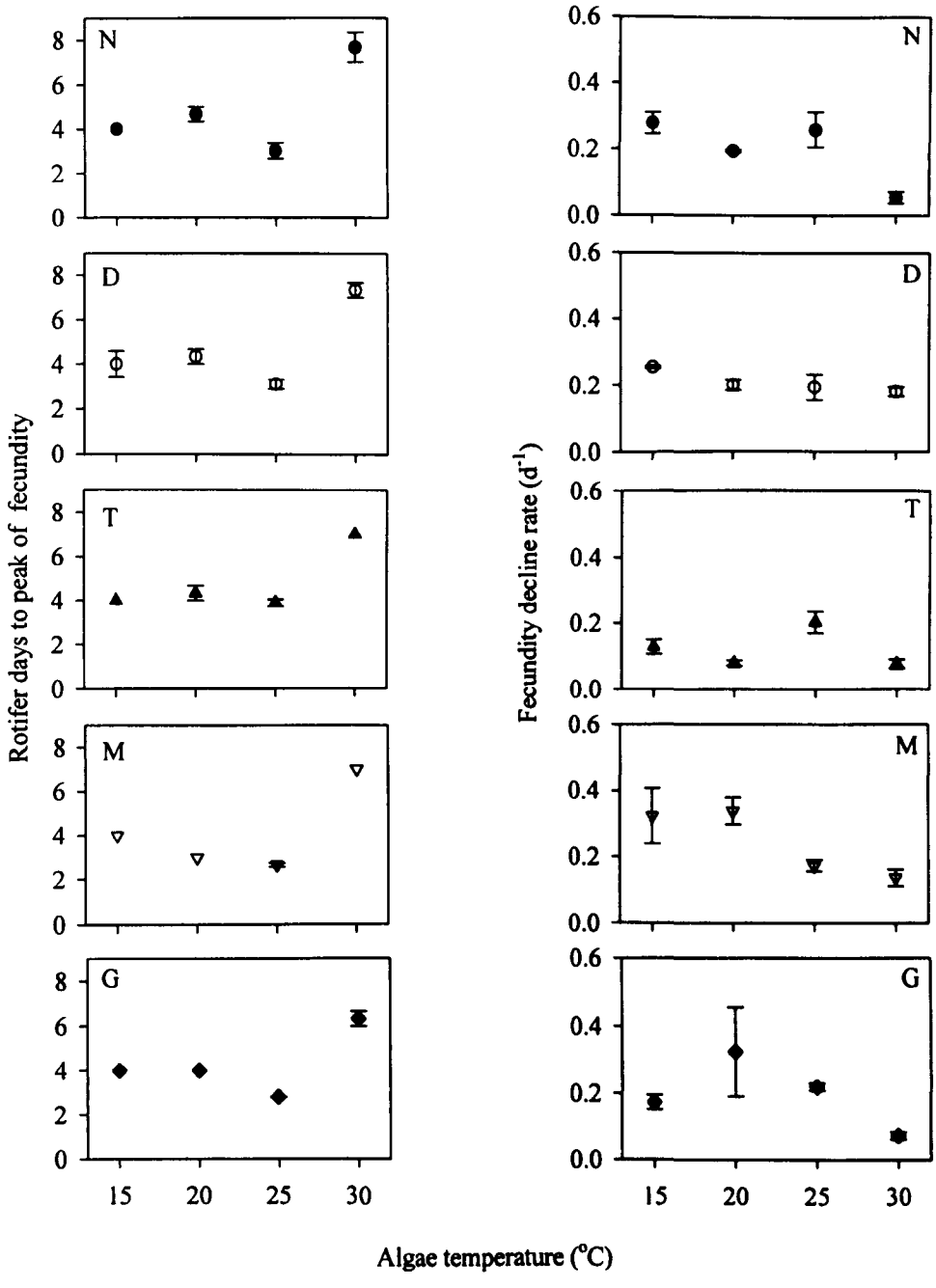

Fig. 5. The days to peak of fecundity, and fecundity decline rate (d^{-1}) of the rotifer *Brachionus plicatilis* cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 Chapter 2 for strain designations). Bars are one standard error ±.

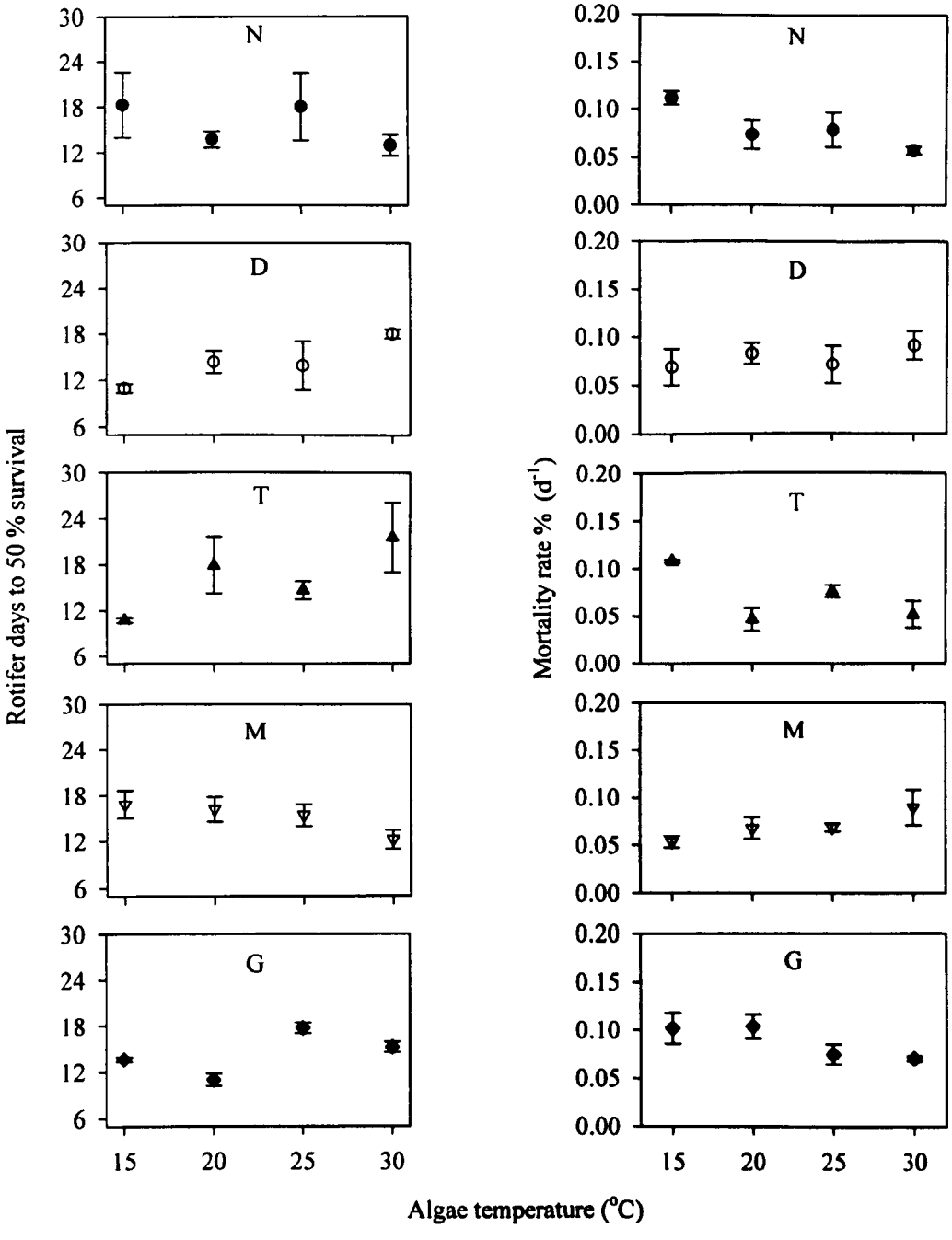

Fig. 6. The days to 50% survival and mortality rate (d^{-1}) of the rotifer *Brachionus plicatilis* cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 Chapter 2 for strain designations). Bars are one standard error ±.

Table 2

Two -way ANOVA and Tukey HSD test for the indicated diet strain and temperature factor on rotifer fecundity and survival parameters. Tukey's test data are ordered from lowest (left) to highest (right); underscoring indicates where there were no significant differences at $p = 0.05$. For all tests degrees of freedom were 3 for temperature, 4 for strain, 12 for the interaction, and 40 for the residual. Data of days to peak of fecundity was x^2 transformed, and fecundity decline rate (d^{-1}) was log transformed.

Factors		ANOVA			Tukey's test
		MS	*F*	*P-level*	
Days to peak of fecundity [a]					
	Algal temperature	38981.4	50.97	9.9×10^{-14}	15 20 25 30
	Algal strain	175.017	2.288	0.077	
	Interaction	74.528	0.974	0.488	
	Residual	76.483			
Fecundity decline rate (d^{-1}) [b]					
	Algal temperature	0.506	3.052	0.039	
	Algal strain	0.063	3.802	0.010	
	Interaction	0.068	4.086	0.0003	
	Residual	0.017			
Days to 50% survival					
	Algal temperature	0.012	1.271	0.297	
	Algal strain	0.004	0.441	0.778	
	Interaction	0.026	2.810	0.007	
	Residual	0.009			
Mortality (d^{-1})					
	Algal temperature	0.001	1.884	0.148	
	Algal strain	0.001	1.553	0.205	
	Interaction	0.001	2.686	0.005	
	Residual	0.001			

4.3.3. Affect of strain and prey growth-temperature on rotifer life history

In general, there were differences in the duration of life phases between the rotifers fed on each of the four *I. galbana* strains and *Nannochloropsis* sp. that were grown at the various temperatures (Fig. 7, Table 3), and there were strong interactions between both factors. Differences occurred independently in all phases of the life histories: duration of embryonic development (De), duration of post-embryonic development (Dj), duration of the interval between eggs (Di), duration of reproductive period (Dr), duration of post reproductive period (Dp), and duration of life-span (Dl) (Fig. 7, Table 3).

Although the prey treatments influence all life history phase durations, overall, the duration of De, Dj, and Di were short (hours) compared to those of Dr, Dp, and Dl (days) (Fig. 7). Therefore, the duration of Dr, Dp, and Dl were sufficiently long to have a marked affect on rotifer population growth, nevertheless of the differences in De, Dj, and Di would still have an effect on rotifer growth regardless of their short periods. The range of variation in Dr, Dp, and Dl were from day 7.35 to day 14, from day 1.22 to day 6.40, and from day 12.22 to day 20.70, respectively (Fig. 7).

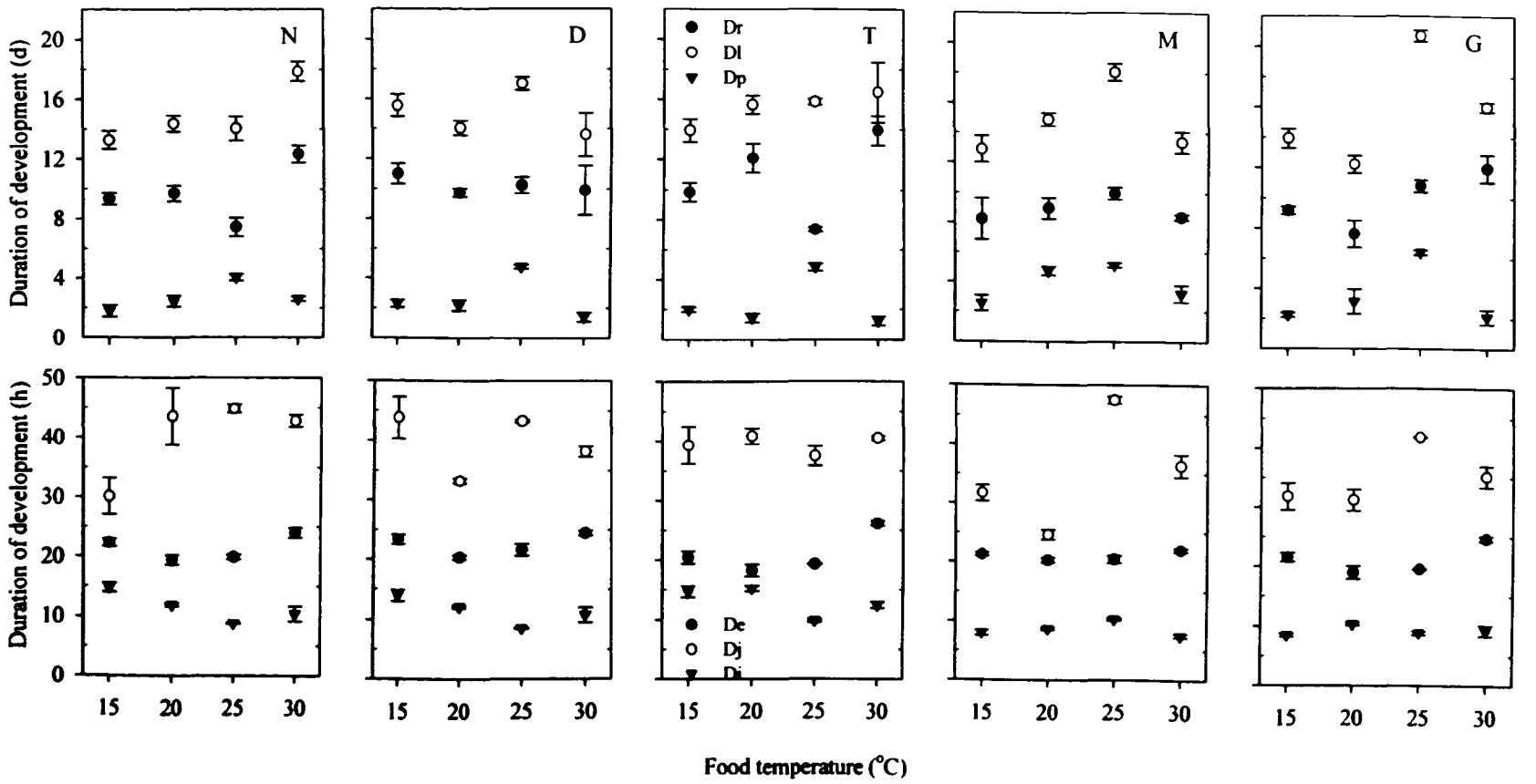

Fig. 7. The duration of different life phases of *Brachionus plicatilis* cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 Chapter 2 for strain designations). Duration of development (h); De, duration of embryonic development; Dj, duration of post- embryonic development; Di, duration of the interval between egg-laying. Duration of development (d); Dr, duration of reproductive period; Dp, duration of post reproductive period; Dl, duration of life-span. Bars are one standard error ±.

Table 3

Two-way ANOVA tests for the indicated diet strain and temperature factor on rotifer life history parameters. Symbols are as described in Table 1, Chapter 2. For all tests degrees of freedom were 3 for temperature, 4 for strain, 12 for the interaction, and 40 for the residual. Data of duration of interval between eggs (Di) was log transformed. Duration of post-embryonic development (Dj) was heterogeneous even after transformation.

Factors	ANOVA		
	MS	*F*	*P-level*
Duration of embryonic development (De)			
Algal temperature	68.26	52.01	7.2×10^{-14}
Algal strain	4.707	3.586	0.0137
Interaction	3.665	2.792	0.0074
Residual	1.313		
Duration of post-embryonic development (Dj)			
Algal temperature	215.95	18.16	1.4×10^{-7}
Algal strain	84.69	7.123	0.0002
Interaction	88.75	7.465	5.8×10^{-7}
Residual	11.89		
Duration of interval between eggs (Di)			
Algal temperature	0.034	18.69	9.8×10^{-8}
Algal strain	0.06	32.65	4.1×10^{-12}
Interaction	0.014	7.54	5.1×10^{-7}
Residual	0.002		
Duration of reproductive period (Dr)			
Algal temperature	13.70	7.946	0.0003
Algal strain	6.076	3.523	0.015
Interaction	9.13	5.294	3×10^{-6}
Residual	1.725		
Duration of post reproductive period (Dp)			
Algal temperature	28.22	73.02	2.8×10^{-16}
Algal strain	4.87	12.6	1×10^{-6}
Interaction	1.427	3.693	0.0009
Residual	0.386		

Duration of life-span (Dl)

Algal temperature	31.85	16.85	3.1×10^{-7}
Algal strain	1.872	0.99	0.423
Interaction	11.98	6.337	4.1×10^{-6}
Residual	1.89		

4.4. Discussion

This research studied the impact of food quality on rotifer growth, i.e., food quality that was affected by two factors: strain/species and prey growth-temperature (see Chapter 3). The results indicated that food quality does have a significant impact on different growth parameters, fecundity, and the duration of different phases. However, overall the effects of these factors are relatively small, and thus it is suggest here that variation in food quality, resulting from both algal strain and temperature has little impact on the productivity of rotifers. Still these small changes can be recognized, so their effects are examined below.

4.4.1. The potential interactive affect of strain/species and prey growth-temperature on rotifer growth

Both the prey-strain and prey growth-temperature may have combined and had interactive affects on rotifer growth. Therefore, the first step in this work was to test for interaction and determine if common trends in responses occurred.

The interaction between prey strain and prey growth-temperature influenced many rotifer growth parameters (Fig. 1, 2, 5, Table 1, 3), but these parameters were affected indirectly. They were mostly affected by a complex of factors within the rotifer population, which explains why in this study there were no obvious trends observed between these parameters and the tested prey factors. This complex of factors, however, has been documented by other researches (Kirk, 1997; Orsenigo et. al., 1998). For example, rotifer dry weight is affected by the amount of elemental compounds, with egg to female ratio, attached eggs, growth or reproductive status of

the population, and eggs formed inside the rotifer body (Scott and Baynes, 1978; Yufera and Pascual, 1989; Aoki and Hino, 1996; Yufera et. al., 1997). In addition, rotifer body volume is related either to size changing during the life cycle (Lubzens and Zmora, 2003), or females developing bigger oocytes that formed eggs inside their bodies (Yufera et. al., 1997).

This interference between growth parameters has also been shown to affect the duration of different stages of rotifer life history. For instance, bigger egg volumes may reduce the time each egg takes to hatch (De) and increase the time spent to produce another egg (Di), where juveniles from large eggs reached maturity earlier (Santo et. al., 2001). Furthermore, the duration of the rotifer's lifetime (Dl) is influenced by the reproduction (Snell and King, 1977). These interactions have been recorded here and in studies involving feeding with different algal diets (Korstad et. al., 1989; Xi et. al., 2001).

The experiment designed to examine rotifer growth in stable conditions with no harsh circumstances showed that prey strain and prey growth-temperature had no affect on rotifer survival. It confirmed the findings of previous studies on the affect of food type on rotifer survival (Planas and Estevez, 1989). Where other factors rather than food quality were shown to influence rotifer survival, e. g., hormones (Gallardo et. al., 1997), ambient temperature (Hirayama and Kusano, 1972; Bosque et al., 2001), salinity (Bosque et al., 2001), food concentration (Schmid-Araya and See, 1991), rotifer population density (Carmona et. al., 1994), and starvation (Yoshinaga et. al., 2001). Therefore, the aim was to investigate any differences that may occur due to prey quality on rotifer production or culture quality.

4.4.2. The affect of prey growth-temperature on rotifer growth

Prey growth-temperature affected two distinct parameters: rotifer growth rate and the time to reach peak fecundity (Fig. 2, 5, Table 1, 2). Specific growth rate decreased when rotifers were fed microalgae grown at warmer temperatures (Fig. 2, Table 1) and the duration of a number of developmental stages increased (Fig. 7). Generally, the decrease in growth rate (Fig. 2) was reflected in an increase in rotifer body volume (Fig. 1), but production, the product of volume and growth rate, generally decreased with increasing prey growth-temperature (Fig. 1, 2). Finally, prey grown at the highest temperature took an increased time to reach peak fecundity (Fig. 5). Thus, overall, this study suggests that an increase in the temperature at which the prey are grown will decrease rotifer production.

Possibly the most important finding of this study is the negative affect of increased prey growth-temperature on rotifer growth both of specific growth rate and in terms of days to peak of fecundity (Fig. 1, Table 1). It is unclear why this occurred, but one possibility is that microalgae cell volume and dry weight decreases with increasing temperature (Atkinson et. al., 2003). Thus, a decrease in prey size may have reduced the rotifer growth rate as has been observed by others (Hansen et. al., 1997). It may be that, although the rotifers were fed at what was assumed to be saturating prey levels (see Materials and Methods), the 3 fold reduction in prey size (Chapter 3) actually brought prey levels to below saturating concentrations in terms of biomass available. Alternatively, the smaller prey may have been more difficult to capture, and more energy was required for food acquisition, even though saturating levels were maintained. However, the present study did not examine the numerical

response (growth rate vs. food levels), which would have allowed a test for this hypothesis. Clearly, this is another area for future study.

Although, an increased prey growth-temperature negatively affected rotifer growth rate and increased the days to reach peak fecundity, overall there may not be a dramatic impact on population growth. The reason for this was that both the number of eggs made in a lifetime and the length of the rotifer lifetime increased with increasing prey growth-temperature (Fig. 2, 7). Thus, from an aquaculture perspective, there need be little concern for which temperature the prey are grown.

4.4.3. The affect of prey strain on rotifer growth

Rotifer growth rate has been shown to vary with food types (Theilacker and McMaster, 1971; Hirayama et. al., 1973; Scott and Baynes, 1978), and in this study prey strains were shown to affect growth rate (Fig. 2, Table 1). Furthermore, many other growth parameters visually appeared to vary with prey strain (Fig.1-7); many of these parameters have been studied by others (Scott and Baynes, 1978; Yufera and Pascual, 1989; Aoki and Hino, 1996; Yufera et. al., 1997) but rarely if ever in the context of food quality. Thus, this study has extended our knowledge on the potential importance of choosing the best prey stains to elicit maximum rotifer growth.

In this study, rotifers fed *I. galbana* T strain had the highest growth rate and overall the highest production (Fig. 2, Table 1). This agrees with the studies of Wedd and Chu (1983), Watanabe et. al. (1983), Helm and Laing (1987), Tiu and Vaughan (1988) , Thinh (1994) and Renaud et. al. (2002) who indicated that *I. galbana* strain T improved the culture of bivalve larvae. This strain is one of the

most popular strains used world-wide (Wedd and Chu, 1983; Rajesh et. al., 2001). Its ability to increase rotifer growth rate may explain the reason for this popularity. Thus, in general aquaculturists might be recommended to rear their rotifers with *I. galbana* strain T if their culture methods match the ones used in this study. However, several aspects of rotifer population growth varied between prey strain and prey temperature (i.e. interaction was significant, Fig. 1, Table 1-3). Thus, aquaculturists must be cautious in making generalities about population responses.

4.5. Application of results to aquaculture

Many of the tested rotifer growth parameters were affected by prey strains and growth-temperatures; the question is, however, how important are these differences in relation to other factors that affect rotifer growth in aquaculture practices? A number of factors have been shown to dramatically affect rotifer growth parameters: starvation and population density (Carmona et. al., 1994; Yoshinaga et. al., 2000); food species, food concentration, rotifer temperature, and rotifer strain (Yufera, 1982; 1987; Yufera et. al., 1983; Schmid-Araya and See, 1991). These factors would overshadow any subtle changes caused by strain or prey growth temperature, as seen in this study. However, if these major, well-studied, factors are controlled, it may be beneficial to consider the prey stain and its growth temperature.

This study did not intend to define the optimum algal diet for rotifer production, but rather to assess if variation in microalgal strains and strain growth-temperature could affect rotifer growth parameters. Nevertheless, data presented here may be useful as guidelines for aquaculturists. For instance, *I. galbana* strain T grown at

25°C elicited one of the highest rotifer population growth responses, and 25°C is the optimum temperature recommended for rotifer growth (Hirayama and Kusano, 1972). Thus, if an aquaculture practice was only able to use 25°C, then it might be recommended to pick *I. galbana* strain T as a diet. Another example would be if a long culture period is required for rotifers, the microalgal prey at high growth-temperature could ensure a steady production for this period. However, if rotifers need to be cultured for a shorter period, the prey grown at low temperature could accomplish high production required for this period. If the same experimental conditions are applied this study can be utilized in case of special aquaculture practice requirements.

4.6. References

Aoki, S., Hino, A., 1996. Nitrogen flow in a chemostat culture of the rotifer *Brachionus plicatilis*. Fish. Sci. 62, 8-14.

Atkinson, D., Ciotti, B.J., Montagnes, D.J.S., 2003. Protists decrease in size linearly with tempeature:*ca.*2.5%$^{o}C^{-1}$. The Royal Science Society 270, 2605-2611.

Bosque, T., Hernandez, R., Todoli, R., Oltra., 2001. Effect of salinity, temperature and food level on the demographic characteristics of the seawater rotifer *Synchaeta littoralis* Rousselet. J. Exp. Mar. Biol. Ecol. 258, 55-64.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal. Biochem. 72, 248-254.

Brown, M.R., Dunstan, G.A., Jeffrey, S.W., Volkman, J.K., Barrett, S.M., LeRoi, J.M., 1993. The influence of irradiance on the biochemical composition of the prymnesiophyte *Isochrysis sp.* (clone T-ISO). J. Phycol. 29, 601-612.

Carmona, M.J., Serra, M., Miracle, M.R., 1994. Effect of population density and genotype on life-history traits in the rotifer *Brachionus plicatilis* O.F. Mueller. J. Exp. Mar. Biol. Ecol. 182, 223-235.

Gallardo, W.G., Tomita, Y., Hagiwara, A., Soyano, K., Snell, T.W., 1997. Effect of dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH), acetone, and ethanol on the population growth, mictic female production, and body size of the rotifer *Brachionus plicatilis* Muller. Bulletin of the Faculty of Fisheries, Nagasaki University. Nagasaki, 15-22.

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Chanley, M.H. (Ed.), Culture of Marine Invertebrate Animals. Plenum Press, New York, pp. 29-60.

Hansen, B., Moller, T., Wittrup, L., 1997. Particle grazing efficiency and specific growth efficiency of the rotifer *Brachionus plicatilis* (Muller). J. Exp. Mar. Biol. Ecol. 215, 217-233.

Helm, M.M., Laing, I., 1987. Preliminary Observations on the Nutritional Value of 'Tahiti *Isochrysis*' to Bivalve Larvae. Aquaculture 62, 281-288.

Hirayama, K., Kusano, T., 1972. Fundamental studies on physiology of rotifer for its mass culture - II. Influence of water temperature on population growth of rotifer. Bull. Jap. Soc. Sci. Fish. 38, 1357-1363.

Hirayama, K., Watanabe, K., Kusano, T., 1973. Fundamental studies on physiology of rotifer for its mass culture. 3. Influence of phytoplankton density on population growth. Source Bull. Jap. Soc. Sci. Fish. 39, 1123-1127.

Hirayama, K., Takagi, K., Kimura, H., 1979. Nutritional effect of eight species of marine phytoplankton on population growth of the rotifer, *Brachionus plicatilis*. Bulletin of the Japanese Society of Scientific Fisheries 45, 11-16.

Kirk, K.L., 1997. Egg size, offspring quality and food level in planktonic rotifers. Freshwat. Biol. 37, 515-521.

Korstad, J., Olsen, Y., Vadstein, O., 1989. Life history characteristics of *Brachionus plicatilis* (Rotifera) fed different algae. Hydrobiologia 186/187, 43-50.

Lubzens, E., Zmora, O., 2003. Production and Nutritional Value of Rotifers. In: McEvoy, L.A. (Ed.), Live feeds in marine aquaculture. Blackwell Science Ltd, Cornwall, pp. 17-52.

Lubzens, E., Tandler, A., Minkoff, G., 1989. Rotifers as food in aquaculture. Hydrobiologia 186/187, 387-400.

Molina Grima, E., Garcia Camacho, F., Sanchez Perez, J.A., Garcia Sanchez, J.L., 1994. Biochemical productivity and fatty acid profiles of *Isochrysis galbana* Parke and *Tetraselmis* sp. as a function of incident light intensity. Process Biochemistry 29, 119-126.

Molina Grima, E., Fernandez Sevilla, J.M., Sanchez Perez, J.A., Garcia Camacho, F., 1996. A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances. Journal of Biotechnology 45, 59-69.

Oie, G., Reitan, K.I., Olsen, Y., 1994. Comparison of rotifer culture quality with yeast plus oil and algal-based cultivation diets. Aquacult. Int. 2, 225-238.

Øie, G., Olsen, Y., 1993. Influence of rapid changes in salinity and temperature on the mobility of the rotifer *Brachionus plicatilis*. Hydrobiologia 255/256, 81-86.

Okauchi, M., Fukusho, K., 1984. Food value of a minute alga, *Tetraselmis tetrathele* , for the rotifer *Brachionus plicatilis* culture. 1. Population growth with batch culture. Bull. Natl. Res. Inst. Aquacult. (Japan) Yoshokukenho. 5, 13-18.

Orsenigo, S., Ricci, C., Caprioli, M., 1998. The paradox of bdelloid egg size. Hydrobiologia 387/388, 317-320.

Planas, M., Estevez, A., 1989. Effects of diet on population development of the rotifer *Brachionus plicatilis* in culture. Helgolander Meeresuntersuchungen 43, 171-181.

Rajesh, K.V., Mohamed, K.S., Kripa, V., 2001. Influence of algal cell concentration, salinity and body size on the filtration and ingestion rates of cultivable Indian bivalve. Indian J. Mar. Sci. 30, 87-92.

Renaud, S.M., Thinh, L., Lambrinidis, G., Parry, D.L., 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 211, 195-214.

Santo, N., Caprioli, M., Orsenigo, S., Ricci, C., 2001. Egg size and offspring fitness in a bdelloid rotifer. Hydrobiologia 446, 71-74.

Schmid-Araya, J.M., See, L., 1991. The effect of food concentrstion on the life histories of *Brachionus plicatilis* (O. F. M.) and *Encentrum linnhei* Sccot. Arch. Hydrobiol. 121, 87-102.

Scott, A.P., Baynes, S.M., 1978. Effect of algal diet and temperature on the biochemical composition of the rotifer, *Brachionus plicatilis*. Aquaculture 14, 247-260.

Snell, T.W., King, C.E., 1977. Lifespan and fecundity patterns in rotifers: the cost of reproduction. Evolution 31, 882-890.

Snell, T.W., Bieberich, C.J., Fuerst, R., 1983. The effects of green and blue-green algal diets on the reproductive rate of the rotifer *Brachionus plicatilis*. Aquaculture 31, 21-30.

Tamaru, C.S., Murashige, R., Lee, C.S., Ako, H., 1993. Rotifers fed various diets of baker's yeast and/or *Nannochloropsis oculata* and their effect on the growth and survival of striped mullet (*Mugil cephalus*) and milkfish (*Chanos chanos*) larvae. Aquaculture 110, 361.

Tamaru, C.S., Murashige, R., Lee, C.S., Ako, H., Sato, V., 1993. Rotifers fed various diets of baker's yeast and/or *Nannochloropsis oculata* and their effect on the growth and survival of striped mullet (*Mugil cephalus*) and milkfish (*Chanos chanos*) larvae. Aquaculture 110, 361-372.

Theilacker, G.H., McMaster, M.F., 1971. Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as a food for larval anchovies. Mar. Biol. 10, 183-188.

Thinh, L.-V., 1994. Potential use of ageing cultures of *Isochrysis aff. galbana* (*Isochrysis* Tahitian, T. Iso) as starter cultures for live algal food production in tropical aquaculture. J. Appl. Phycol. 6, 357-358.

Tiu, A.T., Vaughan, D.E., 1988. Food value of tropical microalgae to bivalve larvae. Journal of Shellfish Research 7, 561-562.

Underwood, A.J., 1997. Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge, 504 pp.

Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional values of live orgainsims used in Japan for mass propagation of fish: a review. Aquaculture 89, 263-272.

Wedd, k.L.s., Chu, F.E., 1983. Phytoplankton as a food source for bivalve larvae. In: Conklin, D. (Ed.), Biochemical and physiological Approaches to shellfish Nutrition. The second International Conference on Aquaculture Nutrition., Louisiana State University, Baton Rouge, pp. 272-291.

Xi, Y., Huang, X., Jin, H., 2001. Life history characteristics of three types of females in *Brachionus calyciflorus* Pallas (Rotifera) fed different algae. Hydrobiologia 446/447.

Yoshinaga, T., Hagiwara, A., Tsukamoto, K., 2000. Effect of periodical starvation on the life history of *Brachionus plicatilis* O.F. Mueller (Rotifera): a possible strategy for population stability. J. Exp. Mar. Biol. Ecol. 253, 253-260.

Yoshinaga, T., Hagiwara, A., Tsukamoto, K., 2001. Effect of periodical starvation on the survival of offspring in the rotifer *Brachionus plicatilis*. Fish. Sci. 67, 373-374.

Yufera, M., 1982. Morphometric characterization of a small-sized strain of *Brachionus plicatilis* in culture. Aquaculture 27, 55-61.

Yufera, M., 1987. Effect of algal diet and temperature on the embryonic development time of the rotifer *Brachionus plicatilis* in culture. Hydrobiologia 147, 319-322.

Yufera, M., Pascual, E., 1984. Influence of diet on egg deposition of the rotifer *Brachionus plicatilis* in culture. Invest. Pesq. (Barc.). 48, 549-556.

Yufera, M., Pascual, E., 1989. Biomass and elemental composition (C:H:N) of the rotifer *Brachionus plicatilis* cultured as larval food. Hydrobiologia 186/187, 371-374.

Yufera, M., Navarro, N., 1995. Population growth dynamics of the rotifer *Brachionus plicatilis* cultured in non-limiting food condition. Hydrobiologia, vol., 313-314.

Yufera, M., Lubian, L.M., Pacual, E., 1983. Effect of four species of marine algae on population growth of two strains of *Brachionus plicatilis* in culture. Invest. Pesq. (Barc.). 47, 325-337.

Yufera, M., Parra, G., Pascual, E., 1997. Energy content of rotifers (*Brachionus plicatilis* and *Brachionus rotundiformis*) in relation to temperature. Hydrobiologia 358, 83-87.

Chapter 5.

Effects of prey growth temperature and prey strain on the biochemical composition of *Brachionus plicatilis*

5.1. Introduction

Much attention is given to the biochemical composition of live-feeds in aquaculture, since successful cultivation and survival of fish larvae depends partly upon the transfer of nutrients from live food (Rainuzzo et al., 1997; Reitan et al., 1997). Among the various species of live food, the rotifer *Brachionus plicatilis* is the most used in rearing larvae of various marine and freshwater fishes, and it is considered a living food capsule that transmits nutrients to the fish larvae (Lubzens et al., 1989; Lubzens and Zmora, 2003). Thus, along with the production of sufficient quantities of rotifers, it is necessary to ensure their proper nutritional quality, to satisfy the needs of the fish larvae.

Brachionus plicatilis can be exclusively grown on microalgae, which are a nutritious food source (Hirayama et al., 1979; Okauchi and Fukusho, 1984; Tamaru et al., 1993). Furthermore, fish larvae that are fed algal-reared rotifers tend to survive better (Scott and Baynes, 1979; Dendrinos and Thorpe, 1987; Mandeville et al., 2001). Thus, attention needs to be paid to the relationship between the rotifer and prey quality.

It is well recognized that there is often a relationship between prey and rotifer biochemical composition (Ben-Amotz et al., 1987). The majority of studies have focused on lipids and fatty acids, as these are important components of the larval fish diet (Ben-Amotz et al., 1987; Fernandez-Reiriz and Labarta, 1996; Lewis et al., 1998; Gallagher et al., 2001). However, carbohydrates and protein may also be important in the larval fish diet (Whyte et al., 1994; Kanazawa, 2003), and few studies have examined these (Scott and Baynes, 1978; Whyte and Nagata, 1990). It

is important to obtain knowledge on all of these biochemical constituents to better understand the benefit of rotifers to the larval fish diet (Kanazawa, 2003).

Another factor that may affect the rotifer's biochemical composition, which has not received attention, is the affect of algal quality. Microalgal quality can be influenced by the strain and growth-temperature (see Chapter 3). It is necessary to study the alternative affect of these diets on rotifer biochemical composition, to choose the most appropriate strain at the most appropriate growth-temperature in order to increase rotifer nutritional value. Therefore, this study investigated the dynamics of lipid, protein and carbohydrate between rotifer and their algal prey; and deduce if these strain- and temperature-induced differences had significant, predictable affects on rotifer "quality".

5.2. Material and Methods

5.2.1. Algal and rotifer stock cultures

Strains of *Isochrysis galbana* from the Isle of Man (M), Tahiti (T), Greece (G), Denmark (D), and one strain of *Nannochloropsis* sp. (N) were obtained from the plankton culture collection at Port Erin Marine Laboratory, Isle of Man (Table 1, Chapter 2). The flagellates were grown in 200 ml flat bottom flasks with f/2 medium (Guillard, 1975). All strains and species were maintained in semi-continuous culture, by transfers every two weeks, at 20 °C, on a 12:12 light: dark cycle, at an irradiance of 100 $\mu E\ m^{-2}s^{-1}$.

The rotifer *Brachionus plicatilis* was obtained from the Larval-Rearing Centre at Port Erin Marine Laboratory, where it was maintained at 25 °C. Rotifers were collected onto a 45 μm mesh and rinsed with filtered seawater (Whatman GF/C) to remove contaminants, prey from the original cultures and to concentrate rotifers. Rotifers were then resuspended in filtered seawater (32 ppt) and fed on a diet of *Nannochloropsis* sp. To ensure uniform food distribution and to prevent sedimentation, gentle aeration was provided. Light conditions were the same as those provided for the flagellates.

5.2.2. Algal culture treatment

The four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) were obtained from stock cultures. Algal strain/species were grown in 3 L round bottom flasks in modified f/2 medium (Guillard, 1975) at four different temperatures. All flasks were maintained in exponential growth phase by semi-continuous culturing on a 24:0 light: dark cycle. An irradiance of 100 μE $m^{-2}s^{-1}$ was provided, which is considered optimal for flagellate growth rate (Brown et al., 1993; Molina Grima et al., 1994; 1996). All cultures were maintained for >10 generations; flasks were suspended in a controlled water bath at 15, 20, 25, and 30 ± 1°C and were gently agitated at least once a day. Each of these flasks was used as a prey treatment for rotifer rearing culture.

5.2.3. *Rotifer rearing culture*

Rotifers were collected from the stock culture onto a 45 μm mesh and rinsed with filtered seawater. Five hundred rotifers were placed in 100 ml flasks (5 prey types x 3 replicates). Algal concentrations were maintained using a Model II Coulter Multisizer, and associated software V.4.1 equipped with a population accessory (Coulter Electronics Ltd., England). To maintain rotifers in exponential growth, to maintain algae concentration, and to increase the volume of the cultures, every day 100 ml of each of the algal culture treatment at ~ 6 x 10^4 cell ml^{-1} was added to the rotifer culture, this food concentration is sufficiently high to maintain maximum growth rate for rotifer following recommendations of Hansen et al. (1997). Rotifers were maintained on their diet for ~7 generations at 25 °C; generation time was calculated following the equation of Hirayama and Kusano (1972). Rotifer growth rate was determined from daily abundance, calculated as the slope of *ln* numbers vs. time in days.

To measure rotifer body volume, 40 active rotifers were randomly selected and examined, from each of the rotifer replicate culture treatment (i.e., fed different algal prey that were grown at different temperatures). These rotifers were placed in a 10 ml settling chamber and were measured using an inverted microscope (Zeiss Axiovert 135tv) equipped with a video camera (JVC model 3-CCD, 750 lines horizontal resolution), attached to a Pentium IIPC with image analysis software (Scion image for windows, Scion corp., MD, USA). For each rotifer, two linear dimensions were measured, and the volume was calculated following the method of Yufera (1982).

The remaining rotifer culture was rinsed with filtered seawater (Whatman GF/C) and collected onto a 45 μm mesh, to remove prey and to concentrate the rotifers. The concentrated rotifers were used to estimate dry weight and biochemical composition (see 2.3 below). For dry weight, the concentrated rotifers were collected on filters and gently washed with a solution of 0.5 M ammonium formate to remove salts; filters were dried at 60°C for 12h before weighting. Due to methodological problem three dry weight data (T, M, G) at 25°C were estimated from the average of the data at 20 and 30°C.

5.2.4. Protein, carbohydrate, and lipid analysis for algal and rotifers

Three replicates, one from each algal flask (see section 2.2 above), were collected for protein, carbohydrate, and lipid determination: 30 ml for protein, 50 ml for carbohydrate, and 100 ml for lipid. Cell concentration was determined by Coulter Counter and was ~10^6 cells ml^{-1}. As for the rotifer biochemical analysis, three replicate of water volumes, from separate rotifers-flasks, each with a ~$1x10^5$ rotifers ml^{-1}, were sampled for chemical analysis. The same analytical methods were performed on both rotifers and algae.

Protein was quantified using the Bradford method (Bradford, 1976), after hydrolysis in NaOH 1N for 1h at 90 °C. Total carbohydrates were quantified as glucose by the phenol-sulfuric acid method (Kochert, 1978). Lipid was extracted following the method of Bligh and Dyer (1959), and quantified by the method of Pande et al. (1963). Calibration curves were made using: bovine albumin as the standard for proteins, D-glucose for carbohydrates, and tripalmitin for lipids.

5.2.5. Statistical analyses

Data trends were assessed by two-way analyses of variance (ANOVA), with prey temperature and strain as the source of variance; rotifer protein, carbohydrate, and lipid per cell, μm^{-3}, or per dry weight were the dependent variables. Data always passed tests for normality. Where data were heteroscedastic, they were transformed (following recommendations of Zar, 1999; see Results for details of transformations). However, in some cases data remained heteroscedastic, and ANOVA was still applied since this technique is robust to departure from homogenous variances (Underwood, 1997).

In two-way ANOVAs, where no interaction occurred (i.e., where trends were apparent), further analysis by Tukey's test indicated where significant differences existed. Where significant interaction was detected, no further statistical analysis on individual cases (e.g., temperature effects on one strain) was conducted. The reason for this was that the purpose of this study was to investigate general trends, rather than to detect specific responses. Thus, rather than producing numerous strain or temperature-specific one-way ANOVA results, data were visually interpreted, using standard error bars as a guideline.

Regression analysis was performed to determine biochemical composition relation between prey-diet and rotifers, and tested using ANOVA. In all analyses, the statistical significance was at $P<0.05$. Statistical analysis used STATISTICA version 5.0. Homogeneity of variance was tested by Cochran's test (Underwood, 1997).

5.3. Results

5.3.1. The interactive effect of prey growth-temperature and strain on rotifer protein, carbohydrate, and lipid content

There were significant interactions between prey strain and prey growth-temperature on rotifer protein and lipid content; no obvious trends were observed in either of these, per individual, μm^{-3}, or per dry weight (Fig. 1, 2, Table 1). There were no significant interactions between prey strain and prey growth-temperature on rotifer carbohydrate content (Fig. 3, Table 1). However, there was no clear trend of either temperature or strain on the biochemical composition: there were strain differences per individual, temperature differences μm^{-3}, and no differences per dry weight (Table 1, Fig. 3). Thus, further statistical analysis was not conducted. In conclusion, there were significant interactions in most responses and trends were deducted visually. Furthermore, when trends did occur (i.e., carbohydrate data) they were not strong and appeared contradictory. Therefore, further analyses were conducted to assess if other trends existed. These analyses used the data from this study and, where possible, larger data set from the literature (Table 2).

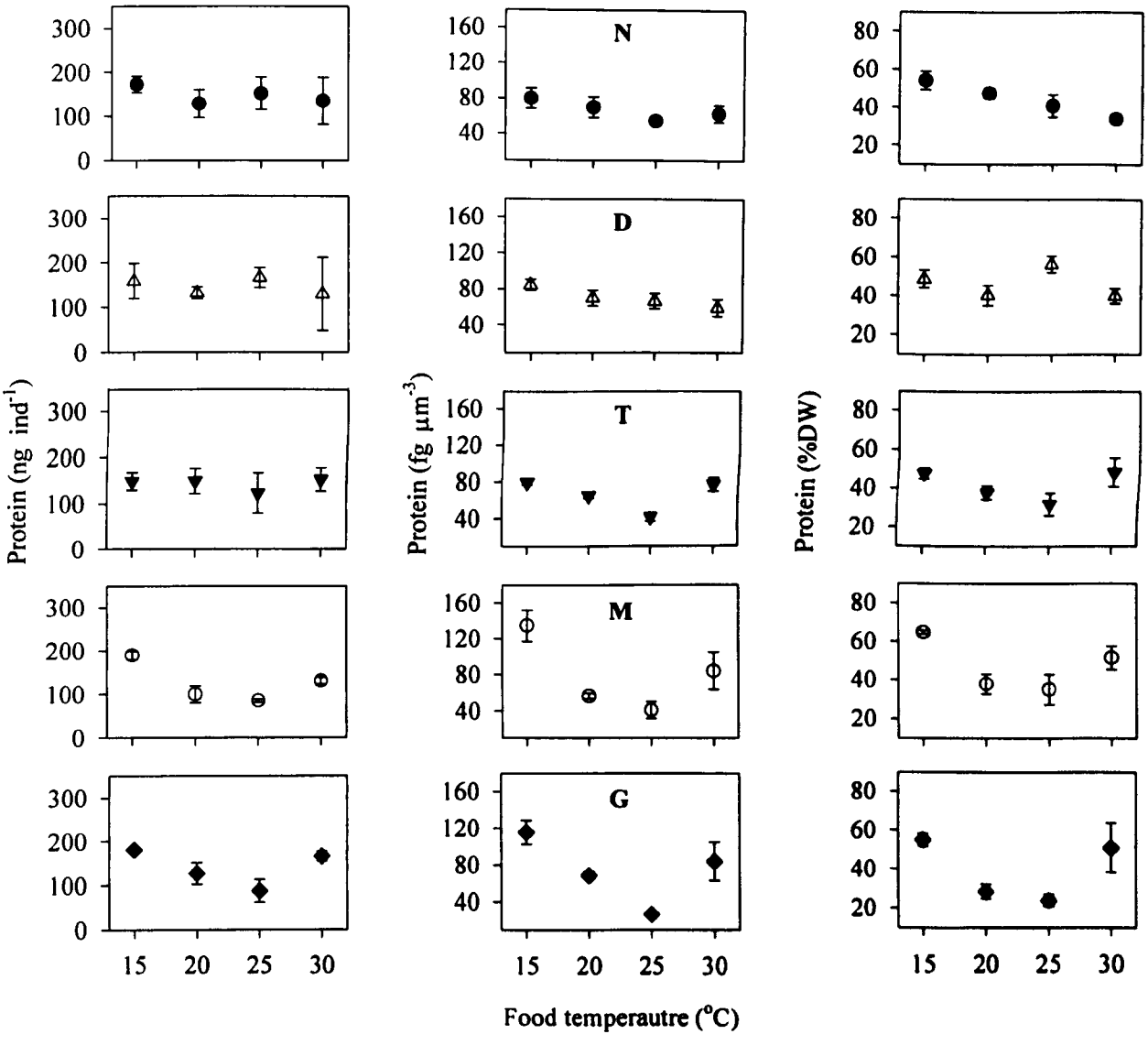

Fig. 1. *Brachionus plicatilis* content of protein: protein (ng ind^{-1}), protein (fg μm^{-3}), and protein (%DW) cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. N (see Table 1 Chapter 2 for strain designations). Bars are one standard error ±.

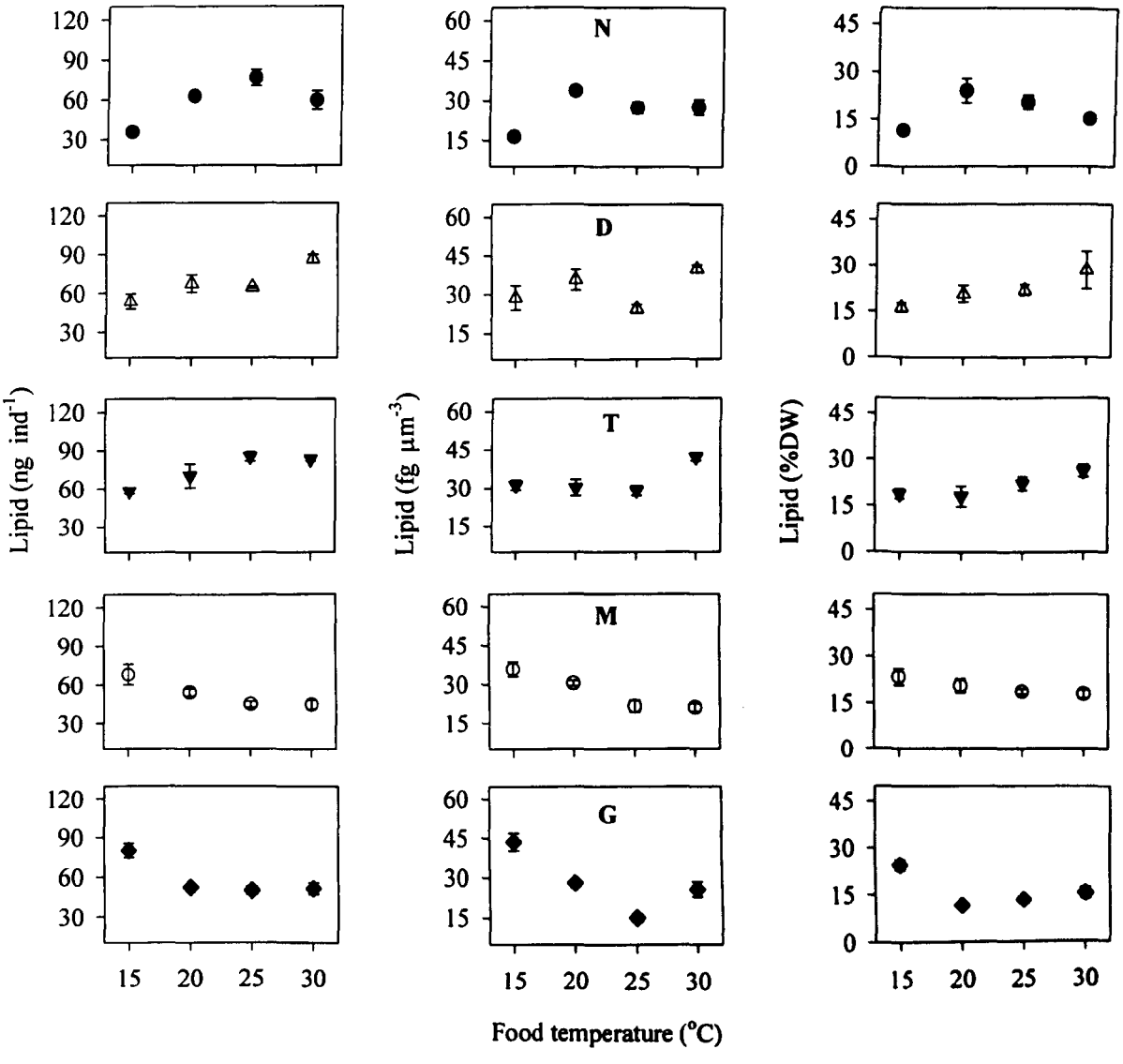

Fig. 2. *Brachionus plicatilis* content of lipid: lipid (ng ind^{-1}), lipid (fg μm^{-3}), and lipid (%DW) cultured on various algae strains: four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 Chapter 2 for strain designations). Bars are one standard error ±.

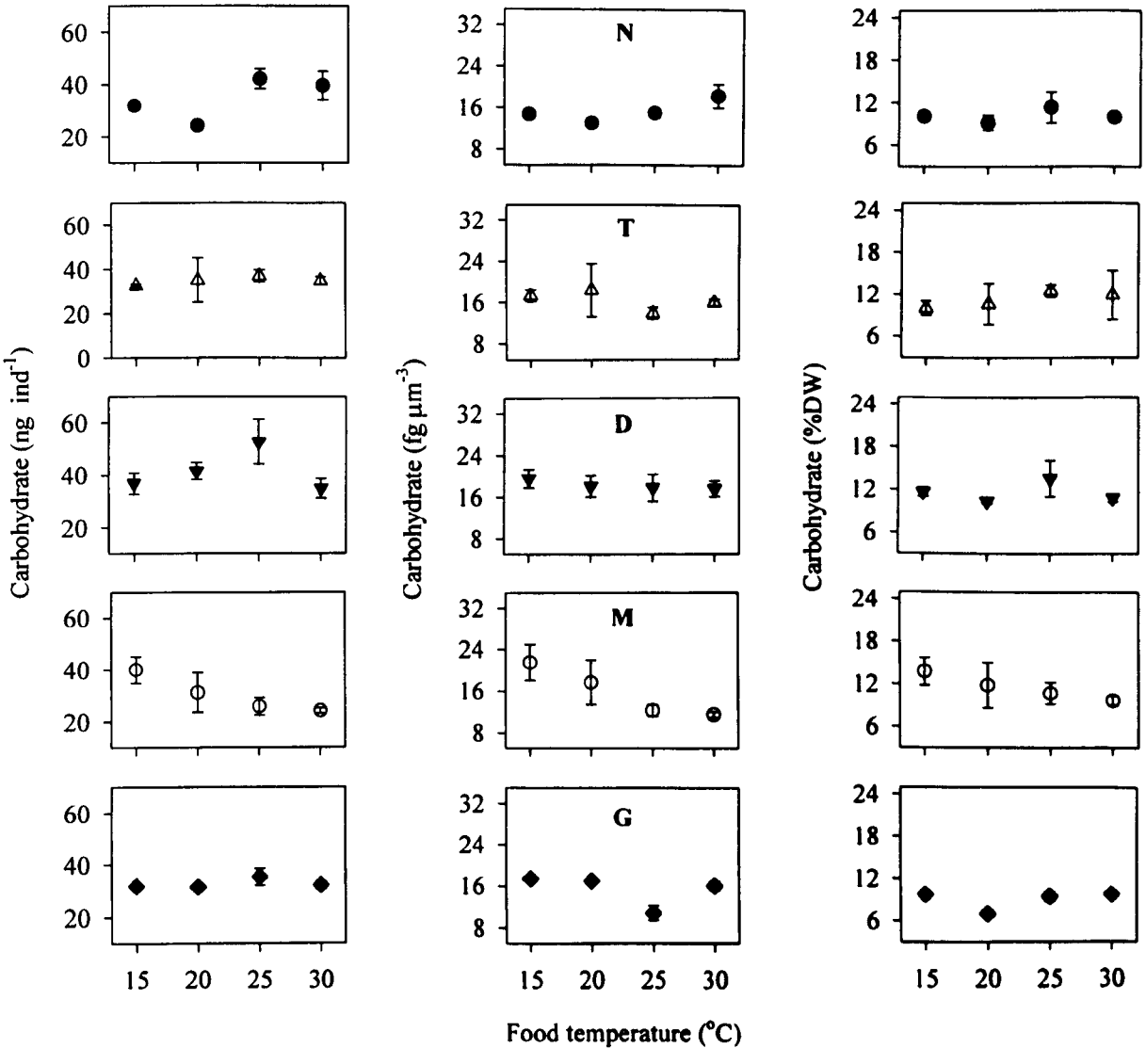

Fig. 3. *Brachionus plicatilis* content of carbohydrate: carbohydrate (ng ind^{-1}), carbohydrate (fg μm^{-3}), and carbohydrate (%DW) cultured on various algae strains (four strains of *Isochrysis galbana* (M, T, G, D), and one strain of *Nannochloropsis* sp. (N) (see Table 1 Chapter 2 for strain designations). Bars are one standard error ±.

Table 1

Two-way ANOVA and Tukey's test for the effect of food strain and growth-temperature on rotifer biochemical compositions. Symbols are as described in Table 1, Chapter 2. Tukey's test data are ordered from lowest (left) to highest (right); underscoring indicates where there were no significant differences at $p = 0.05$. For all tests degrees of freedom were 3 for temperature, 4 for strain, 12 for the interaction, and 40 for the residual. The carbohydrate factor in (fg μm^{-3}) was log transformed; carbohydrate in (%DW) was 1/x transformed. Data of lipid in (fg μm^{-3}) was heterogeneous even after transformation.

Factors		ANOVA			Tukey test
		MS	*F*	*P-level*	
Protein (ng ind^{-1})					
	Algal temperature	13250.61	10.418	$3.4x10^{-5}$	
	Algal strain	180.74	0.142	0.965	
	Interaction	4024.08	3.164	0.003	
	Residual	1271.95			
Protein (fg μm^{-3})					
	Algal temperature	7176.78	21.798	$1.6x10^{-8}$	
	Algal strain	353.1	1.073	0.383	
	Interaction	908.12	2.758	0.008	
	Residual	329.24			
Protein (%DW)					
	Algal temperature	868.99	9.945	$5x10^{-5}$	
	Algal strain	128.06	1.466	0.231	
	Interaction	258.05	2.953	0.005	
	Residual	87.38			
Carbohydrate (ng ind^{-1})					
	Algal temperature	108.818	1.898	0.145	G M N D T
	Algal strain	206.51	3.602	0.013	
	Interaction	112.365	1.96	0.056	
	Residual	57.334			

Carbohydrate (fg μm^{-3})					
	Algal temperature	0.036	4.468	0.008	25 30 20 15
	Algal strain	0.016	1.915	0.127	———
	Interaction	0.015	1.793	0.083	
	Residual	0.008			
Carbohydrate (%DW)					
	Algal temperature	0.001	2.544	0.069	
	Algal strain	0.001	2.51	0.057	
	Interaction	0.0003	0.639	0.796	
	Residual	0.0005			
Lipid (ng ind^{-1})					
	Algal temperature	118.04	1.785	0.165	
	Algal strain	850.54	12.863	$8x10^{-7}$	
	Interaction	697.21	10.544	$6x10^{-10}$	
	Residual	66.122			
Lipid (fg μm^{-3})					
	Algal temperature	$2.1x10^{-10}$	6.575	0.001	
	Algal strain	$2x10^{-10}$	6.235	0.001	
	Interaction	$1.7x10^{-10}$	5.311	$2.9x10^{-5}$	
	Residual	$3.2x10^{-11}$			
Lipid (%DW)					
	Algal temperature	11.143	0.64	0.594	
	Algal strain	69.088	3.965	0.0008	
	Interaction	78.917	4.53	0.0001	
	Residual	17.423			

Table 2

Comparisons between results in rotifer growth, and biochemical compositions parameters. Biochemical compositions, expressed as amount per dry weight (μg mg DW^{-1}), (ng ind^{-1}), and (%DW).

Author	Observations	Growth rate	Dry weight	Proteins		Carbohydrates		Lipids	
		(μ,d^{-1})	(ng ind^{-1})	(%DW)	(ng ind^{-1})	(%DW)	(ng ind^{-1})	(%DW)	(ng ind^{-1})
(Scott and Baynes, 1978)	1. Rotifer fed on:								
	D. tertiolecta cultured at 18C° /23C°	0.4/ 0.45	602 /519	56.8 /57.6		9.1 /3.1		10.2 /5.6	
	I. galbana cultured at 18C°/23C°	0.43	579 /469	53.1 /51.2		3.5 /2.7		11.2 /5.4	
	P. lutheri cultured at 23C°	0.46	474	58.6					
	P. ltricornutum cultured at 23C°	0..54	522	52.1					
	2. Rotifer fed on:								
	D. tertiolecta cultured at 18C° /23C° /28C°	0.35 /0.49 /0.56	596	51.1 /56.1 /54.5		5.5		10.5	
	I. galbana cultured at 18C°/23C° /28C°	0.35 / 0.48 /0.56	369 /874 /514	54.2 /51.9 /50.9		3.8		11.4	
	P. lutheri cultured at 18C°/23C° /28C°	0.26 /0.48 /0.55	414 /704 /535	58.1 /58.8 /51		3.3			
	P. ltricornutum cultured at 18C°/23C° /28C°	0.26 /0.47 /0.58	327 /733 /502	51.3 /57.2 /49.1		5.5		11.4	
	3. Rotifer fed on:								
	D. tertiolecta cultured at 20C°	0.43	603	50					
	I. galbana cultured at 20C°	0.41	583	49.3					
	4. Rotifer fed on *D. tertiolecta* cultured at 25C°		454		214		40		43
(Frolov et al., 1991)	Rotifer cultivated for 20 days at 18-22 C° fed on:								
	Ph. Tricornutum			57.2		26.8		8.7	
	S. cerevisiae			49.2		17.4		11.8	
	N. salina			50.2		20.5		13.6	
	Monochrysis lutheri			48.1		20.7		20.4	
(Frolov and Pankov, 1992)	Rotifer cultivated for 20 days on *Monochrysis lutheri* at 18-22 C° density (5 x10^5)			45.3		21.2		20.1	

Table 2 continue

Author	Observations	Growth rate (μ,d^{-1})	Dry weight (ng ind^{-1})	Proteins (%DW)	Proteins (ng ind^{-1})	Carbohydrates (%DW)	Carbohydrates (ng ind^{-1})	Lipids (%DW)	Lipids (ng ind^{-1})
(Caric et al., 1993)	Rotifer fed on *P. ltricornutum* density (1.5 x10^6)	0.51		42		5.3		16	
	Rotifer fed on *D. tertiolecta* density (4.5 x10^6)	0.28		44.7		5		15.8	
	Rotifer fed on *Nannochloropsis* sp. density (6.2 x10^6)	0.49		34.3		5.6		17.2	
	Rotifer fed on *T. suecica* density (1.3 x10^5)	0.36		41		3.5		12.7	
	Rotifer fed on Nanoplankton density (4.5 x10^6)	0.3		50		4.8		19.4	
	Rotifer fed on yeast density (0.9 x10^5)	0.19		34		4.3		13.5	
(Fernandez-Reiriz et al., 1993)	Rotifer cultivated on yeast	369.5		36.06		16.65		10.48	
	Then enriched by 2 doses of 0.189 of 600 rot ml^{-1}:								
	Protein Selco enriched for 3h / 6h	419.5/ 453.5		34.54/ 38.11		16.02/ 15.50		13.89/ 18.48	
	Dry Super Selco enriched for 3h / 6h	400/ 386.0		34.14/ 37.16		15.45/ 14.20		14.25/17.54	
	Super Selco enriched for 3h / 6h	330.5/ 316.5		36.16/ 39.54		15.80/ 13.03		15.66/ 19.85	
(Olsen et al., 1993)	Rotifer Short-term enrichment food density 0.4 μg ind^{-1}:								
	Super Selco							287.5	
	DHA Super Selco							275	
	Rotifer long-term enrichment fed on yeast+ Selco density 275 μg gDW^{-1}							150	

Table 2 continue

Author	Observations	Growth rate (μ,d^{-1})	Dry weight (ng ind^{-1})	Proteins		Carbohydrates		Lipids	
				(μg mg DW^{-1})	(ng ind^{-1})	(μg mg DW^{-1})	(ng ind^{-1})	(μg mg DW^{-1})	(ng ind^{-1})
(Øie et al., 1997)	Rotifer at20C° fed on yeast+10% Super Selco cultivation conditions:								
	Voume replaced per day20%, Short-term enrichment Protein Selco		502	364	172			182	91
	Voume replaced per day5%, Short-term enrichment DHA Selco		331	336	111			212	70.3
	Voume replaced per day5%, No Short-term enrichment		376	347	137			144	54.2
(Reitan et al., 1997)	Rotifer cultivated for 11 days fed on:								
	I. galbana		620	334	215			129	80
	Tetraselmis sp.		673	317	210			106	71
	P. lutheri		620	332	216			123	76
	Yeast+capelin oil		646	307	205			170	85
(Makridis and Olsen, 1999)	Rotifer fed on *Tetraselimis* sp.:								
	Poorly-fed (μ=0.05)		293					157	46
	Well-fed (μ=0.22)		371					165	61

The first of theses further analyses was designed to test if there was a general relationship between algal and rotifer biochemical content; regressions were preformed, only using the data from this study, based on both compositions μm^{-3} and percent dry weight (Fig. 4). Of these regressions, only one was significant, lipid content per dry weight (Fig. 4f), and this was not a strong relationship. Therefore, it appeared that rotifer biochemical compositions were not clearly related to the prey compositions.

The second of these analyses was designed to test if there were relationships between rotifer body mass (dry weight) and biochemical compositions per individual (Fig. 5). This analysis used the data from this study and those from the literature (Table 2). There appeared to be a relationship between protein per individual and body weight (Fig. 5a), but there were no clear trends between carbohydrate and lipid per individual and body dry weight (Fig.5b, c). Therefore, it appeared that size is not always a good indicator of biochemical composition content in rotifer.

The third of these analyses was designed to test if faster growing organisms invest more biochemical compositions into population growth than storage, and if structural biochemical composition increases with growth rate (Fig. 6). Results of growth rate (see Chapter 4 for details on growth estimates) vs. biochemical compositions revealed only a weak positive relationship between protein μm^{-3} vs. growth rate (Fig. 6a), and no relationships for carbohydrate or lipid μm^{-3} or any relationship based on per individual and per dry weight (Fig. 6b, c, d, e, f, g, h).

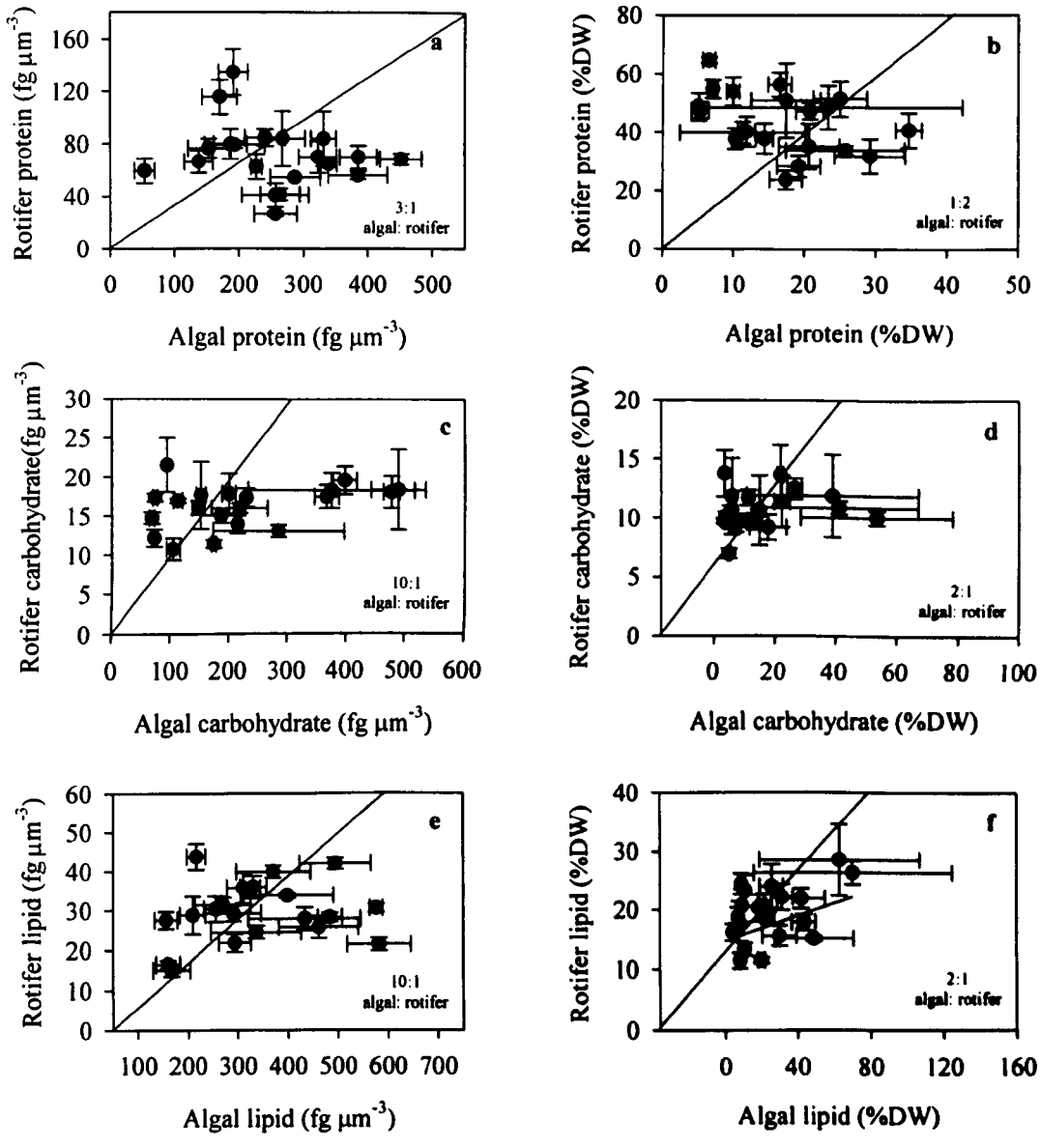

Fig. 4. The biochemical composition content relationship between rotifer *Brachionus plicatilis* and their food strains: **a.** protein (fg µm^{-3}), **b.** protein (%DW), **c.** carbohydrate (fg µm^{-3}), **d.** carbohydrate (%DW), **e.** total lipid (fg µm^{-3}), and **f.** total lipid (%DW). Guides lines show the composition indicated ratio of algal: rotifer. Bars are one standard error ±.

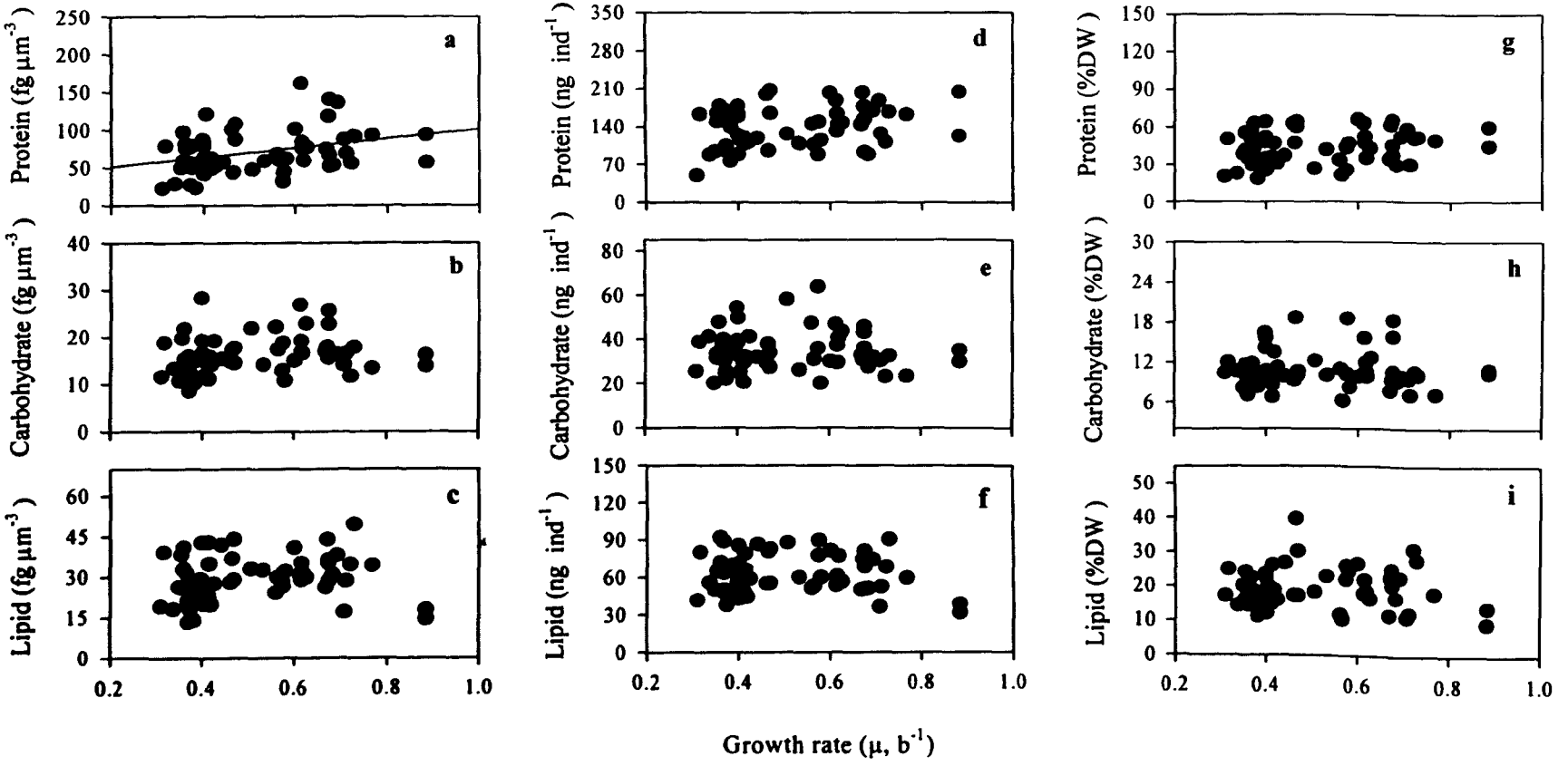

Fig. 6. The biochemical composition content relationship between rotifer *Brachionus plicatilis* and their growth rate: **a.** protein (fg μm^{-3}), **b.** carbohydrate (fg μm^{-3}), **c.** total lipid (fg μm^{-3}), **d.** protein (ng ind^{-1}), **e.** carbohydrate (ng ind^{-1}), **f.** lipid (ng ind^{-1}), **g.** protein (%DW), **h.** carbohydrate (%DW), and **i.** total lipid (%DW). Bars are one standard error ±.

5.4. Discussion

5.4.1. Does rotifer composition reflect that of its prey?

The biochemical composition of the rotifer *Brachionus plicatilis* has been studied intensively to examine the importance of its nutritional quality as a food for larval fish (Watanabe et al., 1983; Lubzens et al., 1989; Øie et al., 1997). This study investigated if prey of varying nutritional composition fed to rotifers, would alter their biochemical composition. Previous work (Chapter 3) indicated differences in the nutritional quality between prey (microalgal) strains and within strains, when they were grown at different temperatures.

There were, in fact, significant differences in the biochemical composition of the rotifers that could be related to prey quality, but these were not predictable (Fig. 1-3, Table 1). Furthermore, there were no strong relationships between prey and predator biochemical composition (Fig. 4). These findings seem surprising, as Ben-Amotz et al. (1987) suggest that the biochemical quality of the prey affects that of the predator. Why then were there no predictable relationships between prey and predator biochemical composition in the present study?

One reason for the lack of a relationship between rotifer and algal prey protein, carbohydrate, and lipid content μm^{-3} could be that these components are not stored straightforward as they may be analyzed and form other elements inside rotifer body. The individual elements that were tested between the rotifer and its prey were, on average, lower in the rotifer than in their food (Fig. 4), suggesting that rotifers might use some of these compounds and this is supported by previous works of Lubzens et al. (1984; 1985), Caric et al. (1993) and Øie and Olsen (1997). Furthermore, the

growth rates of rotifers affect their biochemical composition content and that could be the reason for the lack of a relationship between rotifer and algae in protein and lipid content per dry weight (Caric et al., 1993; Lubzens et al., 1989; Øie and Olsen, 1997), as shown in Chapter 4. Thus, there may be complex interactions between growth/reproduction and biochemical composition that cannot be assessed from data in this study. Clearly, this interaction is an area for future study.

Alternatively, it may be that the biochemistry of the predator does, to some extent, reflect that of the prey, but the resolution of the analysis in this study was insufficient to reveal such relationships. For instance, specific nutrients, such as fatty acids, may be a better means to assess the transformation of dietary components from phytoplankton to rotifers (Abu-Rezeq and James, 1985; Whyte and Nagata, 1990; Frolov et al., 1991; Barclay and Zeller, 1996). However, other specific nutrients have shown no correlation between predator and prey levels (e.g., amino acids, diaclglycerols, and monoaclglycerols; Frolov et al., 1991).

Thus, the conclusion from this study is that the protein, carbohydrate, and lipid concentration in rotifers seems to be affected by prey strain/species and prey temperature. However, there were no clear relationships between prey and predator biochemical composition content.

5.4.2. What else might affect rotifer composition?

It might be expected that organism size is related to the biochemical composition per individual. When the data from this study were combined with those in the literature (Table 2), they fell within the same range. Using the entire data set, there was a relationship between protein per individual and body mass (Fig.

5a), but no clear trends occurred for carbohydrate or lipid (Fig. 5b, c). The reason for this may be that the rotifer lorica is made of structural, heavy proteins, which affect the dry weight of the rotifer (Øie et al., 1994). In contrast, carbohydrates and lipids in rotifers are thought to be used for energy, rather than being permanently stored (Frolov et al., 1991; Caric et al., 1993). Thus, size may not always be a good indicator of biochemical quality.

Growth rate is another factor that may influence biochemical quality; faster growing organisms may invest more energy into growth than storage, and there would thus be inverse relationships between carbohydrate and lipid μm^{-3} vs. growth rate. In contrast, structural protein might be invariant or increase with growth rate. This hypothesis has been supported by observations of a decrease in lipid as a percent of individual dry weight vs. growth rate, where growth rate was altered by diet (Øie and Olsen, 1997) and by decreases in lipid and carbohydrate (but not protein) as a percent of dry weight, where growth rate was altered by growth phase (Caric et al., 1993). However, analysis of growth rate data from this study (see Chapter 4 for details on growth estimates) vs. biochemical composition revealed only a weak, positive relationship between protein μm^{-3} vs. growth rate (Fig. 6a), and no relationships for carbohydrate or lipid μm^{-3} or any relationship based on per individual and per dry weight (Fig. 6b, c, d, e, f, g, h, i). Thus, although this is an attractive hypothesis, it is not upheld by the analysis.

In conclusion, neither organism size nor growth rate can be used reliably to predict rotifer biochemical composition content except for protein under some circumstances. It may be that the interaction between various factors caused these responses. Yet again, this is an area for further study.

5.5. Application of the results to aquaculture

Although there are no clear trends as to why differences in the rotifer biochemical composition occurred, there were differences (Fig. 1, 2, 3, Table 1). These differences could be as large as three fold for protein, and two fold for carbohydrate and lipids. Are these differences important?

Lipid is important and valuable for fish larvae growth and survival (Rainuzzo et al., 1997; Estevez et al., 1999; Sargent et al., 1999), and lipids and protein can be important in the diet of fish, especially when carbohydrates are low (Cowey and Sargent, 1972). For instance, high protein content and a high ratio of protein to lipid in rotifers are advantageous for the growth and survival of turbot larvae during early development stages (Øie et al., 1997). As the nutritional quality of *Brachionus plicatilis* is critical in the transfer of dietary components from phytoplankton to fish larvae (Ben-Amotz, 1984; Watanabe et al., 1978), such two-fold or 3-fold variations may influence aquaculture practices. Thus, these differences may require monitoring.

However, this study has not been able to provide any simple means to predict the biochemical composition of rotifers, based on knowledge of the prey strain/species or prey incubation temperature. Thus, the only recourse this study can offer to aquaculturists, if they are concerned with two- to three-fold differences in rotifer composition, is for them to make these measurements themselves on their own strains at their growth temperatures, or if possible to refer to specific measurements from this study. However, given the wide range of variables that may occur in most practices and the high variation in biochemical parameters (e.g., Table 2), this level of detail may not be considered important.

5.6. References

Abu-Rezeq, T., James, C.M., 1985. Production and nutritional quality of the rotifer *Brachionus plicatilis* in relation to different *Chlorella* cell densities. Annu. Res. Rep. Kuwait Inst. Sci. Res. 10, 30-34.

Barclay, W., Zeller, S., 1996. Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and *Artemia nauplii* by feeding spray-dried *Schizochytrium* sp. J. World Aquacult. Soc. 27, 314-322.

Ben-Amotz, A., 1984. Production of nutritional microphytoplankton for use as food in marine fish hatcheries, 195-200 pp.

Ben-Amotz, A., Fisher, R., Schneller, A., 1987. Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. Mar. Biol. 95, 31-36.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of portion utilizing the principle of portion dye-binding. Anal. Biochem. 72, 248-254.

Brown, M.R., Dunstan, G.A., Jeffrey, S.W., Volkman, J.K., Barrett, S.M., LeRoi, J.M., 1993. The influence of irradiance on the biochemical composition of the prymnesiophyte *Isochrysis sp.* (clone T-ISO). J. Phycol. 29, 601-612.

Caric, M., Sanko-Njire, J., Skaramuca, B., 1993. Dietary effects of different feeds on the biochemical composition of the rotifer (*Brachionus plicatilis* Muller). Aquaculture 110, 141-150.

Cowey, C.B., Sargent, J.R., 1972. Fish nutrition. Adv. Mar. Biol. 10, 383-492.

Dendrinos, P., Thorpe, J.P., 1987. Experiments on the artificial regulation of the amino acid and fatty acid contents of food organisms to meet the assessed nutritional requirements of larval, post-larval and juvenile Dover sole (*Solea solea* (L.)). Aquaculture 61, 121-154.

Estevez, A., McEvoy, L.A., Bell, J.G., Sargent, J.R., 1999. Growth, survival, lipid composition and pigmentation of turbot (*Scophthalmus maximus*) larvae fed live-prey enriched in Arachidonic and Eicosapentaenoic acids. Aquaculture 180, 321-343.

Fernandez-Reiriz, M.J., Labarta, U., 1996. Lipid classes and fatty acid composition of rotifers (*Brachionus plicatilis*) fed two algal diets. Hydrobiologia 330, 73-79.

Fernandez-Reiriz, M.J., Labarta, U., Ferreiro, M.J., 1993. Effects of commercial enrichment diets on the nutritional value of the rotifer (*Brachionus plicatilis*). Aquaculture 112, 195-206.

Frolov, A.V., Pankov, S.L., 1992. The effect of starvation on the biochemical composition of the rotifer *Brachionus plicatilis*. J. Mar. Biol. Assoc. U.K. 72, 343-356.

Frolov, A.V., Pankov, S.L., Geradze, K.N., Pankov, S.A., Spektorova, L.V., 1991. Influence of the biochemical composition of food on the biochemical composition of the rotifer *Brachionus plicatilis*. Aquaculture 97, 181-202.

Gallagher, M.L., Daniels, H.V., Harcke, J.E., 2001. Influence of the enrichment time with algae paste preservations on the fatty acid content of rotifers. World Aquacult. 32, 59-61.

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Chanley, M.H. (Ed.), Culture of Marine Invertebrate Animals. Plenum Press, New York, pp. 29-60.

Hansen, B., Moller, T., Wittrup, L., 1997. Particle grazing efficiency and specific growth efficiency of the rotifer *Brachionus plicatilis* (Muller). J. Exp. Mar. Biol. Ecol. 215, 217-233.

Hirayama, K., Kusano, T., 1972. Fundamental studies on physiology of rotifer for its mass culture - II. Influence of water temperature on population growth of rotifer. Bull. Jap. Soc. Sci. Fish. 38, 1357-1363.

Hirayama, K., Takagi, K., Kimura, H., 1979. Nutritional effect of eight species of marine phytoplankton on population growth of the rotifer, *Brachionus plicatilis*. Bull. Jap. Soc. Sci. Fish. 45, 11-16.

Kanazawa, A., 2003. Nutrition of Marine Fish Larvae. J. Appl. Aquacult. 13, 1-2.

Kochert, G., 1978. Quantitation of the macromolecular components of microalgae. Cambridge University Press, Cambridge, 189-195 pp.

Lewis, T., Nichols, P.D., Hart, P.R., Nichols, D.S., McMeekin, T.A., 1998. Enrichment of rotifers *Brachionus plicatilis* with eicosapentaenoic acid and docosahexaenoic acid produced by bacteria. J. World Aquacult. Soc. 29, 313-318.

Lubzens, E., Zmora, O., 2003. Production and Nutritional Value of Rotifers. In: McEvoy, L.A. (Ed.), Live feeds in marine aquaculture. Blackwell Science Ltd, Cornwall, pp. 17-52.

Lubzens, E., Marko, A., Tietz, A., 1984. Lipid synthesis in the rotifer *Brachionus plicatilis*. In: Sarig, S. (Ed.), German-Israeli Cooperation in Aquaculture Research. European Mariculture Soc., Hamburg (FRG), 5 Mar 1984, pp. 201-210.

Lubzens, E., Marko, A., Tietz, A., 1985. De novo synthesis of fatty acids in the rotifer, *Brachionus plicatilis*. Aquaculture 47, 27-37.

Lubzens, E., Tandler, A., Minkoff, G., 1989. Rotifers as food in aquaculture. Hydrobiologia 186/187, 387-400.

Makridis, P., Olsen, Y., 1999. Protein depletion of the rotifer *Brachionus plicatilis* during starvation. Aquaculture 174, 343-353.

Mandeville, J.M., Watson, M.T., Moran, B.M., 2001. Preliminary studies on optimal rotifer culture diets and alternative replacement diets and enrichments for larval Black Sea bass (*Centropristis striata*). J. Shellfish Res. 20.

Molina Grima, E., Garcia Camacho, F., Sanchez Perez, J.A., Garcia Sanchez, J.L., 1994. Biochemical productivity and fatty acid profiles of *Isochrysis galbana* Parke and *Tetraselmis* sp. as a function of incident light intensity. Process Biochemistry 29, 119-126.

Molina Grima, E., Fernandez Sevilla, J.M., Sanchez Perez, J.A., Garcia Camacho, F., 1996. A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances. J. Biotechnol.45, 59-69.

Øie, G., Olsen, Y., 1997. Protein and lipid content of the rotifer *Brachionus plicatilis* during variable growth and feeding condition. Hydrobiologia 358, 1-3.

Øie, G., Reitan, K.I., Olsen, Y., 1994. Comparison of rotifer culture quality with yeast plus oil and algal-based cultivation diets. Aquacult. Int. 2, 225-238.

Øie, G., Makridis, P., Reitan, K., and Olsen, Y., 1997. Protein and carbon utilization of rotifers (*Brachionus plicatilis*) in first feeding of turbot larvae (*Scophthalmus maximus* L.). Aquaculture. 153, 103-122.

Øie, G., Makridis, P., Reitan, K., Olsen, Y., 1997. Survival and utilization of carbon and protein in turbot larvae (*Schophthalmus maximus* L.) feed rotifers (*Brachionus plicatilis*) with different protein, lipid and protein/lipid ratio. Aquaculture 153, 103-122.

Okauchi, M., Fukusho, K., 1984. Food value of a minute alga, *Tetraselmis tetrathele* for the rotifer *Brachionus plicatilis* culture. 1. Population growth with batch culture. Bull. Natl. Res. Inst. Aquacult. (Japan) Yoshokukenho. 5, 13-18.

Olsen, Y., Reitan, K.I., Vadstein, O., 1993. Dependence of temperature on loss rates of rotifers, lipids, and omega3 fatty acids in starved *Brachionus plicatilis* cultures. Hydrobiologia 255/256, 13-20.

Pande, S.V., Parvin, R., Venkitasubramanian, 1963. Microdetermination of lipids and serum total fatty acids. Anal. Biochem. 6, 415-425.

Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1997. The significance of lipids at early stages of marine fish. Aquaculture 155, 103-115.

Reitan, K.I., Rainuzzo, J.R., Oeie, G., Olsen, Y., 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155, 207-221.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D., 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179, 217-229.

Scott, A.P., Baynes, S.M., 1978. Effect of algal diet and temperature on the biochemical composition of the rotifer, *Brachionus plicatilis*. Aquaculture 14, 247-260.

Scott, A.P., Baynes, S.M., 1979. The effect of unicellular algae on survival and growth of turbot larvae (*Scophthalmus maximus* L.). In: Halver, J.E. Tiews, K. (Eds), Finfish Nutrition and Fishfeed Technology, Heenemann, Berlin, Hamburg, Germany, 423-433 pp.

Tamaru, C.S., Murashige, R., Lee, C.S., Ako, H., 1993. Rotifers fed various diets of baker's yeast and/or *Nannochloropsis oculata* and their effect on the growth and survival of striped mullet (*Mugil cephalus*) and milkfish (*Chanos chanos*) larvae. Aquaculture 110, 361.

Underwood, A.J., 1997. Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge, 504 pp.

Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 89, 263-272.

Watanabe, T., Kitajima, C., Arakawa, T., Fukusho, K., Fujita, S., 1978. Nutritional quality of rotifer *Brachionus plicatilis* as a living feed from the viewpoint of essential fatty acids for fish. Bull. Jap. Soc. Sci. Fish. 44, 1109-1114.

Whyte, J.N.C., Nagata, W.D., 1990. Carbohydrate and fatty acid composition of the rotifer, *Brachionus plicatilis*, fed monospecific diets of yeast or phytoplankton. Aquaculture 89, 263-272.

Whyte, J.N.C., Clarke, W.C., Ginther, N.G., Jensen, J.O.T., Townsend, L.D., 1994. Influence of composition of *Brachionus plicatilis* and *Artemia* on growth of larval sablefish (*Anoplopoma fimbria* Pallas). Aquaculture 119, 47-61.

Yufera, M., 1982. Morphometric characterization of a small-sized strain of *Brachionus plicatilis* in culture. Aquaculture 27, 55-61.

Zar, J.H., 1999. Biostatistical Analysis. Prentice Hall International, INC, New Jersey, USA, 663 pp.

Chapter 6.

Does this study only applied to aquaculture?

6.1. The study from an aquaculture point of view

Forty years ago knowledge of biochemical differences between marine plankton species was relatively sparse (Parsons et al., 1961). Subsequent research has indicated that for a single species the variation in cell composition may differ according to the culture conditions under which they grown (James et al., 1989; Thompson et al., 1991, 1992; Renaud et al., 1999; Tzovenis et al., 1997, 2003). It is clear from the present study that differences between microalgal strains, and also the temperature at which they were grown, not only affect biochemical composition, but also their production (Chapter 3), and that these effects could vary many fold. In turn, their "quality" influences the growth, development, and biochemical composition content of their predator (Chapter 4, 5).

However, several questions remain. How many species are used in aquaculture practices and how many strains of these species have been discovered worldwide? Do they all have sufficiently high production to justify their cost? Do they all behave similarly even under different growth condition? This study may help to answer some of these questions from an aquaculture point of view. The implications of my results for specific aquaculture practices have been shown in the previous chapters. However, my findings have implications for areas other than aquaculture.

6.2. Applied areas other than aquaculture

6.2.1. Ecology

Microalgae are found all over the would. They are mainly distributed in water, but also found on the surface of all types of soil. Although they are generally free-living, some microalgae live in symbiotic association with a variety of other organisms. Many organisms depend on them and they are the main producers in innumerable food webs. Microalgal ecology has been reviewed extensively over the decades, yet only a few researchers have discussed the effect of microalgal strain and their temperature on growth, production, and nutritional "quality"; and the influence of these on the organisms feeding upon them.

Microalgae and zooplankton are exposed to a variety of changes in the environment. Seasonal cycles vary according to the climatic and geographical location of the habitat in which they are growing, and different strains of the same species may respond differently. These organisms have developed mechanisms for sensing and acclimating to changes in their environment (Vonshak and Torzillo, 2004). Response to environmental change is an inherent characteristic of any living organism. Traditionally, temperature has been considered one of the limiting environmental factors that determine the rate of growth and biochemical reactions (Cossins and Bowler, 1987; Atkinson, 1995; Montagnes and Weisse, 2000; Bosque et al., 2001; Montagnes and Franklin, 2001). Strains from different geographical locations are physiologically different (Bouaicha et al., 2001; Delgado et al., 1997), and could vary in their response to any limiting environmental factor.

6.2.2. Industrial production

Today, microalgae are marketed as health food, food supplements, as a source of commercially valuable products, or for therapeutic applications for humans and animals (Dubinsky and Aaronson, 1980; Laguna et al., 1993; Lubian et al., 2000; Sukenik, 1999; Zittelli et al., 1999). They are commonly sold in the form of tablets, powder and liquids. They have been marketed for several years (Becker, 2004), mostly in industrialized countries, where consumers are responsive to "health foods" and where education on the nutritional value of microalgae helps to overcome prejudice. Data on nutritional value, relates mainly to overall chemical composition (protein, carbohydrates, lipids, vitamins, minerals, etc.).

This study has shown that the chemical composition of microalgae is not an intrinsic constant factor, but varies from strain to strain and from batch to batch, the latter potentially depending on environmental factors such as that tested here (temperature). To obtain an algal biomass with a desired composition, the proportion of different algal constituents can be modified to a certain extent by varying culture conditions or changing physical parameters such as temperature, radiation intensity, light, or dark growth. Analyses of gross chemical composition of different microalgal strains under varying culture conditions should be published to draw attention to their differences.

6.2.3. Biotechnology?

The mass culture of microalgae grown in the laboratory or outdoors represents a special environment, where dense suspensions of cells are usually cultivated under conditions of high irradiance, but in reality this may mean low irradiance per cell because of self-shading (Zittelli et al., 2000). These special conditions also involve high concentrations of dissolved oxygen and limited supplies of inorganic carbon (carbon dioxide or bicarbonate) (Zittelli et al., 2004). Therefore, cultivation on an industrial scale critically depends on the interplay of several parameters including: average irradiance per cell, mixing gas exchange (Cheng-Wu et al., 2001; Molina et al., 2001), and temperature (Richmond and Cheng-Wu, 2001; Richmond, 2004). For commercial mass production, the effect of all of these parameters could vary considerably between microalgal strains. Thus strain selection is likely to be of critical importance.

6.3. What is the next step?

Aquaculture and the application of microalgal biotechnology have expanded considerably over the past decade, resulting in an increase in the demand for microalgae (Borowitzka, 1997; Duerr et al., 1998; Wikfors and Ohno, 2001). Many of the microalgae are used in regions other than that in which there were first isolated, and therefore their growth may not be optimal in the local conditions. If they have to be grown under tightly controlled conditions it may

not be cost effective. Because of this, efforts have been made to evaluate the usefulness of existing and newly isolated species of microalgae from local waters (Thinh et al., 1999; Su et al., 2001). Wise governments realized the importance of their microalgae strains for their national wealth (Davy, 1990; 1991a; Cen and Zhang, 1998). So they guided the researchers to investigate the value of their strains (Davy, 1991b; Brown et al., 1997; 1998; Knuckey et al., 2002), and they support the institutes that control their algal culture collections such as the UK National Culture Collection (CCAP).

The present study suggests that as a result of increasing global temperature, i.e. global warming, governments would be well advised to search and scan their local environment for strains that have the potential to optimize their growth and nutritional value under a higher temperature range. There has been work going on in this direction (Brown et al., 1997; 1998; Thinh et al., 1999; Renaud et al., 1999; 2002). However there are many countries where the sea water temperature reaches 40 °C where work still remains to be done (Bengtson, 2003).

Prevention is better than cure; governments need to protect local microalgal strains. A strict law must prohibit untreated waste going into areas that benefit from a rich microalgal diversity. It is suggested that where species are endangered by pollution local strains should be saved in a "strains bank"; this action will help to preserve them for use by the next generation. Such concepts need to put at the top of governments' agenda.

Countries that have a history of success in aquaculture and biotechnology have a duty to pass on their experience to countries that are in need. People that are suffering from serious food shortages in the third world should be taught this technology; they should know how to protect their own strains and benefit from

them. This could be done under the supervision of the United Nations Food and Agriculture Organization (FAO). The knowledge of such an essential technology should be freed from trading benefits, but the rights of governments and researchers should be protected in law.

Aquaculture and biotechnology could be improved. Aquaculture makes up more than 35% of the total 92.6 million tones of fisheries products consumed by humans annually (FAO, 2000). This output could be increased rapidly if every hatchery has the knowledge and experience of some of the most successful organisms in aquaculture. For example, the rotifer *Brachionus plicatilis* has been proven to grow successfully on a diet of different microalgal species and strains. Yet still there are some hatcheries that have not yet discovered the importance of this organism and used it for the production of their fish larvae (Bengtson, 2003).

Microalgae are also particularly valuable for producing products of commercial interest since they may be readily genetically improved. Both modern techniques of genetic engineering and more traditional methods of mutation and strain selection have been suggested as the next step for this purpose (Lopez Alonso et al., 1992, 1993, 1994, 1996).

When a new organism strain or species is discovered for aquaculture and biotechnology purposes, a new technical method is often required and it is advisable to have a standard "menu" and "recipe" with a minimum number of well-established organisms used as a live feed. Therefore, strains around the world need to be "coded" internationally so that confusion between them is avoided. This "code" could give the information on the original geographic location of the isolation and some information on the best growth condition. By this means, the costs of this "coded strain" for the production of live feed culture

could be minimized. As with any other industry it is all a matter of cost and profit.

6.4. References

Atkinson, D., 1995. Effects of temperature on the size of aquatic ectotherms: Exceptions to the general rule. J. Thermal Biol. 20, 61-74.

Becker, W., 2004. Microalgae in Human and Animal Nutrition. In: Richmond, A. (Ed.), Handbook of Microalgae Culture biotechnology and Applied Phycology. Blackwell Science Ltd, Cornwall, pp. 312-351.

Bengtson, D.A., 2003. Status of Marine Aquaculture in Relation to Live Prey: Past, Present and Future. In: Støttrup, J.G., McEvoy, L.A. (Eds.), Live Feeds in Marine Aquaculture. Blackwell, Cornwall, pp. 1-13.

Borowitzka, M.A., 1997. Microalgae for aquaculture: opportunities and constraints. J. Appl. Phycol. 9, 393-401.

Bosque, T., Hernandez, R., Todoli, R., Oltra., 2001. Effect of salinity, temperature and food level on the demographic characteristics of the seawater rotifer *Synchaeta littoralis* Rousselet. J. Exp. Mar. Biol. Ecol. 258, 55-64.

Bouaicha, N., Chezeau, A., Turquet, J., Quod, J.P., Puiseux Dao, S., 2001. Morphological and toxicological variability of *Prorocentrum lima* clones isolated from four locations in the south-west Indian Ocean. Toxicon 39, 1195-1202.

Brown, M.R., McCausland, M.A., Kowalski, K., 1998. The nutritional value of four Australian microalgal strains fed to Pacific oyster *Crassostrea gigas* spat. Aquaculture 165, 3-4.

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151, 315-331.

Cen, F., Zhang, D., 1998. Development and status of the aquaculture industry in the People's Republic of China. World Aquacult. 29, 52-56.

Cheng-Wu, Z., Zmora, O., Kopel, R., Richmond, A., 2001. An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). Aquaculture 195, 1-2.

Cossins, A.R., Bowler, K., 1987. Temperature Biology of Animals. Chapman and Hall, London, 339 pp.

Davy, F.B., 1990. Mariculture in Japan: Development of an industry. World Aquacult. 21, 36-51.

Davy, F.B., 1991a. Mariculture in Japan: Current practices. World Aquacult. 22, 30-36.

Davy, F.B., 1991b. Mariculture in Japan: Biotechnology and the future. World Aquacult. 22, 19-23.

Delgado, M., Garces, E., Vila, M., Camp, J., 1997. Morphological variability in three populations of the dinoflagellate *Alexandrium taylori*. J. Plankton Res. 19, 749-757.

Dubinsky, Z., Aaronson, S., 1980. Review of the potential uses of microalgae, 2nd International Workshop on Biosaline Research, La Paz, Baja California Sur (Mexico), pp. 11.

Duerr, E.O., Molnar, A., Sato, V., 1998. Cultured microalgae as aquaculture feeds. J. Mar. Biotechnol. 7, 65-70.

FAO, 2000. The state of world fisheries and aquaculture, 2000. http://www.fao.org/DOCREP/003/X8002E/X8002E00.HTM.

James, C.M., Al-Hinty, S., Slman, A.E., 1989. Growth and ω3 fatty acid composition of marine microalgae under different temperature regimes. Aquaculture 77, 337-351.

Knuckey, R.M., Brown, M.R., Barrett, S.M., Hallegraeff, G.M., 2002. Isolation of new nanoplanktonic diatom strains and their evaluation as diets for juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture 211, 1-4.

Laguna, M.R., Villar, R., Cadavid, I., Calleja, J.M., 1993. Effects of Extracts of *Tetraselmis suecica* and *Isochrysis galbana* on the Central Nervous System. Planta Med. 59, 207-214.

Lopez Alonso, D., Segura del Castillo, C.I., Molina Grima, E., Cohen, Z., 1996. First insights into improvement of eicosapentaenoic acid content in *Phaeodactylum tricornutum* (Bacillariophyceae) by induced mutagenesis. J. Phycol. 32, 339-345.

Lopez Alonso, D., Molina Grima, E., Sanchez Perez, J.A., Garcia Sanchez, J.L., Garcia Camacho, F., 1992. Isolation of clones of *Isochrysis galbana* rich in eicospapentaenoic acid. Aquaculture 102, 363-371.

Lopez Alonso, D., Sanchez Perez, J.A., Garcia Sanchez, J.L., Grcia Camacho, F., Molina Grima, E., 1993. Improvement of eicosapentaenoic acid content in isolates of *Isochrysis galbana*. J. Marine Biotechnol. 1, 147-149.

Lopez Alonso, D., Segura del Castilo, C.I., Garcia Sanchez, J.L., Sanchez Perez, J.A., Garcia Camacho, F., 1994. Quantitative genetics of fatty acid variation in *Isochrysis galbana* (Prymnesiophyceae) and *Phaeodactylum tricornutum* (Bacillariophyceae). J. Phycol. 30, 553-558.

Lubian, L.M., Montero, O., Moreno-Garrido, I., Huertas, I.E., Sobrino, C., Gonzalez-del Valle, M., Pares, G., 2000. *Nannochloropsis* (Eustigmatophyceae) as source of commercially valuable pigments. J. Appl. Phycol. 12, 3-5.

Molina, E., Fermandez , J., Acien, F.G., Chisti, Y., 2001. Tubular photobioreactor design for algal cultures. J. Biotechnol. 92, 113-131.

Montagnes, D.J.S., Weisse, T., 2000. Fluctuating temperatures affect growth and production rates of planktonic ciliates. Aquat. Microb. Ecol. 21, 97-102.

Montagnes, D.J.S., Franklin, D.J., 2001. Effect of temperature on diatom volume, growth rate, and carbon and nitrogen content: Reconsidering some paradigms. Limnol. Oceanogr. 46, 2008-2018.

Parsons, T.R., Stephens, K., Strickland, J.D.H., 1961. On the chemical composition of eleven species of marine phytoplankters. J. Fish. Res. Bd Can 18, 1001-1016.

Renaud, S.M., Thinh, L.V., Parry, D.L., 1999. The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. Aquaculture 170, 147-159.

Renaud, S.M., Thinh, L., Lambrinidis, G., Parry, D.L., 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 211, 195-214.

Richmond, A., 2004. Handbook of Microalgal Culture Biotechnology and Applied Phycology. Blackwell Science Ltd, Cornwall.

Richmond, A., Cheng-Wu, Z., 2001. Optimization of a flat plate glass reactor for mass production of *Nannochloropsis* sp. outdoors. J. Biotechnol. 85, 259-269.

Su, H.M., Su, M.S., Liao, I.C., 2001. The Culture and Use of Microalgae for Larval Rearing in Taiwan. Taiwan Fisheries Research Institute, 199 Hou-Ih Road Keelung 202 Taiwan.

Sukenik, A., 1999. Production of EPA by *Nannochloropsis*. In: Cohen, Z. (Ed.), Chemicals from Microalgae. Taylor and Francis, London, pp. 41-56.

Thinh, L.V., Renaud, S.M., Parry, D.L., 1999. Evaluation of recently isolated Australian tropical microalgae for the enrichment of the dietary value of brine shrimp, *Artemia nauplii*. Aquaculture 170, 161-173.

Thompson, P.A., Harrison, P.J., Parslow, J.S., 1991. Influence of irradiance on cell volume and carbon quota for ten species of marine phytoplankton. J. Phycol. 27, 351-360.

Thompson, P.A., Guo, M.X., Harrison, P.J., 1992. Effects of variation in temperature. 1. On the biochemical composition of eight species of marine phytoplankton. J. Phycol. 28, 481-488.

Tzovenis, I., De Pauw, N., Sorgeloos, P., 1997. Effect of different light regimes on the docosahexaenoic acid (DHA) content of *Isochrysis aff. galbana* (clone T-ISO). Aquacult. Int. 5, 489-507.

Tzovenis, I., De Pauw, N., Sorgeloos, P., 2003. Optimisation of T-ISO biomass production rich in essential fatty acids. II. Effect of different light regimes on the production of fatty acids. Aquaculture 216, 1-4.

Vonshak, A., Torzillo, G., 2004. Environmental Stress Physiology. In: Richmond, A. (Ed.), Handbook of Microalgal Culture Biotechnology and Applied Phycology. Blackwell Science Ltd, Cornwall, pp. 57-82.

Wikfors, G.H., Ohno, M., 2001. Impact of algal research in aquaculture. J. Phycol. 37, 968-974.

Zittelli, G.C., Pastorelli, R., Tredici, M.R., 2000. A Modular Flat Panel Photobioreactor (MFPP) for indoor mass cultivation of *Nannochloropsis* sp. under artificial illumination. J. Appl. Phycol. 12, 3-5.

Zittelli, G.C., Rodolfi, L., Tredici, M.R., 2004. Industrial Production of Microalgal Cell-mass and Secondary Products- Species of high Potential, Mass Cultivation of *Nannochloropsis* in closed Systems. In: Richmond, A. (Ed.), Handbook of Microalgal Culture Biotechnology and Applied Phycology. Blackwell Science Ltd, Cornwall, pp. 298-303.

Zittelli, G.C., Lavista, F., Bastianini, A., Rodolfi, L., Vincenzini, M., Tredici, M.R., 1999. Production of eicosapentaenoic acid by *Nannochloropsis* sp. cultures in outdoor tubular photobioreactors. J. Biotechnol. 70, 299-312.