

**COPEPOD PRODUCTION: THE INTERPLAY
BETWEEN ABIOTIC ENVIRONMENT, PREY
BIOCHEMICAL COMPOSITION AND
CONSUMERS' REQUIREMENTS**

Dissertation

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“Nothing in biology makes sense, except in the light of evolution”

Theodosius Dobzhansky, 1973

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LIST OF MODEL VARIABLE/PARAMETERS, THEIR DESCRIPTION AND UNITS

Variables	Description	Unit
T	Ambient temperature	K
∂	Activation energy for metabolism	J
b	Energy cost for maintenance	$J.gC^{-1}.d^{-1}$
d	Energy cost for growth	$J.gC^{-1}$
Z_{Lmn}	Minimum lipid content of zooplankton	$g.animal^{-1}$
Z_{Lmx}	Maximum lipid content of zooplankton	$g.animal^{-1}$
Z_L	Required lipid content of zooplankton	$g.animal^{-1}$
h	Lipid content control parameter	K^{-1}
Z_P	Required protein content of zooplankton	$g.animal^{-1}$
Z_H	Required carbohydrate content of adult copepods	$g.animal^{-1}$
β_i	Structural protein, lipid, or carbohydrate requirement of zooplankton for maintenance	$gC.gC^{-1}$
δ_i	Structural protein, lipid, or carbohydrate requirement of zooplankton for growth	$gC.gC^{-1}$
V	Food supply	$gC.L^{-1}$
α_i	Protein-, lipid-, or carbohydrate composition of the food	$gC.gC^{-1}$
Q	Food quality	Dimensionless
I_{mg}	Maximum food ingestion rate	$gC.gC^{-1}.d^{-1}$
I_m	Minimum food ingestion rate	$gC.gC^{-1}.d^{-1}$
Z_{ing}	Food ingestion control parameter	Dimensionless
I_{td}	Operational threshold for food ingestion	$gC.gC^{-1}.d^{-1}$
I_c	Actual food ingestion rate	$gC.gC^{-1}.d^{-1}$
w_{mg}	Maximum prey capture efficiency	$L.gC^{-1}$
w_m	Minimum prey capture efficiency	$L.gC^{-1}$
Z_{ceff}	Prey capture control parameter	Dimensionless
w	Prey capture efficiency	$L.gC^{-1}$
λ_{mg}	Maximum food assimilation efficiency	Dimensionless
λ_m	Minimum food assimilation efficiency	Dimensionless
η	Half saturation constant for substrate assimilation	Dimensionless

LIST OF MODEL VARIABLE/PARAMETERS, THEIR DESCRIPTION AND UNITS

Variables	Description	Unit
λ_i	Chemical-specific assimilation efficiency	Dimensionless
ρ_i	Fraction of assimilate catabolised for maintenance	Dimensionless
γ_i	Fraction of assimilate catabolised to power growth	Dimensionless
x_i	Fraction of assimilate catabolised for neither maintenance nor growth	Dimensionless
F_c or F_i	C or compound-specific defecation rate	$\text{gC.gC}^{-1}.\text{d}^{-1}$
A_c or A_i	C or compound-specific assimilation rate	$\text{gC.gC}^{-1}.\text{d}^{-1}$
M_c or M_i	C or compound-specific catabolism for maintenance	$\text{gC.gC}^{-1}.\text{d}^{-1}$
g_c or g_i	C or compound-specific catabolism to power growth	$\text{gC.gC}^{-1}.\text{d}^{-1}$
X_c or X_i	C or compound-specific catabolism for neither maintenance nor growth	$\text{gC.gC}^{-1}.\text{d}^{-1}$
G or G_i	C or compound-specific growth rate of zooplankton	$\text{gC.gC}^{-1}.\text{d}^{-1}$
K_c or K_i	C or compound-specific gross growth efficiency	gC.gC^{-1}
U_i	Maximum utilisation efficiency for each food chemical constituent	Dimensionless
L_i	Compound-specific potential to limit zooplankton growth	Dimensionless

General Summary

Ecosystems are complex adaptive systems because their living components are capable of adapting to environmental change, be it biotic or abiotic change. Organisms however can experience adverse consequences if environmental change exceeds levels they can tolerate by relying on their adaptive capabilities. Hence, our ability to understand, predict and mitigate the effects of ecological stressors, such as those associated with global climate change, pollution, and harvesting, depends largely on how we incorporate organismic adaptation into ecological investigations.

This is true for trophic ecology as for any other aspect of ecology, because animals demonstrate varying feeding regulation mechanisms in order to satisfy their demands for energy and chemical substances. Most can preferentially feed on prey organisms that promote fitness against disadvantageous ones, and those incapable of prey selection are endowed (by evolution) with physiological capabilities for dealing with disadvantageous food constituents. Hence, trophic adaptation among consumers does not only help define the structure of food webs and thus the complexity of ecological communities, it also determines the fate as well as the ecological transfer efficiency of biomass (and carbon). Adaptive trophic behaviour is however ignored or poorly represented in ecosystem models because a realistic framework, for effecting trophic behaviour, that incorporates relevant natural history details and is capable of providing mechanistic understanding of trophic processes is lacking.

Currently in aquatic ecology, trophic behaviour is assumed to be mainly dictated by food quality (Q), which is determined by employing food quality models (FQMs) that mimic consumers' mechanism for anticipating the fitness consequences for feeding on specific prey items. Heuristically however, progress has been limited because previous FQMs are based on frameworks that *a priori* identify specific food components, usually nitrogen, phosphorus, and/or essential compounds, as pre-eminent or limiting. This suppresses trophic adaptability by negating the effect of habitat conditions (fluctuating temperature, etc), as well as that of consumers' behaviour and physiology on food quality.

This thesis addresses the shortfalls in existing models by proposing a new FQM. The model determines food quality by considering (i) the biochemical characteristics of prey items, (ii) consumers' demand for energy and structural constituents, and (iii) consumers' capacity for food intake and metabolism based on physiological principles. It employs parameters that are

adaptive to the relevant habitat conditions encountered by consumers. The model relies on the balance between the biochemical composition of prey items and consumer's requirements to determine the potential fate and utilisation efficiencies of acquired chemical substances. The yardstick for food quality is the potential growth performance of consumers. The form of the model makes it applicable to all heterotrophs. Here, the focus is on copepod consumers because of the critical role they play in aquatic ecosystems. The model has been used to evaluate the growth performance of *Acartia tonsa* (Copepoda: Calanoida) over wide range of food conditions and the results are consistent with experimental observations. It predicts *Acartia*'s response to changes in prey biochemical composition to be unimodal, with growth being highest only when the biochemical composition of the prey imposes no constrain on egg production. This is consistent with experimental observations. Existing food quality models are incapable of this prediction. Hence, the FQM here is a better alternative, which, when employed could help in understanding substrate acquisition and utilisation for growth and reproduction by heterotrophs.

This was demonstrated by embedding the FQM here in a secondary production model for a consumer whose food intake and metabolic capabilities are bounded within a maximum threshold needed for growth and maintenance when food is "nutritionally good", and a minimum threshold needed for only maintenance when food is "nutritionally poor". The consumer relies on the food quality model to evaluate the growth limiting potentials of individual prey constituents. This information then dictates its regulatory response, in terms of feeding and metabolism, to the available prey. The demonstration was used to re-evaluate the hypothesis that carbon (C), relative to nitrogen (N), is a non-limiting resource for egg production by marine copepods. The results here challenge the hypothesis, and reveal potential causes for carbon limitation that previous models do not predict because they suffer from the above-stated shortfalls. Within the range of ecologically realistic algal C:N ≤ 17 , C-limitation was determined to be higher than that of N, due partly to the low metabolic availability of C associated with cellulose and structural carbohydrates. This result emphasizes the importance of biochemical substances in animal nutrition and production.

The dynamics of zooplankton production may be determined by the interaction between ambient temperature and prey biochemical composition. While this has been documented in experiments, previous zooplankton models contain no explicit description of it. Chapter 4 of this thesis shows how this could be done. It makes the new food quality model adaptive to

changes in ambient temperature by making animals' demand for energy and structural constituents temperature dependent. This was then integrated into a model framework that allows consumers to regulate food ingestion, assimilation and metabolism based on their temperature-specific needs. Using this approach, growth rate, growth efficiency, as well as optimum temperatures for egg production by two copepod species with significantly different ranges of thermal tolerance were realistically simulated. Consistent with the results from other studies, the results here show that the growth response of copepods to changes in ambient temperature is driven mainly by temperature-induced changes in animals' demand for maintenance. This observation emphasizes the cost of maintenance as a major constraint on zooplankton production.

Food quality models suffer from an important practical problem: it is difficult to define the biochemical traits of organisms and how those traits are dependent on the several relevant environmental conditions. To tackle this problem, published data on the proteins, carbohydrates, lipids, essential amino and fatty acids composition of microalgae (68 species belonging to 7 taxonomic classes species) and marine zooplankton (female: 42 species, eggs: 29 species) cultured under diverse conditions were reviewed. From the results, robust parameters, not restricted to specific species or habitat conditions, were determined. They have been employed for this study and could be used by others to characterize the biochemical composition of algae and zooplankton, independent of the environment. In addition, an experiment was conducted to investigate the impact of food availability on the reproductive strategy of copepods, in terms of females' biochemical investment into eggs production. No food availability effect on the biochemical composition of females was observed. However, protein composition of eggs was higher in food-limited females. It has been argued that the production of protein-rich eggs by food-limited copepods is a reproductive strategy for ensuring the survival of offspring during poor feeding conditions.

In conclusion, this thesis provides realistic conceptual and mathematical frameworks for modelling trophic behaviour. It contributes to our understanding of trophic processes and their implications for nutrient cycling by grounding food quality on the behaviour, physiology, and habitat conditions of consumers. The model has been successfully integrated into an egg production model for copepods and implemented under variable temperature and food conditions.

Allgemeine Zusammenfassung

Ökosysteme und ihre lebenden Komponenten können sich an Umweltveränderungen anpassen, egal ob es sich um biotische oder abiotische Faktoren handelt. Wenn die Umwelteinflüsse überhand nehmen, können die Organismen dieses jedoch auch nicht mehr durch ihre Anpassungsfähigkeit ausgleichen. Folglich hängt unsere Fähigkeit die Effekte der ökologische Stressfaktoren (z.B.: Klimaveränderungen, Umweltverschmutzung und Überfischung) zu verstehen, vorherzusagen und abzuschwächen davon ab, wie wir die Anpassungsfähigkeit von Organismen in die ökologische Forschung einbeziehen.

Das gilt sowohl für die trophische Ökologie als auch für jeden anderen Aspekt der Ökologie, denn Tiere können ihrer Futterraufnahme regulieren, um ihr Energie- und Nährstoffbedarf zu decken. Zum Beispiel bevorzugen viele Tiere Beuteorganismen, die ihre Überlebenschancen fördern gegenüber diesen, die sich nachteilig darauf auswirken. Die Tiere, die unfähig sind Beuteselektion zu betreiben, sind (durch die Evolution) mit physiologischen Fähigkeiten ausgestattet worden mit unvorteilhaften Nahrungsbestandteilen zurechtzukommen. Solche Regulationen von Futterraufnahme beeinflussen die Strukturen von Nahrungsnetzen, ökologische Gemeinschaften sowie die Übertragungseffizienz von Biomasse. Allerdings wurde die Akklimatisierung von Futterraufnahme in theoretische Modellen von Ökosystem meistens ignoriert oder schlecht repräsentiert, weil u. a. eine realistische Rahmkonstruktion für biochemische Kontrolle von Nahrungsaufnahme noch nicht vorhanden ist.

In der aquatischen Ökologie wurde angenommen, dass trophisches Verhalten hauptsächlich von Futterqualität (Q) abhängig ist. Infolgedessen werden Futterqualitätsmodelle (FQMs) entworfen um den Beuteauswertungsmechanismus von Räuber zu imitieren. Solche Modelle setzen von vornherein spezifische Futterkomponenten (zum Beispiel Nitrogen, Phosphor und/oder essentielle Nährstoffe) als limitierte Nährstoffe fest. Diese Modellkonstruktion unterdrückt trophische Anpassung, indem es den Effekt von Habitatbedingungen (schwankende Temperaturen usw.) und Räuberphysiologie auf Futterqualität verneint.

Diese Dissertation schlägt ein neues adaptives FQM vor, die die Defekte von bereits vorhandenen Modellen behebt. Um Q zu bestimmen, berücksichtigt das Modell (i) biochemische Charakteristika von Beute, (ii) Räuberbedarf für Energie und Nährstoff sowie (iii) Räuberkapazität für Nährstoffaufnahme und Metabolismus. Es setzt Parameter ein, die fähig sind sich an die relevanten Habitatbedingungen anzupassen. Das Modell benutzt die Balance zwischen dem biochemischen Aufbau der Beute und den biochemische Anforderungen des Räubers, um die Nützung genauso wie die Nutzungseffizienz von

erworbenen chemischen Substanzen zu determinieren. Der Maßstab für Futterqualität ist die potentielle Wachstumsleistung von Räufern. Die Form des Modells macht es auf alle Heterotrophen anwendbar. Hier ist der Fokus auf die Copepoden gerichtet, da sie eine wichtigen Rolle in aquatischen Systemen spielen. Das Modell wurde verwendet, um die Wachstumsleistung von *Acartia tonsa* (Copepoda: Calanoida) über große Nährstoffbereiche auszuwerten und die Resultate stimmen mit den experimentellen Beobachtungen überein. Es prognostiziert *Acartia's* Reaktion auf Veränderungen in dem biochemischen Inhalt der Beute unimodal zu sein, wobei das Wachstum nur am höchsten ist, wenn die biochemische Zusammensetzung der Beute zu keiner Einschränkung in der Eiproduktion führt. Das steht im Einklang mit experimentellen Beobachtungen. Bereit existierende Nährstoffqualitätsmodelle sind außer Stande diese Vorhersage zu machen. Daher ist hier das FQM eine bessere Alternative, die helfen kann, trophische Prozesse zu verstehen.

Hier wurde das demonstriert indem Integration das neue FQM in ein Sekundärproduktionsmodell integriert wurde, für einen Räuber, dessen Nahrungsaufnahme und Metabolismus eine Obergrenze hat, die für Wachstum und Lebenserhaltung benötigt wird, wenn die Futterqualität „gut“ ist und einem Untergrenze, die nur für die Lebenserhaltung gebraucht wird, wenn die Futterqualität „schlecht“ ist. Der Räuber verwendet das Futterqualitätsmodell, um das wachstumslimitierende Potenzial von einzelnen chemischen Inhaltsstoffen der Beute auszuwerten. Dann bestimmt diese Information die Reaktion des Räubers um seine Nahrungsaufnahme und seinen Metabolismus, durch Regulation, zu optimieren. Die Demonstration wurde benutzt, um die Hypothese neu zu bewerten, dass Kohlenstoff (C) im Vergleich mit Stickstoff (N) eine nicht-limitierte Ressource für die Eiproduktion von marinen Copepoden ist. Die Ergebnisse zeigen hier, dass diese Hypothese anzweifelbar ist und machen die möglichen Ursachen von Kohlenstofflimitation deutlich, die die frühere Modelle nicht vorhersagen konnten, weil sie unter der oben genannten Schwäche leiden. Im Bereich der realistischen ökologischen Alge $C:N \leq 17$, wurde die Kohlenstofflimitation höher ermittelt als die von Stickstoff, teilweise aufgrund der geringen metabolischen Verfügbarkeit von Cellulose- und strukturellem Kohlenhydrat-assoziiierenden Kohlenstoffs. Diese Ergebnis unterstreicht die Bedeutung der makromolekularen biochemischen Kontrolle von Zooplanktonproduktion.

Die Dynamik der Zooplanktonproduktion kann durch die Interaktion zwischen Umwelttemperatur und dem biochemischer Inhalt des Futters bestimmt werden. Obwohl dies in Experimenten dokumentiert worden ist, enthalten bisherige Zooplanktonmodelle keine explizite Beschreibung davon. Kapitel 4 dieser Arbeit zeigt, wie dieses getan werden könnte. Es macht das neue Futterqualitätsmodell anpassungsfähig bei Veränderungen in der Umwelttemperatur, indem es den Bedarf für Energie und

strukturellen Substanzen bei den Tieren temperaturabhängig darstellt. Dieses wurde dann in einem Modell integriert, das den Räubern erlaubt, die Nährstoffaufnahme, Assimilation und Metabolismus, basierend auf ihrem temperaturspezifischen Bedarf zu regulieren. Unter Verwendung dieses Ansatzes wurden Wachstumsraten, die Wachstumseffizienz sowie optimale Temperaturen für die Eiproduktion von zwei Copepodensarten mit signifikant verschiedenen Temperaturtoleranzräumen in dem Modell realistisch simuliert. Die Ergebnisse zeigen, dass die Wachstumsreaktion der Copepoden zu den Veränderungen in der Umwelttemperatur, hauptsächlich durch temperaturinduzierte Veränderungen in dem Bedarf der Lebenserhaltung von weiblichen Copepoden, angetrieben wird. Diese Beobachtung betont den Lebenserhaltungsbedarf von weiblichen Copepoden als Hauptbeschränkung der Eiproduktion.

Futterqualitätsmodelle leiden unter einem wichtigen praktischen Problem: es ist schwierig die biochemischen Charakteristika von Organismen zu definieren und wie diese Charakteristika von mehreren relevanten Umweltfaktoren abhängen. Um dieses Problem in Angriff zu nehmen, wurden publizierte Daten von Proteine, Kohlenhydrate, Lipide sowie essentielle Amino- und Fettsäuren von Mikroalgen (68 Arten die zu 7 taxonomischen Klassen gehören) und Meereszooplankton (weiblich: 42 Arten, Eier: 29 Arten) die unter diversen Bedingungen kultiviert worden sind, erneut analysiert. Von den Ergebnissen waren robuste Parameter, die sich nicht auf eine bestimmte Art oder Habitate beziehen, ermittelt worden. Diese wurden in dieser Studie verwendet und könnten bei anderen Studien verwendet werden, um den biochemischen Inhalt von Algen und Zooplankton zu determinieren, unabhängig von Habitatbedingungen. Zusätzlich wurde ein Experiment durchgeführt, um die Auswirkungen von Futterquantität auf die reproduktive Strategie der Copepoden zu untersuchen, im Bezug mit den biochemischen Investitionen von weiblichen Copepoden in der Eiproduktion. Es konnte kein Futterquantitätseffekt beim biochemischen Inhalt der Weibchen beobachtet werden. Allerdings war der Proteininhalt der Eier, die von futterlimitierten Weibchen produziert wurden, höher. Es wurde argumentiert, dass die Produktion von proteinreichen Eiern bei futterlimitierten Copepoden eine reproduktive Strategie für die Gewährleistung des Überlebens der Nachkommen unter armen Ernährungskonditionen ist.

Letztendlich wurden in diese Arbeit realistische konzeptuelle und mathematische Rahmenbedingungen entwickelt, die verwendet werden können, um trophischen Verhalten von Räuber zu modellieren. Es trägt zu unserem Verständnis der trophischen Prozesse und ihrer Auswirkungen auf den Nährstoffkreislauf bei, indem es Verhalten, Physiologie und Habitatbedingungen von Räubern in

Futterqualität integriert. Das Modell wurde erfolgreich in einem Eiproduktionsmodell für Copepoden integriert und unter variablen Temperatur- und Futterbedingungen verwendet.

Outline of publications

Only one publication has been included in this thesis. It can be found under appendix 3.

Title: Food availability effect on reproductive strategy: the case of *Acartia tonsa* (Copepoda: Calanoida).

Authors: Acheampong Emmanuel, Campbell R. W., Diekmann A. B. S, and St. John M. A.

Journal: Marine Ecology Progress Series, a peer review journal (2011: volume 428, pages 151 – 159).

My contribution:

I developed the concept of the study. Diekmann A. B. S. (Universität Hamburg, Germany) helped with the experiment, as well as with the carbohydrate and protein analyses. I did lipid and data analyses, as well as wrote the manuscript with scientific and editorial advice from Campbell R. W. (Prince William Sound Science Center, Cordova, AK, 99574, USA) and St. John M. A. (Universität Hamburg, Germany).

Chapter 1

General Introduction

1.1 Animal nutrition

Marine ecosystems and their services are under pressure due to global climate change (Parmesan and Yohe 2003; Harley et al. 2006), and restructuring caused by pollution (Savage et al. 2002; Gaudry et al. 2007), mariculture (Guo et al. 2009) and harvesting (Frank et al. 2005). Our ability to understand, predict and mitigate the effects of these stressors on marine ecosystems and their key species is dependent, in part, upon the application of ecosystem models (Arrow et al. 2000; St. John et al. 2010). Models are the only investigative tools that allow simultaneous investigation of the impact several factors might have on the past, current and future dynamics of marine ecosystems. This is because, compared with other investigative tools (e.g., direct observation and experiment), models have fewer operational limitations, and as a consequence permit one to realistically describe the combined ecological impact of several conditions, including those outside the natural range of what animals and other key marine organisms normally require for reproduction and continuous survival.

A critical aspect of ecological modelling is predator-prey interactions. This is because species interactions are central to all ecosystems. They define the structure of food webs and thus constitute major component of ecological communities (Petchey et al. 2008). Modelling predator – prey interactions is therefore vital to answering questions about extinctions (van Baalen et al. 2001; Kondoh 2003; Koh et al. 2004), environmental change (Petchey et al. 1999), trophic cascades (Knight et al. 2005), and ecosystem functioning (Pain 2002; Ngai and Srivastava 2006).

Species interactions involve several networks of direct and indirect pathways, usually take place among different organizational and spatial scales, and involve numerous behavioural mechanisms that may produce a high degree of complexity in the dynamics of ecosystems (Paine 1980; Levin 1998). For instance, most consumers usually prey on several to more than 100 prey species from a broad range of trophic types, size-classes, and cell structure, and thus making the trophic positions of organisms difficult to define (Polis and Strong 1996). Even plants do not occupy a single trophic level; many phytoplankton (and some higher plants) are mixotrophic, capable of both autotrophy (self-nourishment through the use inorganic carbon and minerals) and heterotrophy (consumption of other organisms) (Porter et al. 1985; Sanders and Porter 1988). As a result, herbivores may not be strictly primary consumers as the term suggests. Furthermore, omnivory (including intraguild

predation and detritivory), ontogenetic, and environmentally induced diet heterogeneity all obscures the trophic position of consumers (Polis and Strong 1996 and references therein).

As a consequence, determining how consumers derive their nutrition from various resources is a major challenge in ecosystem modelling (see review by Gentleman et al. 2003). Current models account for the diversity in food resources by explicitly describing feeding from multiple resources (e.g. Broekhuizen et al. 1995; Kühn and Radach 1997; Lee et al. 2002; Schrum et al. 2006). These models base consumers' preference for prey organisms (i.e. a prey's contribution to total food intake) on parameters that are either fixed or density-dependent (see for example Schrum et al. 2006). This "black box" approach to modelling trophic interactions predestines the domain of possible dynamical state and patterns in food web structure. This is because they *a priori* specify the functional form of species interactions (i.e. the way intake changes with resource density: Schmitz and Booth 1997), and as a consequence suppress two key aspects of (trophic) complexity, which are non-linearity (i.e. the interaction strength of species is not a simple linear function of species numbers) and adaptability/evolvability (i.e. consumers' ability to change their behaviour to cope with feeding condition) (Levins 1998, 2005; Brown et al. 2002; Proulx 2007).

Experiments have shown that consumers respond, behaviourally as well as physiologically, to the characteristics (including availability) of prey organisms (e.g. Houde and Roman 1987; Darchambeau et al. 2003; Darchambeau 2005; Jones and Flynn 2005). Chemical cues from both predators and preys for instance strongly modify trophic interactions (Turner et al. 2000; Pohnert 2004). Therefore in a multi-prey environment, grazing on any one resource may be affected by the presence of others. This could occur when for example, the processing time for a nutritionally "high quality" prey is retarded by the ingestion of "low quality" ones (Mittra and Flynn 2006, 2007) or when behavioral changes in consumers occur due to the relative availability of both prey types (Paffenhöfer and Van Sant 1985; Verity 1991; Kiørboe et al. 1996). Even in the most simplex ecosystems where there may be only a single prey species (e.g. laboratory studies), the functional feeding response is dependent on prey characteristics (e.g. Houde and Roman 1987). Furthermore, spatial and temporal heterogeneity in environmental conditions may impact consumers' preference for specific food items. This is because consumers "feed to their requirement" (Yearsly et al. 2001) and habitat conditions, such as temperature, strongly influence prey characteristics (e.g. biochemical composition: Kattner et al. 1983; Lynn et al. 2000; Teoh et al. 2004), as well as consumers' requirement (for energy and structural components: Biagini et al. 2000; Hassett and Crockett 2009) and metabolic capabilities (Dutta et al. 2006).

1.2 Food quality models

The disconnect between model formulations and trophic complexity in natural systems weakens the robustness as well as the predictive capabilities of current ecosystem models, and as a consequence exposes them to the perennial criticism that mathematical models have limited utility for ecosystem management (Peterson et al. 2003). Excitingly, the increase in our mechanistic understanding of trophic processes and the enormous increase in computational power over recent decades now allow trophic behavioural complexity to be modelled (e.g. Schmitz and Booth 1997; Mitra et al. 2003; Mitra and Flynn 2007, 2006; Flynn and Mitra 2009). These models explore trophic dynamics by using functions that are individual-based, often spatio-temporally explicit, and operate under settings in which organisms interact *under specific biological rules* of engagement. The engagement rules are usually built into food quality models (FQMs) that mimic consumers' "internal mechanism for anticipating" (*sensu* Holland, 1996, 1998) the fitness consequences for feeding on specific prey items. Generally therefore, FQMs determine the potential utility or nutritional value of prey items to consumers. This information is then employed in ecosystem as the basis for modelling species interactions, total food intake and how that intake is derived from various resources, etc. (see Mitra et al. 2003; Mitra 2006; Mitra and Flynn 2007).

Although the approach is heuristically important (see above references), a framework for food quality that incorporates relevant natural history details and is capable of providing a realistic, mechanistic understanding of prey nutritional value is lacking (St. John et al. 2010 and references therein). Currently in aquatic ecology, optimum foraging theory, OFT (Pyke et al. 1977) and ecological stoichiometry, ES (Sturner and Elser 2002) are the major frameworks for determining prey nutritional value.

OFT aims to explain and predict the pattern of food choice and foraging by animals (Pyke et al. 1977). Its premise is that animals feed to maximize their Darwinian fitness (Stephens and Krebs 1986), which is often defined based on measure(s) that promotes animals' survival in their habitats, such as growth rate, hatching success, developmental time, survival rate, or some other population quantity depending on the ecological scenario (Metz et al. 1992; Kessler and Lamper 2004). Typically, the nutritional currency in OFT is energy (Lehman 1976; Garcia et al. 2007; Visser et al. 2009; Pahlow and Prowe 2010), although protein has occasionally been used (e.g. Taghon 1982). In this respect, models based on OFT evaluate prey nutritional value by assuming *a priori* that a single food component is preeminent for the survival of consumers. While it might be true that an animal may be

limited by a single food component at any one time, the dynamics and time-scale of such a limitation remains an open question (Raubenheimer et al. 2009). At one extreme, single-nutrient limitation might be a perpetual feature of an animal's trophic behaviour. This is what many studies have for example suggested for nitrogen limitation in several species of marine zooplankton (e.g. White 1983; Houde and Roman 1987; Cruz-Rivera and Hay 2000). At the other extreme, limiting substance(s) for animals capable of prey selection might vary over time, depending on the composition of the exploited food resource.

Furthermore, compounds such as proteins, lipids and carbohydrates define the energy contents of organisms. As a consequence, models based on OFT may confound feeding aimed at maximizing the availability of one or more chemical substances, or optimizing their balance, with energy maximization (Raubenheimer et al. 2009). Consequently, OFT does not offer realistic explanation for many ecological phenomena, such as the observation that protein, which is energy-poor compared to other substances, limits trophic behaviour (e.g. Houde and Roman 1987; Cruz-Rivera and Hay 2000), and the fact that animals feed not only for energy, but also for many different chemical substances to satisfy their structural needs (Reiners 1986; Kuijper et al. 2004b).

Stoichiometry on the other hand, refers to the ratio of chemicals in reactions. In biology and ecology, it is, if only for simplicity, usually related to elements. Ecological stoichiometry (ES) has therefore been described as the 'biology of elements' (Sterner and Elser, 2002). ES explains and predicts ecological processes based on the balance of chemical elements within organisms and their environment (Elser 2006). Thus, with the exception of very few (e.g. Anderson and Pond 2000), elements are the preferred nutritional currencies in models for studying aquatic systems (e.g. Mitra et al. 2003; Mitra 2006; Mitra and Flynn 2007). This approach to modelling is heuristically important because it allows biological models to slip seamlessly into models of ocean circulation and biogeochemical cycling, thus enabling the role of biology in elemental (carbon) cycling to be investigated. The state of the art is well illustrated by the organic carbon pump model of Omta et al. (Omta et al. 2009). However, ES has significant biological shortfalls that limit the predictive capability of the models that employ it (see reviews by Tang and Dam 1999; Boersma and Elser 2006; Raubenheimer et al. 2009).

C and non-C elements (e.g. N, P) respectively represent energy and nutrients in ES (Reiners 1986). As a consequence, models based on ES typically determine prey nutritional value by comparing prey elemental composition (mostly nutrient:C ratios) with that of consumers (e.g. Mitra and Flynn 2006; Mitra 2006). A problem with this emphasis on

elements is that it glosses over the fact that most animals are heterotrophic and hence depend on complex organic molecules for nutrition. Thus, predictions based on ES may be reliable only when elemental composition approximates the bio-molecular composition of organisms. In some cases such correlations may exist. For example, nitrogen has been successfully used in many studies as a proxy for protein content of organisms (e.g. Kuijper et al. 2004a; chapters 2 and 3 of this thesis). However in other contexts, elements may not be appropriate proxies for bio-molecules, because functionally distinct molecular complexes can contain similar elements. P for instance occurs in phospholipids that have mainly structural roles in organisms, and in adenosine triphosphate (ATP) that serve as a carrier of energy and information. The form of C in protein and polysaccharide is another clear example. So, an obvious limitation of ES is that it does not properly account for the biochemical characteristics of elements (Tang and Dam 1999). As a consequence, food quality models based on ES do not capture consumers' response, to superfluous supply of nutrients (not carbon) (e.g. Anderson et al. 2005; Mitra 2006), contrary to experimental observations (Augustin and Boersma 2006; Boersma and Elser, 2006 and references therein).

Furthermore, food quality models based on ES usually do not consider consumers' behaviour/physiology when determining prey nutritional value (Mitra 2006, but see Anderson et al. 2005). However, experiments have shown that the usefulness or otherwise of prey organisms may be contingent on consumers' behaviour/physiology. Many animals for example show elasticity in their preference for respiratory substrates (e.g. Mayzaud 1976). This allows preferential catabolism of only food components that occur in excess of their structural requirements, and thus enabling animals to spare limiting substances for vital life processes such as growth and reproduction (cf. protein sparing in animals: McGoogan and Gatlin 1999; Arnould et al. 2001). Consequently, consumers can maximize the usefulness of prey organisms by catabolising only excess substances. However, the gain(s) consumers might derive from substances supplied in excess of consumers' structural requirements is(are) usually not considered in food quality models based on ES. The biological foundation of ES therefore needs to be improved.

In this regard, the dynamic energy budget (DEB) theory (Kooijman 2000) represents an important improvement over the stoichiometric approach to modelling trophic behaviour. Generally, DEB theory describes the rates at which organisms acquire and utilise energy for maintenance, growth and reproduction, while taken into account the chemical composition of organisms and their environments. A fundamental construct within DEB theory is the 'synthesizing unit' (SU), which is a generalization of the classical enzyme concept to complex

reactions involving multiple chemical substances (Kooijman 1998). SU is used in DEB models to predict rates of biochemical reactions, depending on the supply rates of substrates. When substrates have multiple metabolic functions, SU serve as the machinery for simultaneously dealing with different metabolic processes or pathways (see Kooijman 1998 for detailed description of SU). It has therefore been said that DEB theory provides a more specified characterization of organisms than does traditional ES based only on physicochemical principles, and as a consequence provide a powerful means for integrating organismic level processes into ecological ones (Raubenheimer et al. 2009).

DEB theory has experienced many successes (see review by Nisbert et al. 2000), but an improved understanding of food web structure (i.e. why animals select some and not other prey items for consumption) is not among them. I believe the reason for this is that DEB theory does not suggest how consumers evaluate their prey items prior to ingestion. As a consequence, models based on DEB usually employ constant rate for food uptake (e.g. Kuyper et al. 2004a) or assume, as originally suggested by DEB theory, that food uptake rate (i) follows a prey density-dependent functional response and (ii) scales with the size of the consumer (e.g. Pouvreau et al. 2006). As pointed out above, this approach to modelling predator-prey interactions is entirely at variance with the empirical understanding of species interactions in the natural world. Also limiting the utility of DEB theory is its reliance on parameters that are too abstract, and practically impossible to estimate or verify (van der Meer 2006).

As part of this thesis, a new framework that addresses the shortfalls in current approaches for determining prey nutritional value would be proposed, focusing on copepod consumers because of the critical role copepods play in marine ecosystems.

1.3 Why copepods matter

Zooplankton, particularly calanoid copepods, are the most abundant heterotrophic consumers in marine ecosystems (Wilson 1987). Globally, copepods consume approximately 23% of all autotrophic productions in the open ocean (Calbet 2001). Their grazing impact on phytoplankton may be even higher in specific habitats. For example copepods are capable of grazing up to 62% day⁻¹ of the standing crop of phytoplankton in the Gulf of Maine (Durbin et al. 1995) and up to 48% of the daily primary production in Skagerrak (Tiselius 1988). In additions, copepods feed on other animals, such as fish eggs (Yen 1987) and ciliates (Yen 1987; Calbet and Saiz 2005).

Furthermore, copepods serve as very important food source for fish and other aquatic animals (Fjøsne and Gjørseter 1996; Bämstedt 1998; Bämstedt and Karlson 1998;). Juveniles of some exploited fish species (e.g., whiting, *Merlangius merlangus*; bib, *Trisopterus luscious*) even feed sometimes exclusively on copepods (Hamerlynck and Hostens 1993).

Consequently, copepods have much potential to mediate effects that cascade up and down trophic chains. Hence, realistic models for marine ecosystems require appropriate parameterisation for the trophic behaviour of copepods. Achieving this would be critical for our ability to adequately represent zooplankton in biogeochemical models aimed at assessing the flux and sequestration of carbon in marine systems.

1.4 Developing an alternative food quality model

Key to understanding complex behaviour is the interrelationships between microscopic processes and the evolutionary forces that shape them (Holland 1998; Levins 2005). The theory of evolution (Darwin 1859) is based on the assumption that the ultimate goal of an organism is to maximize its inclusive fitness, and an important sub-goal must be the optimisation of the lifetime pattern of food intake, in order to meet the resource demands of survival, growth and reproduction. Animals have thus evolved behavioural and physiological effectors that enable them to link their foraging behaviour to its nutritional and functional outcome (Illius et al. 2002).

This is especially true for the feeding behaviour of copepods. Some can select what they ingest in order to satisfy their specific requirement for chemical substances (e.g. Landry 1981; Cowles et al. 1988; Schultz and Kiørboe 2009), and those incapable of prey selection are endowed with physiological capabilities for dealing with disadvantageous food components (see review by Anderson and Hessen 2008). Copepods can even respond differently towards the same prey species presented with different biochemical constituents (Plath and Boersma 2001; Jones and Flynn 2005). These results show that copepods adapt their short-term food intake to the nutritional value of food resources.

Therefore, in this thesis, an attempt has been made to develop a food quality model that incorporates the behavioural and physiological capabilities of copepods. Individual-based modelling approach has been used here to enable easy accounting for individuality as well as diversity in consumer behaviour, physiology and biochemical requirements. Also, informing the modelling approach is the fact that behaviour at the individual level strongly influences the dynamics of a system at higher organizational levels (populations and communities) (see

for example Hastings 1993; DeAngelis et al. 1993; Durrett and Levin 1994; Schmitz 2008), which I hope would permit integration of the proposed framework into ecosystem models.

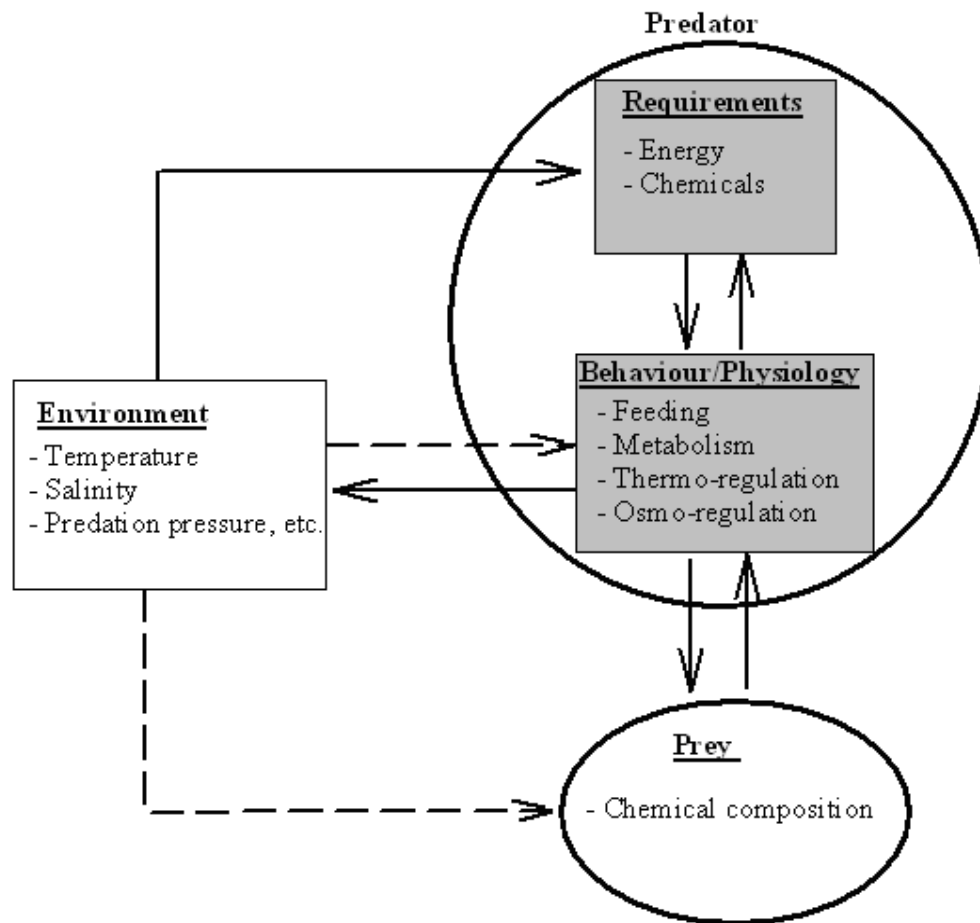


Figure 1. Conceptual scheme for how consumers evaluate the nutritional value of prey organisms. Consumers have habitat-specific requirement for energy and structural composition that is independent of their food environment. They are therefore endowed (by evolution) with behavioural and physiological machinery for satisfying these requirements. Consumers' behaviour/physiology is dictated by their requirements for metabolic substrates as influenced by habitat conditions. So specific habitat conditions confer consumers with specific behavioural/physiological capacity for determining the nutritional value of the prey items. In addition, consumers' capacity for prey capture and processing is a function of prey characteristics. As a result, an interaction between prey characteristics and consumers' behavioural/physiological capacity determines the nutritional value of prey items, with metabolic excesses being returned to the environment via egestion and excretion. Arrows with broken lines indicate known environmental effects (see for example Thompson et al. 1992; Dutta et al. 2006) that are not currently represented in this study (see text for explanation).

1.4.1 Food quality framework

Figure 1 shows the core components of the conceptual framework that underpins the food quality model presented in this thesis. Most generally, these are the consumer, the prey and the environment they share. The framework is inspired by previous attempts at conceptualizing feeding in animals (e.g. Kyriazakis et al. 1999; Raubenheimer et al. 2009).

The environment

This refers to all living and nonliving factors, which influence trophic behaviour of organisms. Abiotic factors are major determinants of prey biochemical characteristics, especially that of those capable of primary production (e.g. Kattner et al. 1983; Mayzaud et al. 1989). In addition, temperature and salinity are major determinants of consumers' behaviour, physiology, life history traits etc. (e.g. Angilletta et al. 2004; Guisande 2006). They also influence animals' demand for specific chemical substances, as well as the metabolic fate of ingested substrate (Anger 1998; Biagini et al. 2000; Hochachka and Somero 2002; Isla et al. 2008).

Furthermore, environmental pressures such as predation, parasitism and diseases play important role in determining food intake (Yearsly et al. 2001; Lafferty et al., 2006). For instance, many species of both marine and freshwater zooplankton avoid visual predators by feeding at night in surface waters and spending much of the day in deep waters, where food availability and nutritional composition are mostly poor (Lampert 1989). Such animals may thus feed not to only satisfy their metabolic requirements in the surface layer but also to obtain resources to power migration across gradients of habitat conditions and enhance survival in food-poor deep waters. As a result, the nutritional value of a prey in the surface layer must be driven by the energetic and physiological costs of migration as well as optimized to account for maintenance and reproductive constraints in the food-poor environment. The resultant selective feeding strategies may as a consequence influence the potential fate of prey items as well as the structure of planktonic communities.

Hence, habitat conditions critical for the survival and reproduction of consumers would be explicitly represented in the model. In general, this means that environmental factors would be represented in the model as variables, and not as constants. This would allow the framework to be employed in research aimed directly at addressing questions pertaining to specific habitat conditions (see chapter 4 for example).

Consumers

In this thesis, individual consumers are characterised by their requirement for energy and structural constituents, as well as by their behavioural/physiological machinery for meeting those requirements. These together define the nutritional state (i.e., all consumer characteristics that affects food consumption and metabolisms) of consumers at any instant.

To ensure reproduction and survival of consumers in their habitat, the nutritional state of animals would be made to reflect prevailing habitat conditions. This would be achieved via model parameters that reflect or are adaptive to habitat conditions (see, for example chapters 4), thus enabling variations in habitat conditions to be factored into the nutritional value of prey organisms.

Due to the strong interdependencies between animals' demand for chemical substances and their behaviour/physiology, a simplifying assumption, inspired by classical ethology (Tinbergen 1963), has been made that ambient conditions influence only consumers' demand for energy and chemical substances, and that changes in behaviour/physiology are just the means through which animals meet their specific requirements. Consequently, animals can change their nutritional state via their habitat-specific requirement for energy and chemical substances. This ultimately influences how consumers judge the usefulness or otherwise of potential prey organisms.

Predators normally draw their food from prey organisms that are available in their ecological range (Ehrlich and Raven 1964). Hence in this thesis, it is assumed that copepods possess "prior knowledge" (*sensu* Kyriazakis et al., 1999) of their food environment and that their feeding and metabolic capabilities have been naturally selected (by evolution) to maximize their fitness (Simpson and Raubenheimer 1995; Sutherland 2005). These capabilities may include specific feeding appendages, gut size, enzymes and/or metabolic physiology. So in this thesis, food quality is determined by accounting for feeding and metabolic capabilities of copepods.

Prey organisms

Prey characteristics are also an important determinant of feeding. While there are several of them (e.g. size, cell structure), biochemical composition may be ecologically the most important. It modifies species interactions (Turner et al. 2000; Pohnert 2004) and can be as important as food quantity for consumers' growth and survival (Müller-Navarra and Lampert 1996; Boersma et al. 2008). Prey biochemical composition can account solely for the efficiency with which consumers utilize food resources (Müller-Navarra et al. 2000), and thus

could significantly influence the mode as well as temporal dynamics of biogeochemical cycling of elements (see chapter 2 of this thesis). As a consequence, prey organisms would be defined only by their biochemical characteristics. Modelling prey biochemical composition is beyond the scope of this thesis. So, changes in prey biochemical composition would not be explicitly modeled here.

1.5 Aims

This thesis aims to:

- a. Develop a food quality model (FQM) that employs habitat-specific (energy and structural) requirements of copepods, as well as the behavioural and physiological capabilities of the same to determine: (i) the usefulness or otherwise of individual chemical constituents of prey organisms, and (ii) the nutritional value of a prey based on its entire biochemical composition (i.e. food quality). The role of FQMs in ecosystem models has been mentioned above (see section 1.1.2). In addition, FQMs could be relied upon to investigate the potential fate of the substances consumers may ingest (e.g. Anderson et al. 2005), as well as to produce forecasts and hindcasts of prey nutritional value if the characteristics of consumers, prey items and vital habitat conditions are known. Thus, FQMs could be integrated into models aimed at investigating the impact of for example global climate change on ecosystem processes.
- b. Investigate the combined effect of temperature and algal biochemical composition on the dynamics of copepod production by implementing FQM in an environment where both prey biochemical composition and temperature are variable. Temperature and prey biochemical composition are two of the most important environmental factors, as both are major determinants of animals' life history traits, habitat selection, etc. (Angilletta et al. 2004; Guisande 2006). Moreover, temperature influences, at least in part, both prey biochemical composition (e.g. Thompson et al. 1992) and the demand for biochemical substances by copepods (; Farkas 1979; Isla et al. 2008; Hassett and Crockett 2009). Therefore, we may better capture the dynamics of copepod production by integrating both temperature and prey biochemical composition into copepod production models. This has hitherto not been done.
- c. Re-examine the hypothesis that marine copepod production is limited by nitrogen (White 1983; Elser and Hassett 1994). Previous studies have examined and confirmed the hypothesis using the framework of ecological stoichiometry (e.g. Touratier et al. 1999; Kuijper et al. 2004a). However, experiments have shown that copepods

generally convert just about 40% of the nitrogen they ingest into eggs, even when they feed on nitrogen-limited diet (Checkley 1980; Kiørboe 1989). This low gross growth efficiency for N contrast strongly with the high efficiency one would normally expect, at least in theory, for a growth-limiting substrate. A re-examination of the N limitation hypothesis using a framework devoid of the shortfalls in ecological stoichiometry (see section 1.1.2) is therefore needed. Establishing which nutrient drives the dynamics of copepod production is an essential pre-requisite for modelling carbon cycling and for assessing how humans impact the dynamics of marine systems. This is because anthropogenic forces such as pollution and mariculture alter the biochemical composition of microalgae (see Savage et al. 2002; Gaudry et al. 2007; Guo et al. 2009), which are responsible for up to 50 % of the primary production in our biosphere (Field et al. 1998), and serve as major source of nutrition for copepods.

1.6 Outline

Chapter 2 presents a mechanistic FQM applicable to heterotrophic consumers in general and copepods in particular. It describes in detail how the behavioural/physiological capabilities of consumers can be utilize to determine the usefulness or otherwise of prey organisms and their chemical constituents. It significantly improves upon existing approach to evaluating prey organisms by for example distinguishing between structural biochemical requirements of different ontogenic stages of consumers, and not restricting respiration to any specific substrates. It captures the observed impact of dietary imbalances on copepod production, regardless of the food component responsible for the imbalance.

Chapter 3 describes how the newly developed FQM food quality model could be embedded into an egg production model for copepods whose capabilities for food intake and metabolism are bounded within a maximum threshold needed for growth and maintenance when food is “nutritionally good”, and a minimum threshold needed for only maintenance when food is “nutritionally poor”. It re-evaluates and challenges the hypothesis that nitrogen generally limits egg production by copepods in marine systems (e.g. White 1983; Elser and Hassett 1994; Touratier 1999; Kuyjper et al. 2004a;). The implications of the results for C-cycling in marine systems have been discussed.

Chapter 4 investigates how temperature and prey nutritional composition may interact to influence copepod production. It relies on a secondary production model within which food ingestion, assimilation and metabolism vary with consumers’ temperature specific demand for energy and structural constituents. The model realistically predicts egg production by calanoid

copepods within the limited range of “biologically relevant” temperatures of 0 to 35° C and algal C:N of 5 to 30. The results indicate that the egg production response of copepods to changes in ambient temperature is driven mainly by temperature-induced changes in animals’ demand for maintenance. The ecological implications of the results have been discussed.

Chapter 5 discusses the modelling approach in this thesis. It emphasizes the importance of correctly defining prey nutritional value and how the predictions of the models here contribute to our understanding of trophic processes. It ends by describing how the food quality model presented here could be implemented in a system involving a rich variety (i.e. higher than those considered here) of chemical substances, prey and predator organisms.

The nutritional quality of prey organisms cannot be assessed without proper parameters for the biochemical need of consumers as well as the biochemical composition of prey organisms. So parameters employed in this study were determined by reviewing published studies on biochemical compositions of zoo- and phytoplankton in order to establish. The results are presented within appendices 1 and 2 for zoo- and phytoplankton respectively. For zooplankton, the review covers only females and their eggs. For phytoplankton, the review covers microalgae cultured at diverse conditions of temperature, light, nutrient richness, etc. Protein, lipid, carbohydrate, amino acid and fatty acid composition of organisms were considered. Appendix 1 shows that the huge differences in biochemical compositions between zooplanktonic organisms could generally be explained by the differences in their dry weights. Appendix 2 shows that the macromolecular composition of algae can be explained by their total carbon contents and that only the essential fatty acid composition of algae differs significantly between algal taxonomic groups. The ecological implications of the results regarding aspects of plankton ecology have been discussed.

In addition to prey nutritional status, prey availability is also an important controlling factor in animal production. In aquatic systems, prey availability changes with ambient conditions. This has not been explicitly considered in the models presented in this thesis due to lack of adequate data on the biochemical response of copepods to prey availability. Therefore, an experiment was conducted to investigate the potential effect of prey availability on the biochemical composition of copepods. The investigated copepod species was *Acartia tonsa* because it (i) has wider global distribution, being common within mid- to low-latitude neretic waters of the world, (ii) is easily maintained in laboratory cultures (Støttrup 2000), and (iii) is dependent on external food supply for survival and successful reproduction because it does not considerably store reserve biomass (Lee et al. 2006). Carbohydrate, protein and fatty acid composition of the copepod (females and eggs) cultured at different food concentrations

were analyzed. Appendix 3 presents the results. They show that food availability is relevant only for the biochemical composition of *Acartia* eggs. The ecological implications of the results *vis-à-vis* aspects of *Acartia*'s reproductive strategy have been discussed.

Chapter 2

Food Quality Model for Heterotrophic Consumers

2.1 ABSTRACT

An ideal prey, with a biochemical composition that satisfies consumers' optimum requirement for survival and reproduction, evidently does not exist in nature. Consequently, animals should be able to anticipate the fitness impact of potential prey organisms in order to ensure ingestion of prey organisms that promote fitness against disadvantageous ones, and/or efficient uptake and utilization of fitness-promoting prey components. In aquatic ecology, food quality models (FQMs) based on ecological stoichiometry are commonly relied upon to evaluate potential prey organisms. Such models lack adaptability since they ignore consumers' behaviour and physiology by *a priori* identifying specific food components, usually N, P and essential compounds, as preeminent. Here, I present an alternative model for food quality (Q). It marries concepts from optimum foraging theory, ecological stoichiometric and geometric frameworks for analysing animal nutrition, and involves parameters that are adaptable to consumers' environment, and bases Q on consumers' capacity for food uptake and metabolic physiology. Uniquely, the model (i) has separate pathways for the utilization of carbon (C) associated with proteins, lipids and carbohydrates, (ii) considers stage-specific structural biochemical requirement of animals, and (iii) does not treat consumers' structural demand for carbon as a "unitary requirement" but discriminates among the required biochemical forms of carbon. The approach is applicable to all heterotrophs. In the example given here the model has been configured to represent the calanoid copepod *Acartia tonsa*. Consistent with experimental observation, but unlike previous models, my model predicts the relationship between Q and food C:N to be unimodal with a maximum Q only at the threshold C:N for biomass production. Results suggest that prey C:N ratios may be irrelevant for food quality due to macromolecular biochemical constraints on the utilisation of chemical elements. This result emphasizes the importance of biochemical substances in animal nutrition and production as well as the necessity of developing food quality models able to adapt to the biochemical needs of the consumer. The model also indicates that the ideal chemical requirement for egg production by *Acartia* may not directly compare with the composition of the same in adult stages of the animals. This underscores the importance of egg production requirements in zooplankton nutrition.

2.2 INTRODUCTION

Animals require a number of different nutrients simultaneously at varying optimal levels for their metabolic growth (Raubenheimer and Simpson 1997). However, the nutrient composition of the available food items can vary substantially between the different prey species and also within a single prey species (Kattner et al. 1983; Morris et al. 1983; Mayzaud et al. 1989). In order to compensate for the lack of sufficient nutrients in a specific prey type, animals demonstrate varying feeding regulation mechanisms in order to satisfy their demands for energy and chemical substances (Illius et al. 2002; Mitra and Flynn 2005). This involves behavioural and/or physiological adjustments to prey biochemical composition, and occurs before, during and/or after food ingestion by consumers. Even in simple systems where there may be single prey species the functional feeding response of the consumer has been shown to be variable, and dependent on the biochemical characteristics of prey items (e.g. Houde and Roman 1987). Thus, animals can and do ingest high quantities of nutritionally poor diets (compensatory feeding; Cruz-Rivera and Hay 2000), engage in omnivory (including intraguild predation and detritivory) (Polis and Strong 1996 and references therein), and/or differentially assimilate prey constituents (Logan et al. 2004) in order to obtain the required balance of nutrients for optimal growth. Furthermore, most animals can select what they ingest in order to satisfy their specific requirement for chemical substances (e.g. Landry 1981; Cowles et al. 1988; Schultz and Kiørboe 2009), and those incapable of prey selection are endowed with physiological capabilities for dealing with disadvantageous food components (see review by Anderson and Hessen 2008). Such an acclimation via modification of feeding and incorporation by consumers would thereby influence food web structures (Paine 1980; Levin 1998) and contribute to the complexity of ecological communities (Levins 2005). Furthermore, adaptive changes in behaviour would determine the fate as well as the ecological transfer efficiency of biomass (carbon) within foodwebs.

Acclimative behaviour is typically ignored or poorly represented in aquatic ecosystem models in part because a realistic framework, for addressing feeding behaviour, is lacking (Raubenheimer et al. 2006). Models for investigating aquatic ecosystems incorporate feeding acclimatization by employing parameters that optimize trade-off between feeding behaviour and the fitness (e.g. growth, reproductive success) of individual organisms (e.g., Mitra et al. 2003, 2007; Mitra 2006; Flynn and Mitra 2009). Such parameters are usually built into functions which could collectively be referred to as food quality modules (FQMs) that mimic consumers' "internal mechanism for anticipating" (*sensu* Holland 1998) the fitness consequences for feeding on specific prey items. This information is then employed as the

basis for modelling species interactions, total food intake, how intake is derived from various resources, etc. Although the approach is heuristically important (see the above reference), a framework for food quality that is capable of providing a general, mechanistic understanding of prey nutritional value is lacking (St. John et al. 2010).

Currently in aquatic ecology, food quality models (FQMs) based on ecological stoichiometry (Sterner and Elser 2002) represent the most heuristic approach for determining prey nutritional value (e.g. Anderson and Pond 2000; Mitra et al. 2003; Anderson et al. 2004, 2005; Mitra 2006; Mitra et al. 2007; Flynn and Mitra 2009). Such models typically divide the chemical constituents of both prey and predators into two major categories: C (for both structure and energy) and a nutrient 'X' (where X is mostly nitrogen, phosphorus and/or essential compounds). They then employ a variant of equation 1 as the base parameter for food quality.

$$Q = \min(\beta_{CX} / \alpha_{CX}, 1) \quad (1)$$

Where β_{CX} and α_{CX} are predator and prey C:X ratios respectively. Other food component, such as toxins and structure are typically not represented within food quality modules. Rather, they are coded as separate constraints within which a consumer has to work in order to achieve its required nutrition. While the merits for emphasizing elements are widely recognized, the conceptual assumptions for this approach to determining prey nutritional value are subject to vigorous debate. Some of the shortfalls associated with approach have been discussed under chapters 1 of this thesis.

In addition, Boersma and Elser (2006) argue that studies that determine prey nutritional value based on equation 1 implicitly assume that a prey containing more X is better (or at least never worse) for the fitness of consumers. In other words, stoichiometric based FQMs implicitly assume that predators incur fitness loss only when their diet contains excess C, but not that of X, and that the consumption of a prey with surplus nutrient X (i.e. food with C:X below that of the consumer; Urabe and Watanabe 1992) does not impair performance. This is well illustrated by the food utilisation model for homeostatic consumers model by Hessen et al. (i.e., figure 1 of Hessen et al. 2004). This assumption is not supported by experimental observation. There are for instance several observations of growth reduction in animals such as *Acartia tonsa* (Augustin and Boersma 2006), *Daphnia magna* (DeMott et al. 1998) and *Penaues monodon* (Plath and Boersma 2001) that feed on relatively X-rich diets. Similarly, animals grow poorly when they feed on diet containing excess C (Checkley 1980; Kiørboe 1989; DeMott et al. 1998). These results suggest that any excess food components can impede consumers' performance, even when that excess component is an essential

compound (see review by Ikawa 2004). A more holistic food quality model, capable of capturing the cost for the consumption of excesses nutrients, is therefore a needed.

Furthermore, most stoichiometric studies ignore the different biochemical characteristics C can exhibit. Non-C elements occur mostly in specific substrates. For example, N occurs in proteins, nucleic acids, and chlorophylls (only in plants). P occurs in nucleic acids (RNA, DNA), phospholipids, and in carriers of energy and information (e.g. Coenzymes, ATP). Consumers' requirement for, as well as the fate of these elements when ingested, can therefore be dictated by the chemical characteristics of the compounds in which they occur (Tang and Dam, 1999). C on the other hand, occurs in every organic compound. Therefore the distribution of C between the demand (by consumers) and supply (by prey) of different compounds may dictate the fate of total ingested C. This is however not considered in most stoichiometric analyses of food quality, even in studies that have considered the biochemical characteristics of specific food components (e.g. Touratier et al. 1999; Anderson and Hessen 2005). As a consequence, existing FQMs do not capture consumers' response, in terms of fitness decline, to superfluous supply of non-C nutrients (e.g. Anderson et al. 2005; Mitra 2006), contrary to experimental observations (Augustin and Boersma 2006; Boersma and Elser 2006 and references therein). This, I believe, represents a drawback on our ability to realistically describe/predict the dynamics of trophic processes. An alternative food quality model, incorporating the biochemical characteristics of carbon, is therefore needed.

To help address these problems, I propose a new model that extends the concept of food quality a step further, to include consumers' maximum capacity for food uptake as well as plastic preference for respiratory substrates. The basis for the model is the food quality model of Anderson et al. (2005). However, I distinguish between stage-specific structural biochemical requirements of consumers. This is in keeping with experimental data demonstrating that structural biochemical requirement of zooplankton varies with their stage of development (Bruce et al. 2005). I also do not treat consumers' structural demand for carbon as a "unitary requirement" but discriminate among the needed biochemical forms of carbon. As a result, the respiratory physiology of my model consumer is not dependent on fixed substrates. Rather, substances are respiration based on the balance between their availability and the requirements (both energy and structural) of consumers. This is in keeping with findings demonstrating that zooplankton exhibit plastic preference for respiratory substrate (Mayzaud 1977; Roman 1983; Anderson 1992). In addition, the utilization of chemical elements in my model follows three different macromolecular pathways, i.e., protein, lipid and carbohydrate utilization pathways. Consequently, chemical elements impact

the fitness of the model consumer via the different compound utilisation pathways, and not on the basis of elemental ratios as in simple stoichiometric models. Using net efficiency at which a prey biomass is converted into that of a consumer as the yardstick for food quality, I have currently configured the model to represent the calanoid copepod *Acartia tonsa*, and discussed the results in context of biochemical constraints on food quality.

2.3 MODEL DESCRIPTION

Overview

Animals require diverse chemical substances (e.g. cholesterol, polyunsaturated fatty acids, amino acids etc.) for survival and successful reproduction. However here, all substances are assumed to belong to one of three major macromolecules: proteins, lipids and carbohydrates. This ensures a simple model structure by decreasing the number of molecules that would otherwise be needed, and yet allows me to realistically account for biochemical constraints in my model. This simplification also agrees with previous observations that the availability of macromolecules determines grazing behaviour (Houde and Roman 1987; Cruz-Rivera and Hay 2000) as well as the fate of food ingested by mesozooplankton (Kuijper et al. 2004a). My goal here is to establish a mathematical framework that could be used to determine the nutritional value of prey organisms prior to ingestion. Hence, my model treats molecular complexes as essential compounds by assuming that they are derived only from dietary sources and can neither be synthesized nor converted into others by the consumers (Anderson and Pond 2000).

As argued earlier in chapter 1, animals' feeding behaviour must reflect both the demand (in terms of consumer's energy expenditure and structure composition) and provision (in terms of food) of their habitat. A realistic food quality model must therefore account for the environmental conditions under which feeding is done. Another goal of this chapter is to establish the mathematical framework for food quality and introduce the parameters it employs. Hence, food uptake capabilities, energy requirement, and the structural requirement of consumers were fixed, but parameterised to reflect habitat conditions of the consumer. Parameters and variables for the model are listed in Tables 1 and 2 respectively.

On the individual level, food quality has been defined as the degree to which the quantity and nutritional composition of a prey meets consumers' nutritional needs (Müller-Navarra 2008). Thus, for an animal whose nutrition depends on for example substances *Y* and *Z*, the ideal supply ratio for *Y* and *Z*, has been given as (Anderson et al. 2005):

$$\text{Ideal prey } Y : Z = \text{Consumer } Y : Z * \frac{\text{maximum } Y \text{ utilisation efficiency}}{\text{maximum } Z \text{ utilisation efficiency}} \quad (2)$$

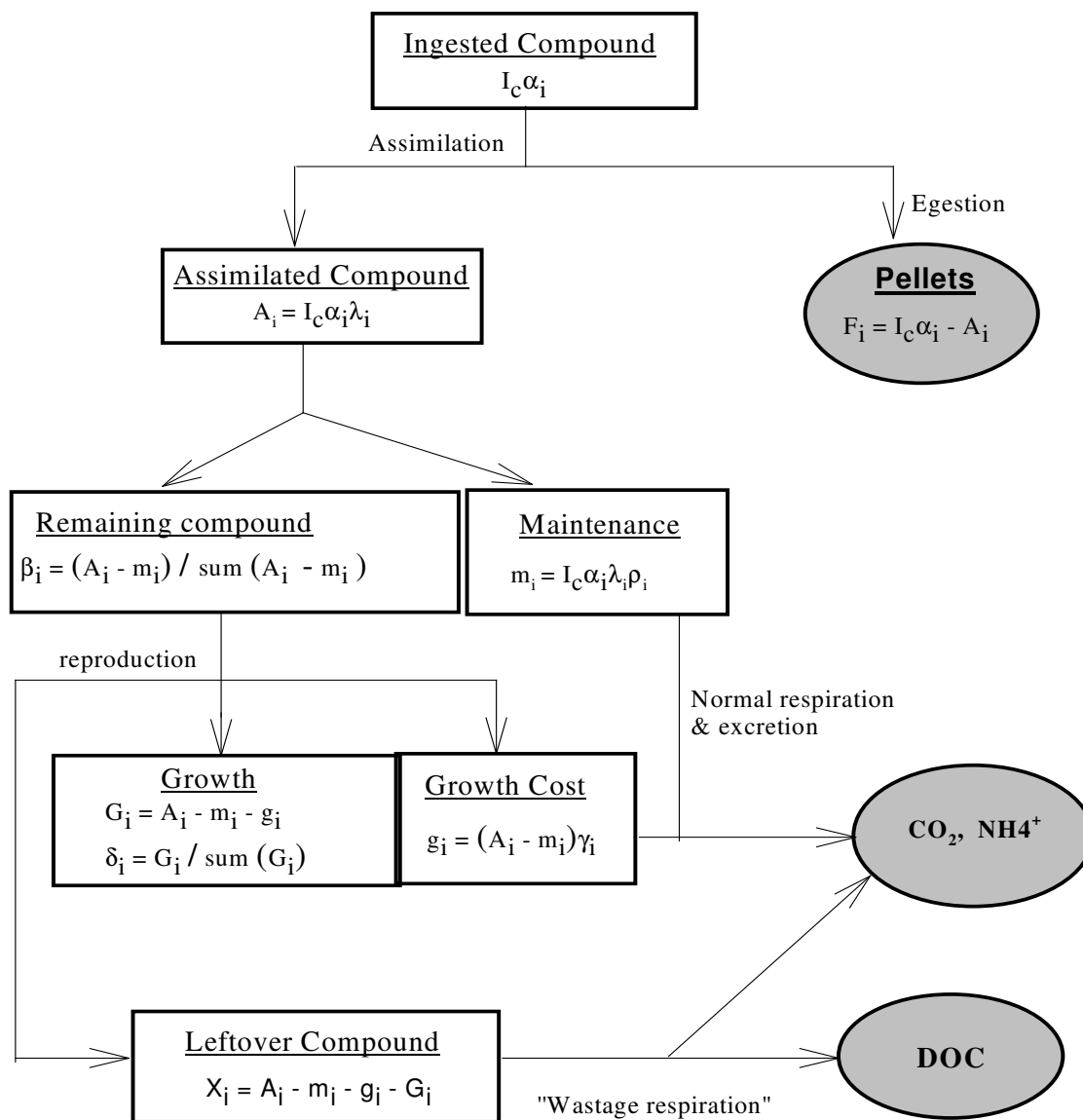


Figure 1. Conceptual scheme for the fate of compounds ingested by consumers. Subscript i represents different chemicals constituting the food. Shaded spheres indicate part of ingested chemicals that may be released to the environment. DOC here means dissolved organic compounds. See Tables 1 and 2, as well as text for symbol definitions. Figure modified from Anderson et al. (2005).

The term “maximum utilisation” in the above formulation signal a dominant paradigm in trophic ecology, which is that animals’ requirement for biochemical substances as well as their physiology for processing the materials they acquire has been naturally selected (by evolution) to maximize their fitness (Stephens and Krebs 1986; Simpson and Raubenheimer 1995; Sterner and Elser 2002). Hence, I have treated food quality as a quantity that is constrained by an interaction between prey biochemical characteristics and consumers’ maximum capacity for food uptake and processing.

Figure 1 shows the model flow diagram. In the model, consumers ingest and assimilate food at maximum rate, while unassimilated substrates are egested as faecal pellets. Assimilated chemicals are first used to meet consumers’ maintenance needs, after which those remaining are used for growth. Only chemicals assimilated in excess of consumer’s structural requirements are respired for energy. Substances remaining after growth are released to the environment in order to satisfy the consumer’s chemical composition requirements. Arguments for the model structure are provided in the following sections.

2.3.1 Uptake of biochemical substances

Where nutrients are limiting to growth, animals can alter the rate of food ingestion (Cruz-Rivera and Hay 2000), and/or assimilation (Logan et al. 2004 and references therein) in order to ensure efficient uptake and retention of needed nutrients. Thus, animals can modulate the cost/benefit (in terms of fitness) for accessing different food items via feeding regulation. Therefore, numerical analysis of food quality requires disabling the animals’ capacity for feeding regulation (see Anderson and Pond 2000; Anderson and Hessen 2005). Otherwise, food quality might represent a point of “best compromise” (*sensu* Simpson and Raubenheimer 1995) between prey biochemical composition and the requirement of consumer, rather than representing the potential maximum effect of food composition on consumers.

Therefore in this model, food quality is determined by using fixed food ingestion rate and assimilation efficiency that reflect consumer’s maximum capacity for food uptake. C is thus ingested at a constant maximum rate, I_c , ($\text{gC} \cdot \text{gC}^{-1} \cdot \text{d}^{-1}$). This C is divided into chemical-specific fractions (α_i , with $\sum_{i=1}^n \alpha_i = 1$) that are subjected to fixed maximum assimilation efficiencies λ_i . The quantity of C assimilated due to each substance (A_i), as well as to all components of the prey (A_c) was thus calculated as follows:

$$A_i = I_c \alpha_i \lambda_i \quad (3)$$

$$A_c = I_c \sum_{i=1}^n \alpha_i \lambda_i \quad (4)$$

where i to n represent the different chemical substances constituting the food in the gut.

Unassimilated fraction of each substance was egested as faecal pellets,

$$F_i = I_c \alpha_i (1 - \lambda_i) \quad (5)$$

Table 1. Model Parameters. See section 2.4 for details on how parameter values were calculated.

Para	Description	Unit	Value	Source
I_c	Maximum C ingestion rate	$\text{gC} \cdot \text{gC}^{-1} \cdot \text{d}^{-1}$	6.88	Besiktepe and Dam, 2002
λ_p	Maximum assimilation efficiency for protein	dl ^a	0.80	Conover, 1966
λ_L	Maximum assimilation efficiency for lipid	dl	0.80	Conover, 1966
λ_H	Maximum assimilation efficiency for carbohydrate	dl	0.80	Conover, 1966
E_p	Energy content of protein	$\text{J} \cdot \text{gC}^{-1}$	32312.64	Estimated
E_L	Energy content of lipid	$\text{J} \cdot \text{gC}^{-1}$	51439.43	Estimated
E_H	Energy content of carbohydrate	$\text{J} \cdot \text{gC}^{-1}$	42922.90	Estimated
β_p	Structural requirement for proteins by female copepods	$\text{gC} \cdot \text{gC}^{-1}$	0.7269	Estimated
β_L	Structural requirement for lipids by female copepods	$\text{gC} \cdot \text{gC}^{-1}$	0.2363	Estimated
β_H	Structural requirement for carbohydrates by female copepods	$\text{gC} \cdot \text{gC}^{-1}$	0.0367	Estimated
δ_p	Structural requirement for proteins by eggs	$\text{gC} \cdot \text{gC}^{-1}$	0.5478	Estimated
δ_L	Structural requirement for lipids by eggs	$\text{gC} \cdot \text{gC}^{-1}$	0.4404	Estimated
δ_H	Structural requirement for carbohydrates eggs	$\text{gC} \cdot \text{gC}^{-1}$	0.0117	Estimated
b	Energy demand for maintenance	$\text{J} \cdot \text{gC}^{-1} \cdot \text{d}^{-1}$	2755.10	Estimated
d	Energy demand for a unit egg production	$\text{J} \cdot \text{gC}^{-1}$	11228.77	Estimated

a: dl = dimensionless

Table 2. Model variables

Variable	Description	Unit
α_i	Molecule-specific fraction of total prey carbon	gC.gC^{-1}
ρ_i	Assimilate fraction catabolised for energy to power maintenance	dl ^a
γ_i	Assimilate fraction catabolised for energy to power growth	dl
x_i	Fraction of assimilate in excess of consumer's maintenance and growth requirements	dl
F_c (or F_i)	Total carbon (or compound) released as faecal pellet	$\text{gC.gC}^{-1}.\text{d}^{-1}$
M_c (or M_i)	Total carbon (or compound) catabolised for maintenance	$\text{gC.gC}^{-1}.\text{d}^{-1}$
g_c (or g_i)	Total carbon (or compound) catabolised for energy to power growth	$\text{gC.gC}^{-1}.\text{d}^{-1}$
X_c (or X_i)	Total carbon (or compound) in excess of consumer's maintenance and growth requirements	$\text{gC.gC}^{-1}.\text{d}^{-1}$
K_c (or K_i)	Carbon (or compound) specific gross growth efficiency	dl
U_i	Compound-specific net utilisation efficiency	dl
Q	Food quality	dl

a: dl = dimensionless

Summing the solutions to equation 5 for different compounds gives the total amount of carbon egested (F_c) by the copepod as

$$F_c = I_c \sum_{i=1}^n \alpha_i (1 - \lambda_i) \quad (6)$$

Quantitative investigation of the exchange of biochemical substances between individual organisms and their environment involves, implicitly or explicitly, the derivation of biochemical budgets. At their simplest, biochemical budgets comprise an equation that relates intake to the post-ingestive fate of the ingesta, with the latter often partitioned into several compartments (e.g. Simpson and Raubenheimer, 1995). That is, intake of a substance is driven by the sum of the different requirements for it. i.e.

$$A_i = r_1 + r_2 + r_3 + \dots + r_N \quad (7)$$

Where r_1 to r_N represents the various uses for which a substance is required. Here two main uses for biochemical substances by consumers are considered following Anderson et al. (2005), these being maintenance and growth.

2.3.2 Maintenance

I follow the so-called dynamic energy budget (DEB) approach (Kooijman 1998) and assume that the mass of a consumer consists of “reserve biomass” from which resources are extracted for metabolism, and a “structural biomass” that is permanent and has an associated maintenance cost, which is met using the content of the reserve biomass. Hence in my model, substrates assimilated by consumers are directed into a reserve biomass before being utilised for the needs of the consumer. For a consumer assimilating i to n different chemical substances, the carbon content of this reserve biomass is divided into molecule-specific fractions, β_i (gC.gC^{-1}) such that:

$$\sum_{i=1}^n \beta_i = 1 \quad (8)$$

It is practically difficult, if not impossible, to distinguish the biochemical composition of reserves from that of structural biomass of mesozooplankton (Nisbet et al. 2000). In addition, the composition of animals’ structural biomass sometimes assumes the role of reserve biomass by serving as the source of metabolic substrates. For example, copepods reproducing during inadequate food supply (Alonzo et al. 2000) and starvation (Ederington et al. 1995) rely upon their own structural biomass as the source of the proteins and phospholipids incorporated into eggs, as these compounds are not stored in large amount by copepods (Sargent and Falk-Petersen 1988; Alonzo et al. 2000). Hence in this model, I do not distinguish between the biochemical composition of reserve and structural biomass. Rather, a balance between the biochemical composition of consumers’ reserve and structural biomass is assumed. Within the model, this is achieved by applying the phenomenon of substrate sparing (McGoogan and Gatlin 1999; Arnould et al. 2001) for any compound supplied below consumers’ structural requirement. Thus, consumers were allowed to show plasticity in their preference for metabolic substrates based on the balance between substrate supply and the composition of their structural biomass.

For determining resource utilisation for maintenance, I employ an intermediate complexity approach to model parameterisation (e.g. Anderson et al. 2005; Hannah et al., 2010) by separating maintenance into two components, these being the energy (b ; $\text{J.gC}^{-1}.\text{d}^{-1}$) and structural requirements (β_i ; gC.gC^{-1}) of consumers. Here, b covers all the energy costs

associated with maintenance. This may include thermoregulation (Bennett and Ruben 1979), ion transport (Milligan and McBride, 1985), protein/biomass turnover (Mente et al. 2002) etc. β_i represents carbon-specific fraction of different chemicals in consumers' biomass and is employed to ensure that the need by animals for specific substances (such as lipids for cold temperature adaptation: Nanton and Castell 1999; Farkas 1979) is not compromised during catabolism of assimilates for maintenance energy.

For a consumer ingesting a prey particle containing a number of different biochemical substances, the required intake to meet only b can be calculated as

$$I_c = \frac{b}{\sum_{i=1}^n \alpha_i \lambda_i E_i} \quad (9)$$

where E_i represents chemical-specific energy content in J (g C)^{-1} . At this ingestion rate, there would be no extra substrate for investment into growth. As a result, an ingestion rate that is high enough to meet both maintenance and growth needs of the consumer is assumed here. Following this assumption, I could describe maintenance metabolism of consumers by using the following equations:

$$b = I_c \sum_{i=1}^n [\alpha_i \lambda_i \rho_i E_i] \quad (10)$$

$$\beta_i = \frac{I_c \alpha_i \lambda_i (1 - \rho_i)}{I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i)} = \frac{\alpha_i \lambda_i (1 - \rho_i)}{\sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i)} \quad (11)$$

where ρ_i represents the fraction of each assimilate that could be respired for energy without adversely affecting the biochemical composition of the consumers. This is in keeping with the stoichiometric axiom that animal's use of nutrients is tightly coupled with the nutrient ratios of its biomass (Sterner and Elser 2002; Grover 2003). So in the model, the consumer is made to respire chemicals assimilated in excess of requirements (i.e. with $\rho_i > 0$), while retaining limiting ones (with $\rho_i = 0$). As a result, maintenance budget for each chemical and total food intake is determined using equations 12 and 13 respectively.

$$m_i = I_c \alpha_i \lambda_i \rho_i \quad (12)$$

$$m_c = I_c \sum_{i=1}^n \alpha_i \lambda_i \rho_i \quad (13)$$

where m_i and m_c respectively represents that rate at which a chemical and total carbon is catabolised for maintenance.

2.3.3 Growth

After maintenance, remaining substrates (i.e. $I_c \alpha_i \lambda_i (1 - \rho_i)$ for each assimilate) are used for growth. Here too, an intermediate complexity approach has been taken by separating consumer's requirement for growth into energy (d) and structural components (δ_i). d represents the energy costs for producing a new zooplankton biomass. Therefore, total energy requirement for growth at any temperature could be described using the following equation:

$$E_g = Gd \quad (14)$$

Where, E_g is the rate of energy consumption ($\text{J.gC}^{-1}.\text{d}^{-1}$) for total growth (G ; $\text{gC.gC}^{-1}.\text{d}^{-1}$).

Conversely, δ_i defines the fraction of the new biomass that must be derived from individual

compounds (hence, $\sum_{i=1}^n \delta_i = 1$). It is employed to ensure that specific structural biochemical

requirement for new biomass formation is met during growth. The rate with which a biochemical substance is converted into new biomass could therefore be described using equation 15, where G is the growth rate.

$$G_i = G\delta_i \quad (15)$$

Depending on the balance of the biochemical substances remaining after maintenance, growth could be limited by any substances, which could lead to excesses in others. To ensure growth maximization, only part (γ_i , dimensionless) of substrates that are in excess of the specific structural needs of the animals are catabolised for energy to power growth. So for limiting substances, $\gamma_i = 0$, while $1 < \gamma_i > 0$ for non-limiting substances. For each chemical substance however, the actual magnitude of γ_i depends on the extent to which zooplankton can convert substrates remaining after maintenance into growth while satisfying both energy (d) and structural requirements (δ_i) for new biomass production. Growth ceases when any of these conditions is not met. When this is reached, the value of γ_i could be used to calculate the quantity of individual substances and total C that could be respectively respired for growth:

$$g_i = I_c \alpha_i \lambda_i (1 - \rho_i) \gamma_i \quad (16)$$

$$g_c = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) \gamma_i \quad (17)$$

Where, g_i and g_c indicate the rates ($\text{gC.gC}^{-1}.\text{d}^{-1}$) at which each chemical and total carbon are respectively catabolised to power growth.

Given x_i as the proportion of individual substances that would remain when growth ceases due to imbalance(s) in the diet of the zooplankton, the quantity of individual substances and total C that may neither be used for maintenance nor growth could be calculated respectively as

$$X_i = I_c \alpha_i \lambda_i (1 - \rho_i) x_i \quad (18)$$

$$X_c = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) x_i \quad (19)$$

where X_i and X_c represent that rate at which a chemical and total carbon may be in excess of animals' requirement for both maintenance and growth. Within the zooplankton community, the fate of these nutritional excesses varies widely. They can be respired through increased bodily activity or elevated metabolism (Plath and Boersma 2001; Fu and Xie 2004; Jeyasingh 2007). They can also be stored where an animal has a capacity for doing so, as shown for lipid storage in some copepods (Sargent and Falk-Petersen 1988). Also, based on observations involving terrestrial invertebrates (Zanotto et al. 1997; Trier and Mattson 2003), it has been suggested that aquatic invertebrates may rid themselves of excess components of their food via respiration decoupled from biochemical/mechanical work, the so-called "wastage respiration" (Anderson and Hessen 2008). Here, the aim is to establish a food quality model that could be used to evaluate prey items before being consumed. Accordingly, I assume that food that is in surplus to that required for both maintenance and growth has no nutritional value but is voided via "wastage respiration". As a result, dividing equations 18 and 19 respectively by $I_c \alpha_i$ and I_c gives the fraction of each ingested chemical and total C that are voided to satisfy the structural biochemical requirement of animals.

The difference between the amount of food assimilated by an animal (equation 3) and the sum of the amount respired for energy to power maintenance (equation 12), and growth (equation 16) as well as those voided to satisfy the animals' biochemical composition requirement (equation 18) must equal the quantity of each chemical that is converted into new biomass as given by equation 15. This, when simplified becomes

$$G \delta_i = I_c \alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i - x_i) \quad (20)$$

Summing the solutions to equation 20 for different chemicals gives the total rate for new biomass production as

$$G = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i - x_i) \quad (21)$$

Dividing equation 21 with I_c gives the fraction of total ingestion that is converted into growth (i.e., gross growth efficiency, K_c). After growth, the ratio between equation 28 and 29 must equal the composition of individual substances required for the new biomass as

$$\delta_i = \frac{I_c \alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i - x_i)}{I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i - x_i)} = \frac{\alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i - x_i)}{\sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i - x_i)} \quad (22)$$

with the total energy requirement for biomass production (equation 14) being

$$E_g = Gd = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) \gamma_i E_i \quad (23)$$

Hence, the process of growth within the model is described using equations 21, 22 and 23.

2.3.4 Food Quality

The model uses equations 21, 22 and 23 as conditions to guide how individual substances are either respired for energy to power growth or wasted in order to met consumers' chemical composition requirements for egg production. When these conditions are met together with the requirements for maintenance (i.e. equations 10 and 11), equation 21 can be rewritten as equation 24 to express intake of individual substances in terms of their potential utility to the consumer (cf. equation 3 of Simpson and Raubenheimer 1993).

$$I_c \alpha_i \lambda_i = \frac{G \delta_i}{(1 - \rho_i) (1 - \gamma_i)} \quad (24)$$

x_i has been intentionally excluded from equation 24 because zero utility has been assumed for excess food components. It then follows that the composition of each substance in the food ($I_c \alpha_i$) could be expressed in terms its metabolic availability (via assimilation efficiency, λ_i) and how it is used for maintenance (via ρ_i) and biomass production (via $G \delta_i$ and γ_i) using equation 25.

$$I_c \alpha_i = \frac{G \delta_i}{\lambda_i (1 - \rho_i) (1 - \gamma_i)} \quad (25)$$

As a consequence, the net efficiency (U_i) at which an ingested substance can potentially be converted into new biomass by animals could be determined as

$$U_i = \frac{G \delta_i}{I_c \alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i)} \quad (26)$$

This is akin to the maximum utilization efficiency of Anderson et al. (2005) and accounts for consumers' capability for dealing with excess food components as argued above in the introduction.

Following these assumptions, the net utilisation efficiency for substances supplied in excess of the consumer's composition requirement will be lower than those supplied in lower amounts, and that theoretically for individual substrates, the value of U_i lies between 0 and 1. U_i equals 1 means a chemical occurs below consumer's structural composition requirement and hence may not be respired but retained for biomass production. On the other hand, $0 < U_i < 1$ means a chemical occurs in excess of consumer's requirement and so a portion of it may not be used by the consumer. Hence, the potential (L_i) for a chemical to limit the growth of a consumer could be calculated as the difference between its current U_i value and the theoretical maximum (cf. equation 3 of Anderson and Pond, 2000) using equation 27.

$$L_i = \min(U_i, 1) \quad (27)$$

In a multi-chemical system, individual substances may have different limiting potentials. While some will be in excess (i.e. non-limiting with $L_i < 1$), others will be limiting (with $L_i = 1$), but their occurrence would not be mutually exclusive. The question is which of them could be responsible for the quality of the prey?

The answer to the above question depends on how food quality is measured. If growth rate is the yardstick, then substrate(s) supplied below the requirement of the consumer will control food quality (i.e. limiting substance(s), see Anderson and Pond 2000). This is because their limitation means a reduction in the number of new biomass that could otherwise be produced. However, a reduction in growth rate does not necessarily equal a reduction in growth efficiency. Limiting substrates would usually have higher specific-potential growth efficiency (i.e. U_i values of, or closed to 1), and so would have relatively less adverse effect on the efficiency with which a prey could be used for growth. In contrast, the impact of non-limiting or excess composition of the prey on consumer's growth efficiency could be profound. It should be noted that these substances needed not be only useful chemical substances whose composition in the prey exceeds consumers' requirement, but could also represent toxic substance that could not be disposed via standard respiration or indigestible components of the diet (e.g. cellulose and mucus). These can cause prey rejection, decrease ingestion via physical obstruction (e.g. by mucus), or decrease enzyme efficiency for the utilization of limiting compounds (Malej and Harris 1993; Van Donk et al. 1997). In addition, excess substances could serve as substrates for synthesizing limiting ones where the capacity for adequately doing so exists, thus determining the extent to which the rate-reducing impact

of limiting compounds can be decreased. The net result of all these could be a strong dependence of consumers' growth efficiency on the excess composition of their food.

Prey species vary in size and with it their absolute composition of chemical substances (see appendix 2), which means the quantity of substrates they can provide individual⁻¹ for new biomass production by consumers could differ between prey species. This weakens growth rate as an appropriate yardstick for food quality. This is because the quantity of biomass that could be produced (individual prey item)⁻¹ by a consumer with disabled machinery for feeding regulation, would not be comparable. However, the potential efficiency with which prey biomass can be converted into that of the consumer would depend on whether the composition of the prey allows the biomass production machinery of its consumer to function maximally. This borders not only on the amount of substrates (individual prey item)⁻¹ but on whether substrates occur in the proportions ideally required by consumers. Where this does not occur, production efficiency, which drives carbon transfer between trophic levels could be reduced, and with it the efficiency of an entire system.

Therefore, using the net efficiency at which a prey biomass is converted into that of a consumer as the yardstick, food quality (Q) has been defined here as

$$Q = \min(L_i, L_j, \dots, L_n) \quad (27)$$

This way, I implicitly ensure that substrates actually used for production contribute positively towards the quality of a prey, while unwanted or an excess of useful chemicals decreases food quality. The consequence will be high quality values for prey with low composition of excess substances and vice versa.

Similar to other models (e.g., Kuijper et al. 2004a), I do not fix the maximum efficiency at which a chemical assimilate is used for growth. Rather, net utilisation efficiency for a prey is driven by the need to satisfy the structural and energy requirements of the consumers. Hence, when a biochemical substance is limiting, its respiration for maintenance and growth as well as its wastage (i.e. ρ_i , γ_i and x_i) would be zero in accordance with the model assumptions and thus results in relatively higher specific net utilisation efficiency for limiting than non-limiting substances. Therefore, if $0 < Q < 1$, which should occur when prey nutritional imbalance necessitates substrate wasting (i.e. $x_i > 0$), the chemical substance with the highest net utilisation efficiency was considered as the source of the limiting carbon, L_c (Anderson and Pond, 2000).

$$L_c = \max(U_i, U_j, \dots, U_n) \quad (28)$$

Conversely, when $Q = 1$, the prey could be considered to be an ideal one, because its nutritional status may be just enough to meet the requirements of the consumer. In other words, the consumer could achieve the theoretically possible maximum net use efficiency (of 100%) for individual biochemical substances because wasting of compounds to overcome nutritional imbalance in the prey would not occur. When $Q = 0$, prey could be considered to have no nutritional value for growth.

2.4 PARAMETER DETERMINATION AND MODEL IMPLEMENTATION

Parameter determination

Experiments investigating the effect of food composition on zooplankton production typically measures only egg production rate (e.g. Checkley 1980; Kiørboe 1989; Augustin and Boersma 2006). Thus for my results to be comparable, I assume that egg production is the only form of growth in this study. Consequently, β_i is calibrated to represent the biochemical composition of female copepod, while δ_i represents that of copepod eggs. As model copepod, *Acartia tonsa* was chosen, as it is one of the most intensively studied copepods. Unlike other copepods (e.g. *Calanus hyperboreus*), *Acartia* adults have considerably less capacity for substrate storage (Sargent and Falk-Petersen 1988) and so could be assumed to maintain constant biochemical composition independent of feeding conditions. These adults also do not undergo significant structural (exoskeleton) growth (Miller et al. 1977), thus justifying my assumption of egg production as the only form of growth. To ensure the model predictions are realistic and reflect the behaviour of the copepod, all model parameters (Table 1) were set based directly on published experimental data.

In an experiment involving adult *Acartia tonsa*, Besiktepe and Dam (Besiktepe and Dam 2002) measured an ingestion rate of $28.9 \mu\text{gC}\cdot\text{individual}^{-1}\cdot\text{day}^{-1}$. Per unit C of the copepod, this rate of food ingestion is equivalent to $6.88 \mu\text{gC}\cdot\mu\text{gC}^{-1}\cdot\text{day}^{-1}$ (assuming the carbon weight of adult *Acartia* is $4.2\mu\text{g}$, Kiørboe 1989). This, to my knowledge, is the highest ingestion rate ever reported for *Acartia tonsa*. So it was used for I_c in my model. Maximum food assimilation by the copepod was set at 80% based on data from Conover (1966). Hence, the fraction of each ingested substrates that could be assimilated by the copepod was fixed at 0.8. Noting that animals assimilate different substances at different efficiencies, the reasons for my approach as well as the complications arising from it will be discussed later.

Energy content (kcal g^{-1}) of 4.1 for protein, 9.3 for lipid and 4.1 for carbohydrates was assumed, corresponding to the values used by Anderson (1992) when investigating the influence

of food C:N ratio on the growth of *Acartia*. In addition, a fixed molecular configuration for proteins ($C_{59}N_{16}H_{94}O_{19}S_{0.5}$, 1346g, Vollenweider 1985), lipids ($C_{18}H_{36}O_2$, 284g) and carbohydrates (CH_2O , 30g) (Anderson 1992), was assumed, consistent with previous studies (Kuijper et al. 2004a). Following these assumptions, energy content per gram carbon of proteins, lipids and carbohydrates was determined to be 32312.64, 51439.43 and 42922.90 J $(gC)^{-1}$ respectively (given 1 cal = 4.1876 J).

Compound composition of female *A. tonsa* and its egg were estimated using the equation below:

$$\log Y = a \cdot \log D - \log Z \quad (29)$$

Where D represents organism's dry weight (ng individual⁻¹), Y is its total content (ng individual⁻¹) of protein (P), lipid (L) or carbohydrates (H). Assumed dry weight for *Acartia* eggs and females were 120 and 10500 ng respectively. These were determined based on the C weights of 0.046 μ g and 4.2 μ g respectively for eggs and females (Kiørboe 1989) and an assumption that C constitutes 40% of copepod dry weight (Bämstedt 1986). Equation 29 was determined based on published biochemical composition data on females (42 species) and eggs (29 species) of marine (mostly copepods) zooplankton. Details of the review can be found within appendices 1 and 5 of this thesis. The constants, *a* and *Z*, for the relationship are listed in Table 3.

Table 3. Constant values for describing the relationship between dry weight (ng individual⁻¹) and biochemical content (ng individual⁻¹) of zooplankton.

Stage	Biochemical substance	Constant values for equation 29	
		<i>a</i>	<i>Z</i>
Female	Protein	0.95	1.83
	Lipid	0.14	1.23
	Carbohydrate	1.21	2.44
Eggs	Protein	0.96	1.07
	Lipid	0.12	0.60

Using these constants, the relative chemical composition of female *Acartia*, expressed as percentage of total dry weight, was 45.62 % protein, 10.38% lipid, and 3.10 % carbohydrates. These give a total biochemical composition of 59.10% in females. The remaining 40.9% of female dry weight was taken to be substances not currently represented in the model, such as chitin. Due to inadequate published data on the carbohydrate content of

copepod eggs, carbohydrate content of *Acartia* eggs was calculated as the difference between egg dry weight and the sum of its protein and lipid contents. Based on this approach, protein, lipid and carbohydrate composition of eggs were determined to be 62.64, 35.12 and 1.78 % respectively. These weights were converted into C, assuming C constitutes 53%, 75% and 40% respectively P, L, and H content of zooplankton (Ventura 2006). The result was a P:L:H carbon mass ratios of 0.73:0.24:0.04 for *Acartia* females (i.e. $\beta_P : \beta_L : \beta_H$) and 0.55:0.44:0.01 for eggs (i.e. $\delta_P : \delta_L : \delta_H$).

Consistent with previous studies (Anderson 1992), I relied on oxygen consumption by *Acartia* to determine energy requirement. Energy demand for maintenance, b , was determined based on the rate of oxygen consumption by starving copepods. Thor (Thor 2003) measured the respiration rate of starving as well as non-reproducing adult *A. tonsa* to be 0.4 nLO₂ individual⁻¹min.⁻¹. This converts into maintenance energy consumption rate of 2755.10 J.gC⁻¹.d⁻¹ based on the assumed carbon content of *Acartia* and the conversion factors 22.4 L (mol O₂)⁻¹ and 0.45 J (μmol O₂)⁻¹ (Karjalainen et al. 2003). Conversely, energy requirement (d) for growth was determined based on the volume of oxygen consumed by feeding and growing animals. In the same experiment, Thor (Thor 2003) measured an increase in *A. tonsa* respiration from 0.4 to 0.95 nLO₂ individual⁻¹min.⁻¹ and an average egg production rate of 1.23 eggs female⁻¹hour⁻¹ as food (*Rhodomonas baltica*) was provided to starving individuals. This increase in oxygen consumption was assumed to represent respiration due to growth-related processes. Also, using the O₂ to energy conversion factor and assuming C constitutes 40% of egg dry weight, the demand for energy to power egg production was calculated to be 11228.77 J.gC⁻¹. *Rhodomonas sp.* is considered a good prey for zooplankton production (Jónasdóttir 1994; Jónasdóttir and Kiørboe 1996; Koski et al. 1998; Tang et al. 2001; Sigsgaard et al. 2003; Klein Breteler et al. 2004) and so the data extracted from Thor's work potentially reflects optimum energy consumption by *Acartia*.

Model implementation

In my model, *Acartia* acquires its nutrition from a 3-dimensional biochemical field made up of different combinations of protein, lipid and carbohydrate molecules. For each dimension, a grid resolution of 0.005 was used. This means that the relative contribution (i.e., α_i) of a chemical to the total biochemical field changed from a minimum (= 0; prey does not contain the chemical) to a maximum composition (= 1; prey contains only that chemical) over unit steps of 0.005. The different combinations of compounds were taken to represent different prey items. The model consumer was then made to feed and grow on each prey in

accordance with the model procedure. The equation that describes food quality (i.e. equation 25) is not linear as it can only be solved by simultaneously satisfying the conditions for maintenance and growth. An iterative approach was used to estimate how the acquired substrates could be used for maintenance and growth or voided in order to satisfy the required structural biochemical composition of the copepod.

In the model, chemicals assimilated by consumers were first used to satisfy maintenance needs, after which those remaining are used for growth. To power maintenance, a fraction ρ_i of each assimilate could be respired. This is specified by equation 10. It is also a condition in my model that catabolism of assimilates does not have to compromise consumer's structural biochemical requirement for maintenance. This is specified by equation 11. So a rule in the model is that $1 > \rho_i \geq 0$, depending on the availability of each compound. The model assumes that consumers require a specific fixed fraction (in terms of C) of each chemical substance in their structure. Consequently, the sum of the solution to equation 11 must always equal one as given by equation 8. Only three different compounds (i.e., proteins, lipids and carbohydrates) are considered in this study. As a result, equations 10 and 11 actually constitute three independent equations with three unknown ρ_i values. Assuming j takes the value of compound i , and k the value of the other two compounds, ρ_j can be determined from equation 10 as

$$\rho_j = \frac{b/I_c - \sum_k \alpha_k \lambda_k \rho_k E_k}{\alpha_j \lambda_j E_j} \quad (30)$$

Inserting the above equation into equation 11 and rearranging gave the relation below.

$$\alpha_k \lambda_k \rho_k + \beta_k \sum_k \alpha_k \lambda_k \rho_k \left(\frac{E_k}{E_j} - 1 \right) = \alpha_k \lambda_k - \beta_k \left(1 - \frac{b}{I_c} \right) - \alpha_k \lambda_k \quad (31)$$

This constitutes two equations with two unknowns from which ρ_k could be explicitly calculated. Afterwards, the remaining ρ_i value was determined by inserting the determined ρ_k values into equation 30. When ρ_i estimated for a substance is less than zero, the substance was considered to have been supplied below the structural requirement of the consumer. For such chemicals, ρ_i was set to zero, while consumer's energy demand for maintenance was fulfilled by respiring only non-limiting substance(s).

After maintenance the remaining compounds were used for egg production. Here, equations 21 and 23 respectively specify total growth rate and the amount of energy needed to

power it. In both equations, γ_i and x_i respectively represents the fraction of assimilates that could be catabolised for, and without production. For mathematical convenience, the iteration procedure does not distinguish between these forms of respiration. Instead, it treats them as a single parameter ϕ_i (i.e. $\phi_i = \gamma_i + x_i$). Consequently, equations 21, and 23 could be rewritten as equations 32 and 33 respectively.

$$G = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) (1 - \phi_i) \quad (32)$$

$$\delta_i = \frac{\alpha_i \lambda_i (1 - \rho_i) (1 - \phi_i)}{\sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) (1 - \phi_i)} \quad (33)$$

Since $\phi_i = \gamma_i + x_i$, the total amount of energy produced during egg production could be equal to or greater than the total amount actually needed for only egg production. Consequently, equation 23 could be rewritten as below.

$$Gd \leq I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) \phi_i E_i \quad (34)$$

The model assumes that consumers require a specific fixed fraction of each chemical substance in their eggs. It was therefore essential that compounds remaining after all respiratory processes meet the assumed chemical composition requirements for egg biomass as specified by equation 22 (also rewritten as equation 33). Hence, for any number of compounds considered,

$$\sum_i^n \delta_i = 1 \quad (35)$$

Consequently, equations 32, 33 and 34 constitute four independent equations with four unknowns, i.e., G and one ϕ_i for each of the three substances considered here. These were solved by first substituting equation 32 into 34 to eliminate G . The resulting equation was then solved for one value of ϕ_i by following the approach described above for maintenance metabolism. Afterwards, the solution was used with equation 33 to obtain two equations with two unknowns, these being the other ϕ_i values. These two equations were solved simultaneously. Hereafter, the remaining ϕ_i was found by using either equation 32 or 34.

It is widely recognized that consumers utilise chemicals supplied in excess of their structural requirements for a number of different processes (see review by Anderson and

Hessen 2008). I therefore assume that it is advantageous for consumers to utilise excess substrate for, than without, production. Consequently, when the amount of energy produced during growth metabolism was equal to that needed for powering only new biomass production, it was taken that $\phi_i = \gamma_i$ and $x_i = 0$. Conversely, when growth metabolism produced more energy than needed to cover the costs of egg production, ϕ_i was divided between γ_i and x_i . Here, a simplifying assumption was made that substances are catabolised to power growth based on their energy contents and availability. Hence, energy contribution of each biochemical substance for only new biomass production could be described using the relation below:

$$I_c \alpha_i \lambda_i (1 - \rho_i) \gamma_i E_i = Gd \frac{I_c \alpha_i \lambda_i (1 - \rho_i) \phi_i E_i}{I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) \phi_i E_i} \quad (36)$$

From equation 36, γ_i and x_i could be calculated using equations 37 and 38 respectively.

$$\gamma_i = Gd \frac{\phi_i}{I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) \phi_i E_i} \quad (37)$$

$$x_i = \phi_i - \gamma_i \quad (38)$$

Finally, the values for the parameters ρ_i , γ_i and x_i attained from this iterative process were then inserted in the appropriate equation to calculate the amount of each substrate that may be respired for maintenance and growth as well as those that may be voided. Compound-specific potential growth rates and efficiencies as well as food quality were similarly determined.

2.5 RESULTS

Model Validation

The usefulness of the approach presented here depends on the model producing biologically realistic results. The yardstick for a prey's quality is the growth performance of its consumer. In a laboratory study, Kiørboe (1989) observed that *Acartia*'s growth decreases with increasing C:N ratio of its food, which suggests a decrease in food quality with increasing algal C:N. To demonstrate the validity of the assumptions underlying this model as well as the realism in its predictions, the predicted prey potential quality and growth efficiencies have been compared with Kiørboe's observation.

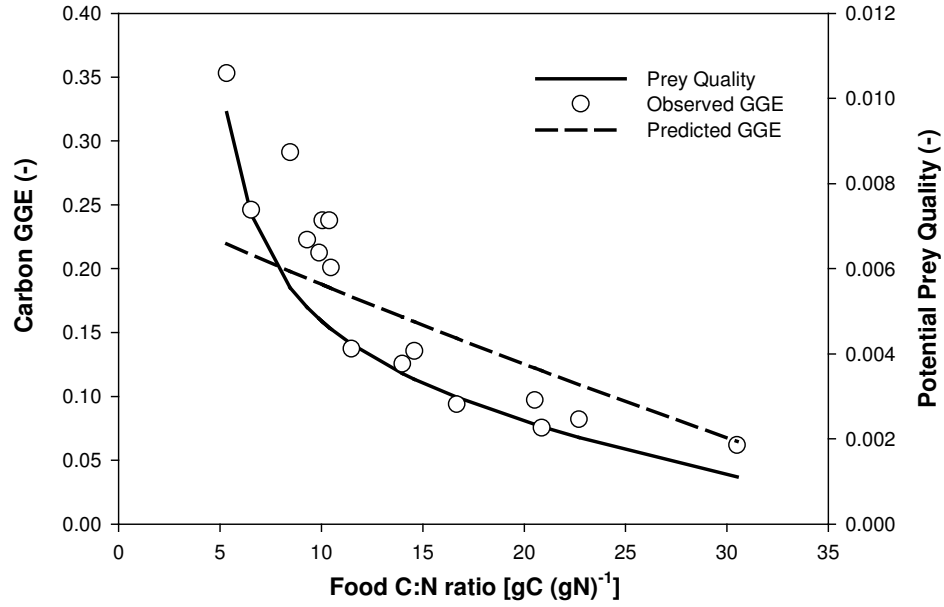


Figure 2. Food quality and carbon gross growth efficiency (GGE) for *Acartia*. Observed data were extracted from Kiørboe (1989).

In order to do so, I first converted the algal C:N ratios in Kiørboe's report from mass ratios (M_{cn}) into molar ratios (F_{cn}) using a conversion factor of 1.67 (i.e. molar mass of N divided by that of C). Protein (α_p), lipid (α_L), reserve carbohydrate (α_{H1}) and structural carbohydrate (α_{H2}) fractions of prey total C were then calculated using the appropriate equation below (Anderson 1994):

$$\alpha_p = \frac{P_{cn}}{F_{cn}} \quad 39a$$

$$\alpha_{H1} = 0.0232 * F_{cn} + 0.0118 \quad 39b$$

$$\alpha_L = 0.141(1 - \alpha_{H1}) \quad 39c$$

$$\alpha_{H2} = (1 - \alpha_{H1})(1 - 0.141) - \alpha_p \quad 39d$$

Where P_{cn} represents C:N ratio (by moles) of protein. This was taken to be 3.7 (Vollenweider 1985). The solutions to equations 39a-d gives the contribution of the respective compounds to total prey C based on the molar ratios of algal C and N. Mass C to mass N ratio equivalents of the calculated fractions were obtained by multiplying each estimate by M_{cn} / F_{cn} . I did not distinguish between reserve and structural carbohydrates and so their sum was taken to represent prey carbohydrate content (i.e. $\alpha_H = \alpha_{H1} + \alpha_{H2}$).

The comparison of model predictions and experimental observations are shown in figure 2. The model wrongly estimates carbon gross growth efficiency (GGE) at almost all algal C:N. This was expected partly because the carbon ingestion rate used in the model was ~5x more than what was experimentally observed ($1.35 \mu\text{gC} \cdot \mu\text{gC}^{-1} \cdot \text{day}^{-1}$ on average: Kiørboe 1989). In addition, the model does not currently couple assimilation of biochemical substances with their requirements by the consumer (for that see chapter 3 of this thesis). However, useful conclusions can be drawn from the predicted prey potential qualities. The model predicts a decline in food quality with increasing algal C:N. This is because increasing proportions of the ingested carbon could not be used by the consumer due to excesses in the prey's composition of carbohydrates. The resulting relationship between prey quality and algal C:N content is supported by the experimentally observed decrease in growth (Figure 2; regression of food quality versus observed C GGE, $r^2 = 0.86$, $p < 0.0001$). The food quality formulation presented here is therefore realistic and justified.

Food Quality

The effect of changing prey composition of protein, lipid and carbohydrate on food quality is shown in Figure 3. Food quality varied between 0, indicating that the prey had no nutritional value for growth, and the highest value depending not only on the proportion of a compound in the food (α_i) but also on how the rest of the prey's biomass ($1 - \alpha_i$) was distributed between the remaining compounds.

The nature of the relationship between variations in part of the prey biomass constituted by a compound and the highest food quality values was Gaussian-like with intermediate α_i values that elicited maximum food quality of 1 (Figure 3B-D), and a maximum consumer gross growth efficiency of $60 \pm 1\%$ (Figure 4E).

The balance of compounds, enough for the consumer to achieve this maximum growth efficiency was predicted to range between 43.5 and 64.5% of protein, 34.0 and 52.5% of lipid, and 1 and 20.5% of carbohydrate (Figure 3). At these prey nutritional levels, food quality was 1 and no waste of C was predicted (Figure 4C); thus, the release of assimilated compounds to satisfy consumer's composition requirements did not occur. Consequently, growth was maximized, causing the respiration of relatively more carbon to power it (Figure 4D).

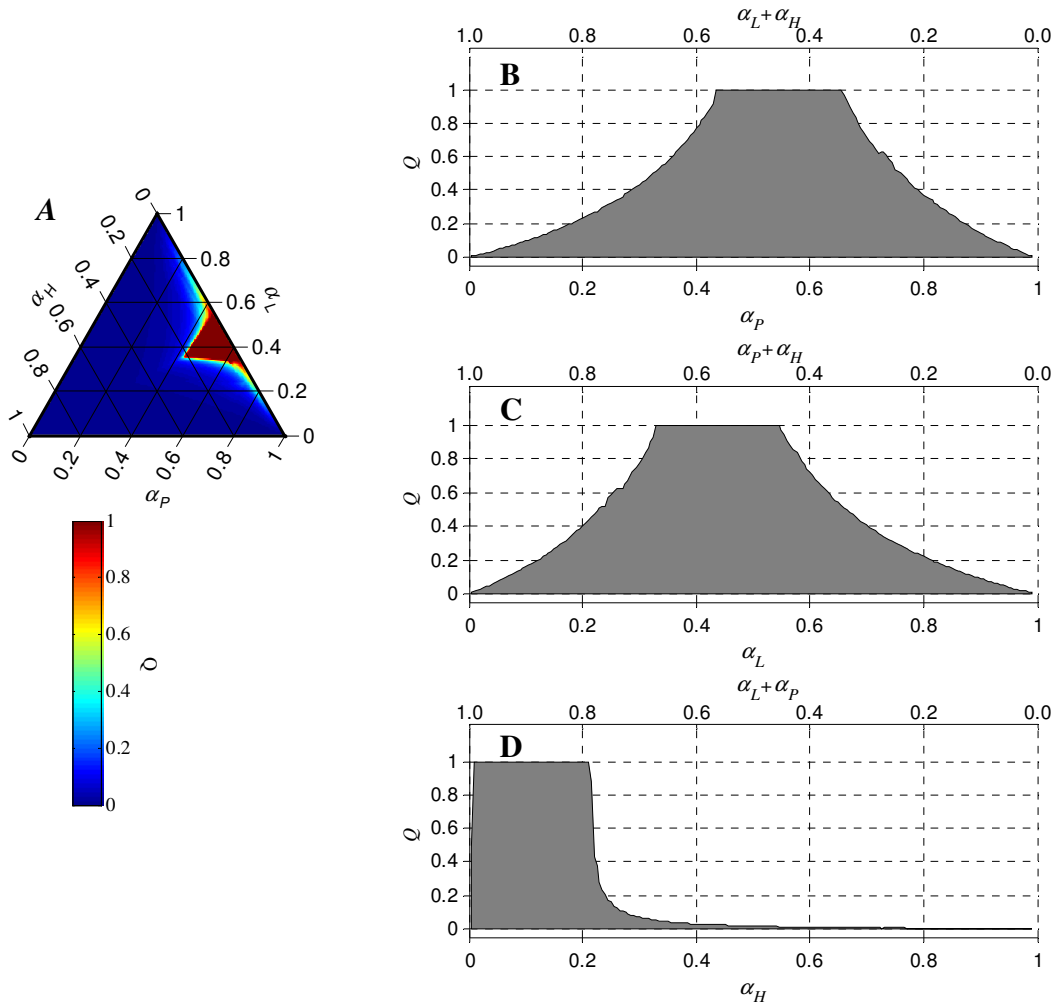


Figure 3. A: Predicted effect of prey's relative composition of protein (α_p), lipid (α_L) and carbohydrate (α_H) on food quality (Q). For each point within the triangular space, $\alpha_p + \alpha_L + \alpha_H = 1$. A wide relative composition range (i.e. $0 \leq \alpha_i \leq 1$) for the individual compounds was used to enable easy illustration. The yardstick for food quality is the maximum net efficiency with which a prey biomass is converted into that of consumers (see equation 27 and text associated with it). Food quality equals 1 means the composition of the food allowed the model copepod to attain maximum growth (see Figure 4). On the other hand, prey has no value for growth when food quality is zero. Subplots **B**, **C** and **D** show 2-dimensional reductions of the triangular plot. For each compound, α_i values at which $Q = 1$ indicate end of limitation by that compound (see Figure 4). Note: model is not defined for $\alpha_p + \alpha_L + \alpha_H \neq 1$. This is not visible from the figure due to the high resolution employed for the definition of individual α_i (see section 2.4).

Table 4. Normalized sensitivity (S) of the ideal balance of biochemical substances to model parameters for *Acartia* production. See Table 1 for definition and nominal values of parameters. Parameters were increased (P1) and decreased (P2) by 10%. $S > 1$ means parameter increased α_i by a fraction of $S - 1$; $S < 1$ means parameter decreased α_i by a fraction of $1 - S$; S is negative if the direction of response (e.g., increase) opposes the direction of parameter change (e.g., decrease); values of $S = 0$ indicate the requirement for a compound is independent of a parameters. Subscripts P, L and H represent protein, lipid and carbohydrate respectively.

Parameter	Change	S Value		
		α_p	α_L	α_H
I_C	P1	-0.05	-0.18	1.48
	P2	-0.05	0.06	0.00
λ_p	P1	-0.20	0.24	0.06
	P2	-0.64	0.72	0.41
λ_L	P1	0.48	-0.70	0.67
	P2	0.44	-0.59	-0.27
λ_H	P1	0.06	-0.03	-0.32
	P2	-0.05	0.24	-1.10
b	P1	-0.12	0.02	0.77
	P2	-0.13	0.07	0.52
d	P1	-0.07	-0.11	1.17
	P2	-0.10	0.05	0.42
E_p	P1	0.05	-0.08	0.14
	P2	-0.13	0.21	-0.29
E_L	P1	0.07	-0.20	0.64
	P2	0.03	-0.02	-0.09
E_H	P1	0.14	-0.11	-0.36
	P2	-0.01	0.12	-0.67

Carbon used for maintenance was however relatively low when prey's nutritional status was ideal relative to the consumer's requirement. This was because here, only energy-rich lipids were predicted to be mostly respired for maintenance (Figures 4B and 5A). Most of the carbon respired for growth came from protein, with carbohydrate and lipid contributing relatively less when prey was "ideal" because these fractions were of lesser availability

(Figures 4D and 5B). For each compound in the prey, the levels (α_i) at which food quality was highest and stable ($Q = 1$) indicated the end of limitation by that compound (Figure 3B-D).

To determine whether consumers' structural composition requirements alone (β_i and δ_i) set these levels, the mean of the predicted ideal prey's content of protein (= 0.52), lipid (= 0.41), and carbohydrate (= 0.07) were compared with their respective counterparts in the consumer (t-test, $\alpha = 0.05$, $df = n-3$; where n equals 314 and represents the different compound combinations that gave $Q = 1$). Carbohydrate composition in the ideal prey was similar to the required composition of the same in both stages of the consumer (i.e. α_H equals β_H and δ_H , $p > 0.05$). Also the predicted ideal protein and lipid composition of the prey were similar to their required compositions in *Acartia* eggs ($p > 0.05$). However, there were significant differences between the ideal protein and lipid composition of the prey and their respective requirement in adult tissues of the consumer ($p < 0.05$). While lipid content of the ideal prey was more than that of the female copepod, protein composition of the ideal food was significantly less than that of adult *Acartia*.

The biochemical composition of the ideal food was determined not only by *Acartia*'s structural requirement but by other model parameters as well. A sensitivity analysis was therefore conducted to determine how the predicted ideal requirement for individual substances was influenced by other model parameters. In order to do so, a sensitivity value, S , was calculated using equation 40 (Haefner 2005), by arbitrary altering parameter values by $\pm 10\%$.

$$S = \frac{(R_a - R_n) / R_n}{(P_a - P_n) / P_n} \quad (40)$$

Where R_n was the ideal requirement for a given compound for the standard case with parameter value P_n , and R_a is the requirement for the case when the parameter is given a new value P_a . The results suggested high sensitivity of the ideal requirements for individual biochemical substances to model parameters (Table 4). This means the nutritional value of potential prey organisms can be influenced by grazing rate (I_c), assimilation efficiencies (λ_i), energy densities of the acquired substance (E_i), and consumer's energy requirements (b and d), which is consistent with the results of other model studies (e.g. Anderson and Hessen 2005).

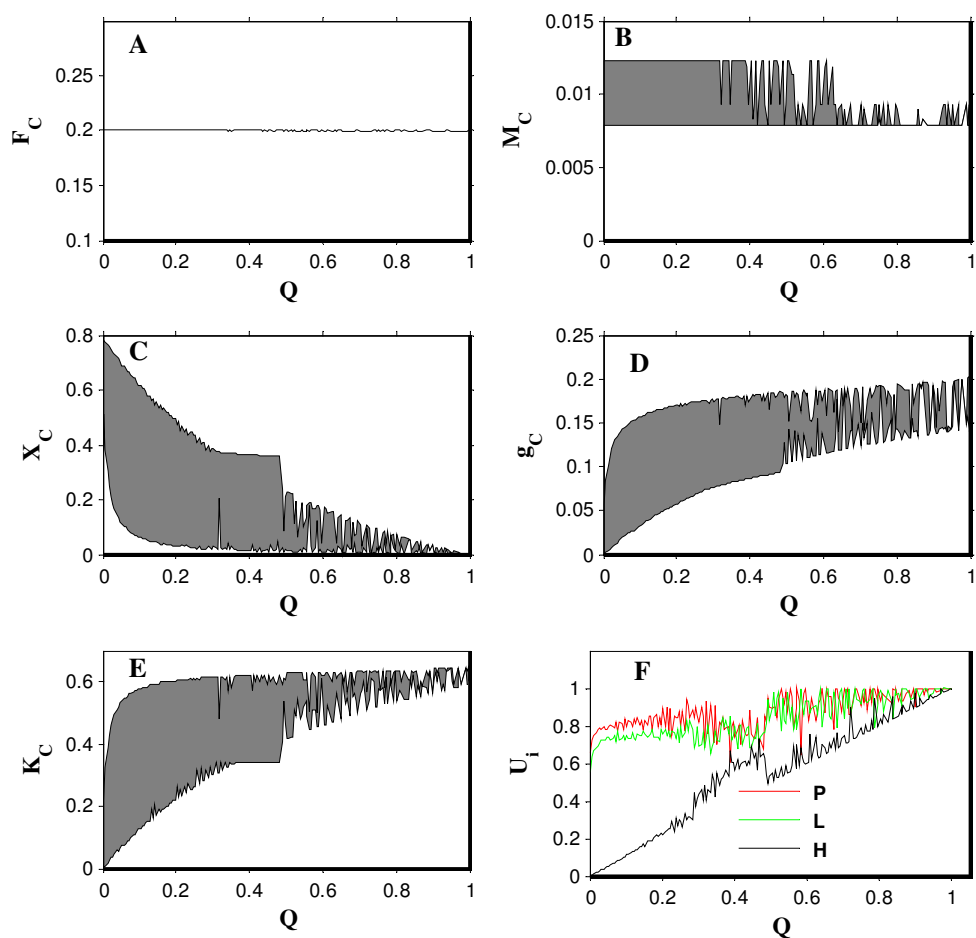


Figure 4. The relationship between food quality (Q , dimensionless) and the proportion of total ingested C released as faecal pellet, F_c (A), used for maintenance, M_c (B), catabolised without production in order to satisfy consumer's structural composition requirement, X_c (C), respired to power growth, g_c (D), and incorporated into eggs, K_c (E). The model incorporate plastic preference for metabolic substrates by consumers, hence the variations in the fate of C ingested by animals. Subplot F shows the relationship between food quality and the net utilization efficiency (U_i) of protein (P), lipid (L), and carbohydrate (H) components of the food. At each food quality, growth-limiting chemical(s) is/are those with highest U_i values. At $Q = 1$, growth is highest, with ~60% of ingested food being converted into eggs while U_i for all compounds is 1 (i.e.100%), because no carbon was voided ($X_c = 0$).

Effect of prey composition on carbon use

As shown above, my model consumer assigns food quality values to different combinations of protein:lipid:carbohydrate from 3-dimensional biochemical field (i.e., Figure 3A), based on its requirements (both energy and structural) and maximum capacity for food uptake and processing. Therefore, at any biochemical composition of the prey, the fate of ingested C was plotted against food quality to examine the effect of prey's nutritional composition on how a consumer may potentially distribute C between its various requirements. Figure 4 shows the results.

I assumed constant assimilation efficiency for biochemical substances (Table 1; see also model description for justification of the approach) and relied on substrate voiding (X_c) to control excess carbon. As a consequence, the fraction of total ingested carbon released as faecal pellets was independent of food quality (Figure 4A). The contribution of individual compounds to the total faecal pellet carbon was therefore equal to their composition in the food (results not shown).

Maintenance represented a relatively smaller part of the overall carbon budget of the model *Acartia* (max. just over 1.25%). On average, when food quality was good, relatively less carbon was predicted to be respired for maintenance than when food quality was poor (Figure 4B). The model employed here makes the percentage of ingested carbon used for maintenance dependant upon the mixture of compounds respired.

Energy density of lipid was approximately 1.2 times that of protein and carbohydrate (Table 1). As a consequence, the highest (>1.2) and least (<0.8) percentage of ingested carbon predicted to be respired for maintenance occurred when the bulk ($\geq \sim 80\%$) of the carbon respired was protein and lipid respectively (Figure 5A).

Where there was no chemical limitation on the utility of a prey to the consumer (i.e. $Q = 1$), no biochemical substance was voided (i.e. $X_c = 0$), and a higher carbon use for growth and growth-specific respiration was predicted. When the consumer became limited by the biochemical composition of the food (i.e. $Q < 1$), growth and carbon respiration for growth decreased while excretion of excess biochemical substances increased (Figure 4). Carbon demand to power growth dominated the carbon respiration budget, increasing with food quality because egg production increased. This made standard catabolism of carbon higher when the consumer accessed an ideal prey than when the prey was sub-optimal. Again, due to the energy density differences between biochemical substances, the percentage of ingested carbon respired to provide energy for growth depended on the mixture of the compounds respired (Figure 5B). On average, the highest (above 17%) of ingested carbon

predicted to be respired for growth occurred when only protein was the dominant respiratory substrate.

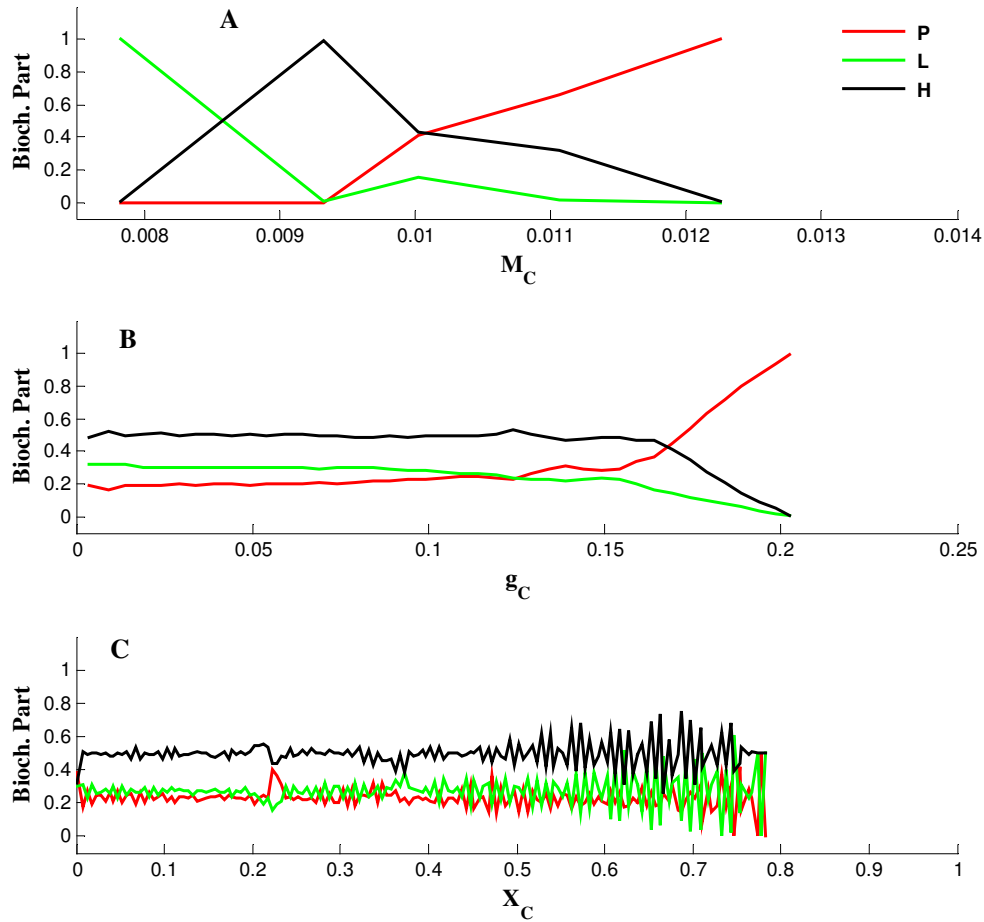


Figure 5. Relative contributions of protein (P), lipid (L), and carbohydrate (H), to total amount of carbon respired for (A) maintenance, M_C , (B) to power growth, g_C , and (C) voided, X_C , to satisfy structural biochemical composition requirement of copepods.

Fate of individual food components

Figure 5 shows the average contributions of individual food components to total carbon catabolised to power maintenance and growth, as well as those voided in accordance with both maintenance and growth requirements of the model copepod. Figure 6 shows the relationship between the proportion of prey biomass constituted by individual compounds and their predicted fate. The model approach presented here allows respiration of compounds for maintenance only if their supply exceeds their required compositions in adult tissues. As a

consequence, proteins, lipids and carbohydrates were not used for maintenance until their composition in the food exceeded levels assumed for female copepods (Figure 6Ai-Ci). Once these were exceeded, individual compounds were respired for maintenance at rates that depended on the balance of biochemical substances in the food.

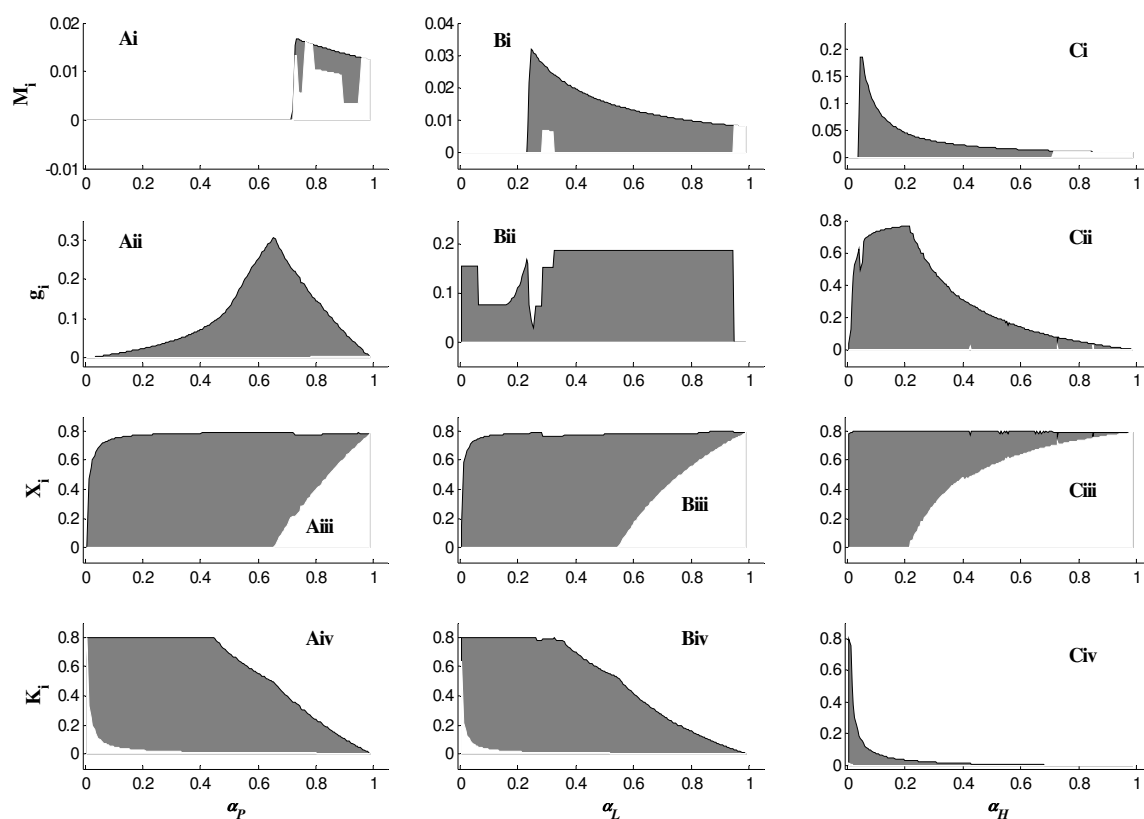


Figure 6. Relationship between the potential fate of individual substances, i , and their respective proportions (α_i) in the food. Left (A), middle (B) and right (C) panes represents protein, lipid and carbohydrate respectively. M_i , g_i , X_i and K_i represents the proportion of total ingested compound used for biomass maintenance (i), respired for energy to power growth (ii), voided to satisfy consumer's composition requirement (iii), and converted into eggs (iv). Fate of substances varied between a minimum (lower edge of area plot), and a highest value (upper edge of area plot) depending not only on the proportion of a compound in the food (α_i) but also on how the rest of the prey's biomass ($1-\alpha_i$) was distributed between the remaining compounds. Substances were assimilated with a fixed assimilation efficiency of 0.8. As a consequence, the proportion of individual substances released as faecal pellets was constant at 0.2 and independent of α_i value (results not shown).

Previous studies have shown that environmental factors, such as temperature, are major determinants of prey biochemical composition (Thompson et al. 1992) and modifiers of animals demand for energy (Gaudy et al. 2000; Ikeda et al. 2001) and structural constituents (Hassett and Crockett 2009). So one can for example expect environmentally induced changes in consumers' maintenance energy budget to significantly impact the availability of resources for growth, if habitat-induced change in prey biochemical composition is not commensurate with consumers' requirement. To avoid this, the model approach here was chosen to implicitly make the use of biochemical substances for growth (i.e. for egg and growth-specific energy production) dependent on the similarities (or otherwise) in the relative composition of individual biochemical substances required in egg tissues and those remaining after maintenance (see model description). As a result, respiration of individual compounds to provide energy for growth differed from that for maintenance (Figure 5). Mostly, carbohydrate contributed the bulk of the carbon respired to power growth. This moderated the cost (in terms of carbon) for egg production, as carbohydrate is relatively energy rich. Lipid and carbohydrate were predicted to be respired for growth even when they constituted relatively low proportion (less than 1%) of the food biomass (Figure 6 Bii, Cii). On the other hand, protein was significantly catabolised only when its composition in the prey exceeded ~10 % (Figure 6Aii).

Here, biochemical substances were assumed to constitute a fixed proportion of egg biomass. Therefore independent of their composition in prey, compound-specific contribution to growth was constant, being equal to the proportions assumed respectively for each egg constituent (results not shown). As a result, the fraction of individual substances converted into eggs decreased when their proportion in the prey increased (Figure 6 Aiv-Civ). While carbohydrate-specific growth declined at a faster rate, the rate at which protein- as well as lipid-specific growth decreased was relatively slow due to the high protein and lipid demand the model set for egg production. Consequently, the net efficiency with which substrates were used for egg production (calculated using equation 26) was predicted to vary, being 100% for all biochemical substances only when there was no biochemical limitation of the consumer (i.e. food was ideal) (Figure 4F). Potentially, any substrate can limit growth when its availability does not meet the requirement of the consumer (Sternern and Elser 2002). The model presented here predicts protein and lipids as the dominant growth-limiting compounds because of their relatively high requirement for growth. However, instances of carbohydrate limitation as well as co-limitation by multiple compounds were also predicted (Figure 4F).

Voiding of any compound depended on the extent to which that substance was in excess of requirements. The relatively high protein and lipid requirements of the model consumer almost always resulted in protein and/or lipid limitation. As a result, these substances constituted relative little of the total C wasted (Figure 5C). Conversely, voiding of carbohydrates was relatively high because the model consumer required relatively low amount of it in its structure. The results also show that compounds could be wasted, even if they constituted very little of the diet if the food is nutritionally imbalanced (Figures 4C and 6Aiii-Ciii). Generally, an increase in the proportion of a compound in the prey led to an increase in its excretion. This is because the assumption of constant assimilation efficiencies meant an automatic increase in the metabolic availability of individual biochemical substances, sometimes to unwanted levels, whenever their composition in the food increased.

2.6 DISCUSSION

By marrying concepts from optimum foraging theory (Stephens and Krebs 1986), ecological stoichiometry (Pond and Pond 2000; Sterner and Elser 2002) and geometric analysis of animal nutrition (Simpson and Raubenheimer 1995), the model presented here provides an improved framework for evaluating prey organism. It considers consumers' capacity for both food intake and metabolism. Growth is estimated based on the need to satisfy both the structural (i.e. chemical composition) and energy requirements of consumers. Here, I discuss how the implementation of the framework I have suggested contributes to our understanding of ecological processes.

Model output

A dominant paradigm in feeding ecology is that animals feed to maximize their fitness (Yearsly et al. 2001; Raubenheimer et al. 2009). Here using egg production (growth) efficiency as a proxy for fitness, I assess the viability of my macromolecular food quality model. The highest carbon-specific gross growth efficiency predicted by the model was ~60.1% (Figure 4E). This is above the highest growth efficiency of 48% observed for the model consumer (Broglia et al. 2003) but agrees well with the upper limits of 60 (empirical data compiled by Moloney and Field 1989) and 70% (theoretical estimation based on 90% assimilation efficiency: Bämstedt et al. 1999) generally known for copepods. The food quality formulation presented here is therefore realistic.

In my simulations, food ingestion and substrate assimilation efficiencies as well as energy expenditure for maintenance and unit egg production were assumed constant.

Furthermore, I assumed that, the chemical composition of the consumer was constant. Hence, the predicted maximum growth efficiencies are outcomes of the interplay between the standard respiratory physiology of the model consumer and the composition of its food. The composition of the prey that elicited maximum gross growth efficiency in the consumer was 43.5 – 64.5% protein, 1 – 20% carbohydrate and 34 – 52.5% lipid (Figure 3). This balance of compounds defined a biochemical space within which growth was limited by no chemical substance, comparable with threshold ratios of Urabe and Watanabe (Urabe and Watanabe 1992) or intake target of Simpson and Raubenheimer (Simpson and Raubenheimer 1993). My results therefore suggest that copepods can achieve comparable maximum growth without regulating food acquisition, biosynthesis, or energy expenditure, even when they access nutritionally distinct diets. Based on these results and as supported by laboratory observations (DeMott et al. 1998; Plath and Boersma 2001; Fu and Xie 2004; Jeyasingh 2007), models that ignore consumers' ability to physiologically acclimate to prevailing feeding conditions risk errors in predicting the dynamics of the systems modelled. Thus there is necessity to include the influence of respiratory physiology when modelling the response of species to the effects of external stressors. Results here also provide supporting evidence for the call to re-evaluate the ecological impact of prey chemicals that occur in surplus of consumers' structural needs (Hessen and Anderson 2008). In my model, these excess substances were used as respiratory substrates.

Following this approach, the model predicted that, maximum egg production and hence food quality declines once excess prey constituents exceed demand for respiratory substrates by consumers. This occurred regardless of the substance responsible for the nutritional imbalance (Figure 3B-C). Hence excesses as well as limitation in any food constituent can be seen to adversely affect consumer's growth. This finding is consistent with experimental observations in both aquatic (Boersma and Elser 2006 and references therein) and terrestrial ecosystems (Raubenheimer and Simpson 2004). Furthermore, the model predicts that egg production among copepods can be simultaneously limited by multiple substances (Figure 4F), as shown by previous studies (Martin-Creuzburg et al. 2009). Protein and lipid were predicted as the dominant growth-limiting compounds, which is consistent with the view of protein/N (e.g. Checkley 1980; Kiørboe 1989) and lipid limitation in marine copepods (e.g. Støttrup and Jansen 1990; Jónasdóttir 1994). The prediction of carbohydrate limitation is also consistent with the dictates of stoichiometric theory (Sterner and Elser 2002). Hence, the model framework I have suggested has demonstrated the ability to address the effect of excess nutritional substances on the dynamics of growth in marine zooplankton.

My approach distinguishes the structural biochemical requirements of females from that of their eggs. The justification is that biochemical requirements for egg production may differ from that of maintenance (Koski 1999), as eggs require specific compounds for processes such as hatching that do not occur in females. Here as there were significant structural requirement differences between females and their eggs, the amount of substances needed for maximum egg production was not significantly influenced by female's structural requirements (e.g., compare α_p at $Q=1$ in Figure 3 with β_p in Table 1). This however does not mean that the representation of adult's requirement for chemical substances was of no significance. Respiration of chemical substances for maintenance energy was partly set by the need to satisfy the structural composition requirement of adults, thus implicitly ensuring that other vital non-energy requirements of adults, which could be lipids for cold temperature adaptation (Farkas 1979; Nanton and Castell 1999), or protein (amino acids) for osmolarity (ion transport) regulation (Bryant et al. 1975; Anger 1998; Hochachka and Somero 2002), were not compromised during maintenance respiration. Substrates were, therefore, not catabolised for maintenance energy until their relative supply had exceeded females' structural requirement (Figure 6), with protein sparing being the highest as it constituted the bulk of females' biomass (Table 1). These predictions compare well with those of models (e.g., Kuijper et al. 2004a) that are based on dynamic energy budget theory and its "synthesizing unit" (SU) representation of the classical enzyme concept for processes involving multiple chemical substances (Kooijman 1998). As this model, SU can be applied to ensure that substrates required for biomass formation are not catabolised for energy (Kuijper et al. 2004a). The SU concept, however, involves parameters that are practically difficult to determine (van der Meer 2006). Conversely, the parameters needed for my model can be measured using existing laboratory techniques. I therefore recommend my framework as an alternative approach to modelling substrate utilisation for growth and reproduction by consumers.

Moreover, the approach provided the consumer with important physiological advantages. For example, the relative protein composition of the ideal food was less than the relative content of the same in adults, although the model sets higher protein requirements for adults than eggs (see Table 1). In terms of C, protein by virtue of its low energy density made energy production more expensive (Figure 5). A lowered protein composition in the ideal diet allowed the consumer to acquire enough of energy-rich lipids to meet its energy requirements. As a consequence, protein was not markedly respired for maintenance when prey was ideal. This caused a reduction in the amount of total carbon used for maintenance when prey was

ideal (Figures 4B and 5A). However, most of the proteins spared from catabolism were not incorporated into eggs as they were constrained by the assumed structural requirement for egg production (i.e., δ_i). As a consequence, maximum growth of the consumer (i.e. GGE ~ 60%) was attained by respiring mainly proteins (Figures 4D, 4E and 5D). This finding is similar to previous studies showing that zooplankton can respire a substantial portion of the proteins they acquire for energy (Blazka 1966; Kuijper et al. 2004a). It also explains why food quality increased with carbon catabolism (Figure 4D), a result that compares with the experimental observation of higher carbon respiration by copepods that feed on prey with the biochemical composition conducive for egg production (Thor et al. 2002).

Clearly an animal's structural requirements play crucial role in determining the total quantity of C it releases to the environment. From my simulations, carbon respiration for growth increased with Q (Figure 4D), similar to what has been reported by others (e.g. Kiørboe et al. 1985; Touratier et al. 1999; Thor et al. 2003; Kuijper et al., 2004a; Jansen and Hessen 2007). Furthermore, the maximum fraction of ingested carbon respired for maintenance and growth was approximately 20% (Figure 4B, D), which compares with results from other studies (Kiørboe et al. 1985; Kuijper et al. 2004a). The total amount of voided carbon varied with prey biochemical composition, amounting to up to 78% of the ingested carbon when prey was nutritionally too poor to allow egg production (Figure 4). This suggests that copepods can enhance the rate of recycling of substances held within prey items that are nutritionally poor via ingestion, assimilation and catabolism. It can however be said that the cycling of different substances (e.g. lipids, carbohydrates and proteins) may not be similarly impacted by copepods. For example, my results suggest that relatively little protein and lipid was unused and released to the environment (Figure 5). Although these results are self-evident because of the relatively high structural demand the model sets for these substances, the model approach provides a physiological evaluation of the process in the context of the entire carbon budget of the consumer, based on a realistic representation of *Acartia*'s metabolic capacity. The results suggest that most of the protein and lipid zooplankton acquire from their prey may be (i) incorporated into zooplankton biomass (here via egg production) or (ii) released (here via X_i , M_i , and g_i) as a metabolic by-product, possibly in the form of ammonia (for only proteins), DOC (dissolved organic carbon) and/or CO₂ (Darchambeau et al. 2003). In my model, carbon associated with carbohydrate constituted most of the voided carbon. This agrees with experimental observations where carbohydrate assimilation efficiency among copepods is generally low when algae are carbohydrate-rich (Anderson 1994).

The macromolecular composition of the prey impacts upon utilization efficiency of different biogeochemically relevant substances as shown by experimental results and predicted by this model. As yet this is not accounted for in the modelling of marine ecosystems or biogeochemical fluxes and its incorporation in these models will increase their ability to describe ecosystem and biogeochemical dynamics.

Comparison with current food quality models

Most models for food quality (e.g. Mitra et al. 2003; Mitra 2006; Flynn and Mitra 2009) have emphasized elements, and the determination of consumers' ideal requirement for individual elements, the so-called threshold elemental ratios, TER (Urabe and Watanabe 1992; Sterner and Hessen 1994; Anderson and Hessen 1995). Here I illustrate how the macromolecular framework I have suggested is advantageous over these stoichiometric food quality models. In order to do so, the elemental composition of the "artificial food" (Figure 3A) provided to the model consumer was determined. Stoichiometric studies suggest that N, relative to C, is a limiting resource for the production of marine zooplankton (Elser and Hassett, 1994). Hence, only C:N would be considered here. Following previous studies (Thomas and Krauss 1955; Kuyper et al., 2004a) I assume that N occurs only in proteins. Food C:N ratio was therefore calculated by simply dividing the protein fraction of total prey carbon (α_P) with protein C:N ratio (by mass), which was assumed to be 3.2 (Vollenweider 1985). Although this is a minimalist view of prey composition, it allows bounds to be set on the expected C:N stoichiometry based on the abundance of organic macromolecules (i.e. proteins, lipids, and carbohydrates).

To determine C and N limiting potentials (using equation 27), net utilisation efficiencies (U_i) were first estimated for each element by dividing element-specific growth rates with the difference between the rates with which each was assimilated and used for standard respiration (i.e. expanded equation 26). For C, growth, assimilation and respiration rates were simply the sum of the respective rates for the individual compounds. Total ingested N equalled I_c divided by food C:N ratio. N-specific assimilation, respiration and growth rates depended on that of protein. These were estimated by dividing the respective rates for protein by the assumed protein C:N ratio.

Figure 7 shows the results for food C:N ratios ≤ 35 . Results are not shown for food C:N > 35 as they were not significantly different from those for food C:N = 35, and in any case such high values are likely only in detritus.

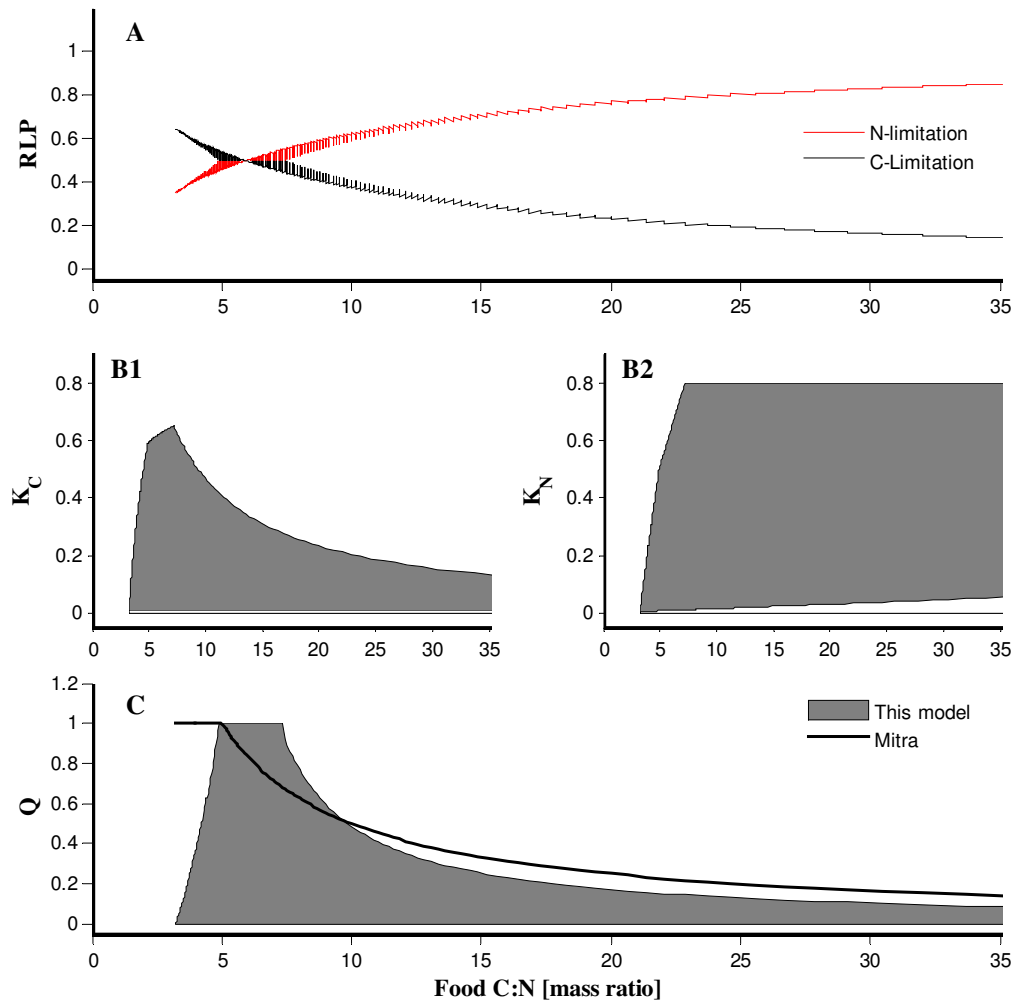


Figure 7. A: The influence of prey nutritional status on the relative limiting potentials, RLP, of C and N. $RLP = L_i / (L_C + L_N)$, with L_i being limiting potential of either C or N. See text for how food C:N content and L_i were calculated. Where $RLP = 0.5$ for both elements, food C:N indicate the threshold ratio for *Acartia* egg production. B: The effect of food C:N on gross growth efficiency for carbon (K_C) and nitrogen (K_N). In my model N-specific growth is dictated by that of protein, while that of C was influenced by every component of the food. Consequently, nutrient-specific gross growth efficiency varied with how food composition was distributed between different substances (i.e. $\alpha_P : \alpha_L : \alpha_H$). C: Comparison of this model with the stoichiometric approach to defining food quality exemplified by equation 1. For implementing equation 1, C:N ratio of 5.66 was used based on the assumed biochemical composition of adult *Acartia* (Table 1). This C:N ratio for *Acartia* tissues is comparable with what has been used in other studies (e.g. Touratier et al. 1999; Kuijper et al. 2004a; Mitra 2006)

C:N ratio of *Acartia* eggs was used. Based on the relative compositions of protein assumed for eggs (Table 1), a C:N ratio of 5.66 was calculated for *Acartia* egg. This is comparable to the ratio used in other models (Touratier et al. 1999; Kuijper et al. 2004a; Mitra 2006).

C limited egg production when food C:N was < 4.96 . Conversely, N limitation occurred at food C:N > 7.36 (Figure 7A). Hence, the balance of C and N sufficient for the consumer to achieve maximum growth (i.e. threshold elemental ratio, TER; Urabe and Watanabe 1992) lies between these two thresholds (Figure 7C). This is similar to the threshold C:N range of 3 to 5 determined for *Acartia* by Touratier et al. (Touratier et al. 1999).

On either side of the C:N threshold range, maximum growth and hence maximum food quality declined. This is consistent with the statement by Boersma and Elser (Boersma and Elser 2006) that the response of animals to changes in the elemental ratios in their food should be unimodal, with maximum growth only at the threshold elemental ratio. Such a response has been observed in a number of animals including insects (Frost and Elser 2002), gastropods (Elser et al. 2005), crustaceans (Plath and Boersma 2001) and fish (Vielma et al. 2002). Several explanations, including experimental artefacts, and changes in feeding behaviour (Dy Penaflores 1999; Tan et al. 2001; Plath and Boersma 2001), have been suggested for the phenomenon. As shown in Figure 7, nutritional imbalance alone could cause the phenomenon to occur when an animal has a rigid structural composition requirement for chemical substances. Although, I have not found any report of *Acartia* showing unimodal growth response to changes in food C:N ratios, the copepod grows poorly when its diet contains either excess C (Kjørboe 1989) or N (food C:N = 4.49) (Augustin and Boersma 2006). The results here are therefore consistent with the existing observation. In addition, they show that prey elemental ratios may be irrelevant for food quality due to macromolecular biochemical constraints on the utilisation of carbon (Figure 6). This is because unlike nitrogen and phosphorus that dominantly occur in only few compounds, carbon occurs in every organic compound. Therefore, the impact of C on consumers' fitness (here egg production efficiency) may be dictated by how it is distributed between both the supply (by prey) of, as well the demand (by consumers) for different compound. While this is widely recognized, it has hitherto not been explicitly considered in most models, including studies that incorporate the molecular biochemical characteristics of food constituents (Touratier et al. 1999; Anderson et al. 2004b; Anderson and Hessen 2005).

Stoichiometric elemental based food quality models incorporate respiratory physiology of zooplankton in different ways (Anderson et al. 2005; Anderson and Hessen

2005). These studies serve as the basis for the framework I have suggested here. However, my approach has important differences that must be emphasized. First, it distinguishes between structural biochemical requirements of female copepods from that of eggs production. The justification is that eggs need specific compounds, such as polyunsaturated lipids (Xu et al. 1994, 1993), for development and so their biochemical constituents may differ from that of adults (see Koski 1999 for the C:N differences between *Acartia* female and egg tissues). Furthermore, some non-growth processes in adults require specific compounds, such as lipids for cold temperature acclimation (Farkas 1979; Nanton and Castell 1999), and amino acids for osmolarity regulation (Bryant et al. 1975; Anger 1998; Hochachka and Somero 2002). Where the supplies of these compounds are inadequate, the non-growth requirement of the consumer could impact egg production (see chapter 4 of this thesis). Distinguishing between the biochemical requirements for adult and egg tissues therefore makes this model more realistic. The second difference between my model and that of Anderson et al. is that my model does not (but Anderson et al. do) treat consumers' structural demand for carbon as a "unitary requirement". Rather, it discriminates among the biochemical forms of C required by animals. Finally, the utilization of carbon in my model follows three different macromolecular pathways, i.e., protein, lipid and carbohydrate utilization pathways. Conversely, in previous models, two pathways for carbon utilization were considered. These were protein and non-protein pathways (Anderson et al. 2005). Furthermore, in previous models, the quantity of nutrient excreted has either been fixed (as done for phosphorus by Anderson and Hessen 2005) or respiration substrates are restricted to lipid and carbohydrates (Anderson et al. 2005). Here, the phenomenon of substrate sparing, known mainly for proteins (e.g. McGoogan and Gatlin 1999; Arnould et al., 2001), was applied to any compound supplied below consumer's structural composition requirement (β_i and δ_i) in order to maximize growth. In other words, the model consumer was allowed to consider the fitness costs for respiring individual food components (*sensu* Simpson and Raubenheimer 1995) in its prey evaluation scheme. Moreover, by restricting respiration to excess prey constituents, it was ensured that fitness (her growth) gains that could be derived from nutritional excesses were factored into the prey evaluation scheme of the consumer.

The significance of these differences can be appreciated by comparing the results of this model (Figure 7B) with Figures 2C and 2D of Anderson and Hessen (2005; not shown here). My model predicts a reduction in the growth of copepod once a diet becomes nutritionally imbalanced, irrespective of the food component responsible for the imbalance. As a result, nutrient-specific gross growth efficiency (K_C and K_N) could be lower than their

respective maximums when food C:N \neq TER. This occurred even when a nutrient was limiting in the diet. This is consistent with current knowledge (Boersma and Elser 2006). However, despite its reasonable parameterisation of physiology, the model of Anderson and Hessen (2005) does not predict a decline in carbon gross growth efficiency when food composition is $<$ TER. In this regard, the behaviour of their model is comparable with that of other food quality models that evaluate prey organisms based on a simple elemental stoichiometric disparity between consumers and their prey as given by equation 1 (e.g. Mitra et al. 2003; Mitra 2006; Flynn and Mitra 2009).

Figure 7C shows how this approach for evaluating potential prey organisms compares with the one I have suggested. Both predict a decline in food quality when food C:N is greater than TER. However, unlike my model, the stoichiometric approach assigns food quality values of 1 to all diets with C:N-ratios \leq TER. In other words, the approach forces model consumers to “perceive”, and probably respond, similarly to all potential prey with C:N ratios \leq TER. This may not be an issue when such models are applied in ecosystems in low and medium latitude waters as copepods inhabiting these systems have low lipid content, and so tend to generally have C:N ratios that may be lower than that of seston (Bämstedt 1986). However, the applicability of the simple elemental stoichiometric disparity approach to describing food quality may be of limited in higher latitude ecosystems. This is because high-latitude copepods have high lipid reserves (Tande 1982; Gronvik and Hopkins 1984; Reinhardt and Van Vleet 1986), and so tend to have C:N ratios that could be as high as 15 (Bämstedt 1986). For such copepods, a reliance on the stoichiometric approach for prey evaluation would make them incapable of differentiating between most prey organisms, considering that the natural uppermost C:N ratio is \sim 17 for algae (Geider and Roche 2002). In contrast, the approach suggested here allows realistic adaptation of food quality to prey biochemical composition (Figures 3 and 7). It is therefore recommended as an alternative to the current approach to describing food quality in ecosystem models.

Conclusion

Ultimately, the structure and functioning of marine ecosystems is defined by the transfer of autotrophic production to higher trophic levels and selective consumption of these autotrophs by predators. Hence, feeding regulation via modification of grazing and food incorporation by predators is critical for understanding and predicting the dynamics of ecosystems. However, attempt to integrate such functional responses into marine ecosystem and biogeochemical models suffer from a lack of simple mechanistic framework describing

how consumers determine the nutritional value of prey items before being consumption. To address this issue, I provide a mathematical framework with food quality being contingent upon molecular biochemical requirements, as well as the physiology and food uptake capabilities of consumers. The model realistically captures metabolic costs for the consumption of excess nutrients. It also illustrates that prey elemental ratios (here C:N) may be irrelevant for the growth of consumers due to macromolecular biochemical constraints on the utilisation of chemical elements.

Finally, it is worth commenting on the parameterisation of the model. The costs for maintenance and a unit growth were assumed to be constant, dictated only by abiotic conditions, but independent of the available food. They were calculated based on O₂-consumption by animals. At the cellular level, respiration is driven by both energy demand and the supply of substrate. O₂-consumption rate can therefore vary with the respiratory substrates (Kiørboe et al. 1985). The parameterisation of such process goes beyond the scope of the present study, thus the assumption of constant b and d .

Feeding-related energy costs such as those associated with food ingestion and assimilation was not explicitly represented in the model. However, this was not expected to affect the model results as this is captured in the calculation of the cost for growth. Moreover, the cost for feeding by *Acartia* is generally low, being just about ~1 % of total SDA (specific dynamic action; Kiørboe et al. 1985). Hence, the inclusion of feeding cost in the model parameterisation could have little impact on the general predictions of the model.

Naturally, food availability varies both in space and time. The maximum rate for food ingestion (I_c) may thus vary with food availability, being high when food is abundant and low at low food densities (Tirelli and Mayzaud 2005). Furthermore, λ_i varies with food availability (Tirelli and Mayzaud 2005). Thus the maximum feeding capacity of the consumer could be impacted by food availability. Here, this is not considered because maximum food availability was assumed.

Furthermore, food ingestion and assimilation varies with prey type (see Besiktepe and Dam 2002). For example in algae, carbohydrate occurs in structural and so-called “reserve” forms, which are assimilated at different efficiencies by copepods (Head 1992; Anderson 1994) thereby impacting the metabolic availability of the compound. In my approach, prey structural or reserve components were not considered. Rather, the “artificial food environment” from which the modelled consumer preyed (Figure 3A) was designed to examine the effect of substrate availability, independent of prey type or form of the substrate.

Animals can avoid nutritional excesses by balancing how they assimilate substrates from ingested materials against their requirements (For example see DeMott et al. 1998). This can be achieved by regulating digestive enzymatic activity (Landry 1983; Hassett and Landry et al. 1984; Darchambeau 2005) and/or the retention time of food in the gut (Santer and Van Den Bosch 1994; Darchambeau 2005;). Here, I followed established method (e.g. Anderson et al. 2005) of relying on the catabolic physiology of animals to ensure the removal of excess chemical substances. Results of several experimental studies support this approach (Darchambeau et al. 2003; Jansen et al. 2006; Jansen and Hessen 2007). Moreover, the approach lays the foundation upon which the model could be improved to allow consumers the option of either storing excess assimilates or using them as substrates for biosynthesis.

Furthermore, β_i and δ_i may not be constant as assumed in the model. In spite of the many similar assumptions in the literature (e.g. Touratier et al. 1999; Anderson and Hessen 2005; Anderson et al. 2005), the evidence is inconclusive. Most studies investigate the elemental composition of zooplankton and the data show variations in the C:N ratio of marine zooplankton (e.g. Tande 1982). As well, differences in the protein composition of eggs produced by *Acartia* under different food concentrations have been observed (see appendix 3 of this thesis). On the other hand, reports of zooplankton maintaining a constant chemical composition in the face of changing environmental conditions exist (e.g. Hessen 1990; Sterner et al. 1993; Sterner and Schulz 1998). Whether zooplankton maintain a rigid chemical composition or not, a biochemical composition that maximizes fitness (e.g. viable egg production) under specific ambient conditions is plausible. The assumed constant values of β_i and δ_i are my attempt at representing this occurrence in nature.

Though evidently simplistic, the assumptions herein are necessary to allow the potential full impact of prey's nutritional composition on the consumer to be modelled. The focus here was to develop a mathematical formulation for Q based on consumers' requirements (i.e. b , d , β_i , and δ_i) as well as food uptake and physiological capabilities (i.e. I_c , λ_i , ρ_i , and γ_i) for which parameterisations can readily be obtained. These variables are known to be influenced by habitat conditions such as temperature (Gaudy et al. 2000; Isla et al. 2008), salinity (Biagini et al. 2000) and food availability (Appendix 3 of this thesis). Therefore, further work is needed to explicitly represent habitat conditions critical for the survival and reproduction of consumers within my model to enable Q to be adaptive to the habitat specific needs of consumers (see chapter 4 for temperature effect on Q).

Chapter 3

Egg production by calanoid copepods: limitation by nitrogen or carbon?

3.1 ABSTRACT

It is well known that egg production of calanoid copepods correlates with carbon (C) and nitrogen (N) contents of algae. However, resolution of which of these elements actually limits copepod egg production is still being debated. Here, this issue has been re-examined using a new model and egg production by *Acartia tonsa* as a test case. The model incorporates stage-specific structural demand for chemical substances by animals, discriminates among the required macromolecular forms of C, and considers food quality dependent regulation of food ingestion, assimilation and metabolism. In comparison with literature data, the model realistically predicts the rate of egg production, as well as C- and N-specific gross growth efficiencies of egg production by *Acartia* in response to changes in algal C:N. Consistent with experimental observation, N assimilation efficiency was predicted to be high, at ~0.9 on average. Consequently, most algal N could be integrated into (i) new herbivore biomass (via egg production) that could be transferred to higher trophic levels via secondary consumption or (ii) metabolic by-products such as ammonia that could be re-assimilated by phytoplankton. Conversely, C assimilation efficiency decreased from 0.66 to 0.16 with increasing algal C:N. This suggests that, more algal C may be potentially transferred out of the euphotic epipelagic zone as faecal pellet, especially when algal C:N is high (i.e. poor quality food), as part of marine snow if faecal pellets are not re-ingested or broken up by other organisms. My results suggest that carbon predominantly limits zooplankton production due partly to the low metabolic availability of C associated with algal cellulose and structural carbohydrates. This finding contradicts previous predictions that N, rather than C limits the growth of marine zooplankton.

3.2 INTRODUCTION

The emergence of the branch of ecosystems ecology entitled “ecological stoichiometry” and described as the “biology of elements” (Sterner and Elser 2002) has reinstated the importance of the balance of chemical elements within organisms and their environment (Reiners 1986; Elser 2006). Thus, with the exception of very few examples (Anderson and Pond 2000), models for studying the effect of chemical elements on zooplankton production typically divide the chemical constituents of both prey and predators into two major categories: carbon (C; for both structure and energy) and nutrient elements (mostly nitrogen, N, and/or phosphorus, P; Elser and Hassett 1994; Touratier et al. 1999; Hessen et al. 2004). They then determine the limiting element for predator

growth through comparison of the ratios of C and nutrient elements in consumer and prey, often taking into consideration element-specific utilization efficiencies.

By employing stoichiometric arguments, some researchers have suggested that the production of marine copepods is limited by inadequate dietary supply of N (Elser and Hassett 1994; Touratier et al. 1999). This is based in part on observations demonstrating that the carbon to nitrogen ratio of zooplankton biomass is lower than that of seston (Redfield et al. 1966; Elser and Hassett 1994). Further cited evidence for N-limitation comes from experiments that show that the rates of grazing (Checkley and Entzeroth 1985; Houde and Roman 1987; Cowles et al. 1988) and reproduction (Checkley 1980; Kiørboe 1989) by marine copepods are directly correlated with the amount of food N.

However, marine copepods generally convert about 40% of the N they ingest into eggs, even when they feed on N-limited diet (Checkley 1980; Kiørboe 1989). For instance, *Acartia tonsa* converts a mere 8% of the N it ingests into production throughout its life cycle (Jones et al. 2002). This low gross growth efficiency for N contrasts strongly with the high efficiency one would expect, at least in theory, for a growth-limiting substrate (e.g. Anderson and Pond 2000). Also, it is not in keeping with experimental observations demonstrating that the utilization efficiency for growth-limiting chemicals is high among zooplankton (e.g. DeMott et al. 1998).

Consequently, Anderson and Hessen (1995) challenged the view of N as a dominant limiting resource for the production of marine copepods based on results from a theoretical model. Their model predicted that the minimum food C:N ratio (in moles) which allows for the complete utilization of N by copepod is above 10. This threshold food C:N ratio is lower than the C:N ratio of typical prey items available to and consumed by marine copepods (Geider and Roche 2002). For this reason, Anderson and Hessen (1995) concluded that marine copepods should commonly be limited by C, rather than by N. This assertion is supported by experimental data demonstrating that marine copepods produce more eggs when feeding on relatively C-rich prey (C:N ratio 10–15) than when feeding on prey with C:N substantially less than 10 (Boersma and Elser 2006). Furthermore, some N-deficient algae may also be rich in cellulosic and structural carbon (Van Donk et al. 1997; Alderkamp et al. 2007), which could reduce carbon assimilation efficiency (Reinfelder and Fisher 1991; Van Donk and Hessen 1995) and thereby enhance C-limitation of zooplankton grazing on nutrient-deplete algae. Evidence also suggests that biochemical substances that may co-vary with N may limit the production of marine copepods. As an example, a number of experiments have demonstrated correlations between copepod growth (including egg production) and prey content of the amino acid arginine, and the phospholipids 20:5(n-3) (eicosapentaenoic acid, EPA) and 22:6(n-3) (docosahexaenoic acid, DHA) (Kleppel et al. 1998; Klein Breteler et al. 2005). These

constituents have been termed essential biochemicals for copepod production, as they are required for vital physiological processes (e.g. membrane biosynthesis), but are not or are inefficiently synthesized by most zooplankton (Müller-Navarra 2008). Hence, inadequate supply of essential biochemical substances may limit copepod production (Anderson and Ponds 2000) and thereby constrain the limiting impact of elemental N (and that of other chemical elements) on copepod productions.

However, the model of Anderson and Hessen (1995) predicted that nitrogen gross growth efficiency (N GGE) for egg production by copepods increases with increase in food C:N. This is contrary to experimental data demonstrating that nitrogen gross growth efficiency for egg production is nearly constant and independent of food C:N (Checkley 1980; Kiørboe 1989; cf. Jones et al. 2002 which shows a decrease in N GGE for copepod population with a decline in prey N:C).

Therefore, the suggestion that N may not limit the production of marine copepods was re-evaluated and refuted by subsequent models that could realistically reproduce the observed relationship between food C:N and nitrogen-specific growth of copepods (e.g. Touratier 1999; Kuyper et al. 2004). These authors argue that the high structural demand for protein by marine copepods makes it more likely for N (derived predominantly from prey protein; Geider and Roche 2002) to limit copepod production. Indeed, limitation by N is believed to be widespread in the animal kingdom because of the high structural demand for protein by animals (White 1983).

Within the zooplankton community, laboratory measurements indicate that protein constitutes the major structural component (~64 – 24 %) of calanoid copepods (Ventura 2006); it constitutes above 55% (on average) of the eggs produced by marine copepods (Guisande and Harris 1995; Drillet et al. 2008; appendix 3 of this thesis). Furthermore, results from experimental studies suggest that protein biosynthesis is the dominant energy consuming process involved in new biomass formation by copepods (Whiteley et al. 1996; Thor 2000) and could alone account for over 80% of the total energy needed for egg production by copepods such as *Acartia tonsa* (Kiørboe et al. 1985), *Calanus finmarchicus* (Bämstedt et al. 1999). Thence, the structural demand for protein, and thus N, by the copepods would appear to support the argument of possibility of N-limitation within the marine copepod populations.

However, there are good reasons for one to also conjecture that high structural demand for protein by marine copepods could also make N-limitation of the animals unlikely. This is because results from experimental studies suggest that copepods are endowed (arguably by natural selection) with several behavioural strategies for satisfying their high structural demand for protein. First, they can preferentially ingest protein-rich (or low C:N) food or modify grazing rate to

optimise protein intake (Houde and Roman 1987; Cowles et al. 1988; Cruz-Rivera and Hay 2000). Secondly, they are more efficient at assimilating protein, and hence N from the food they ingest for metabolism. For instance, copepods have been found to assimilate as much as 90% of the nitrogen from the ingested prey (Landry et al. 1984; Hassett and Landry 1988). In addition, copepods can alter the efficiency at which they assimilate ingested chemicals (Logan et al. 2004), thus optimising the metabolic availability of nutrients needed for vital life processes. Also, copepods like many other animals can preferentially catabolise other compounds (e.g. carbohydrates) for energy and thus spare protein for growth when dietary supply of protein is low (Roman 1983; Anderson 1992; McGoogan and Gatlin 1999; Arnould et al. 2001). Given the above repertoires of acclimative behaviour and physiologies, and the apparent “wasting” of N by marine copepods, one could question whether marine copepods are adapted to living in N-poor environment and therefore N-limitation of their production is unlikely?

In chapter 2 of this thesis, a generic multi-substrate food quality model (FQM) was developed with simple but biologically realistic parameters for investigating the influence of food biochemical substances on the growth of zooplankton. The model incorporates reasonable parameterisation of animals’ metabolic physiology and, unlike previous models (e.g. Anderson and Hessen (1995), captures the growth impact of any nutritional imbalance in consumers’ diet. However, FQM determines food uptake rate by using constant parameters that reflect animals’ maximum capacity for food ingestion and assimilation. It therefore predicts only the growth-limiting potentials, and not actual limitation by individual food chemical constituents, as it negates animals’ ability to mitigate or avoid dietary biochemical limitation via feeding regulation (cf. stoichiometric modulation of predation, Mitra and Flynn, 2005). So the aim of this study is to demonstrate the usefulness of FQM by integrating it into a secondary production model modified from that of Mitra (2006), to (i) determine the actual limiting impact of food C and N on the growth of marine copepods, and (ii) examine the relationship between prey nutritional status and the metabolic fate chemical elements ingested by the animals.

Here, the model consumer evaluates the growth-limiting potentials of food biochemical constituents based on its specific requirements (both energy and structural) and maximum capacity for food uptake and metabolic physiology by employing FQM prior to food consumption. This information then dictates consumer’s response, in terms of food ingestion, assimilation and metabolism, to the available prey. Actual growth limitations by elements are assessed only when growth ceases due to biochemical imbalance(s) in consumer’s diet. So unlike previous models (Anderson and Hessen 1995; Touratier 1999; Kuyper et al. 2004), food ingestion rate and assimilation efficiency in this study are not constant, but vary with the composition of the food.

Table 1. Model Parameters. See section 3.4 for details on how parameter values were determined.

Parameter	Description	Unit	Value	Source
I_{mg}	Food ingestion rate to satisfy both maintenance and maximum growth	$\text{gC} \cdot \text{gC}^{-1} \cdot \text{d}^{-1}$	6.88*	Besiktepe & Dam 2002
I_m	Food ingestion rate to satisfy only maintenance	$\text{gC} \cdot \text{gC}^{-1} \cdot \text{d}^{-1}$	b	Fitted
w_{mg}	Food capture efficiency for grazing to satisfy both maintenance and maximum growth	$\text{L} \cdot \text{gC}^{-1}$	2500	Støttrup & Jansen 1990
w_m	Food capture efficiency for grazing to satisfy only maintenance	$\text{L} \cdot \text{gC}^{-1}$	b	Fitted
λ_{mg}	Food assimilation efficiency to satisfy both maintenance and maximum growth	dl	0.95*	Besiktepe & Dam 2002
λ_m	Food assimilation efficiency to satisfy only maintenance	dl	b	Fitted
b	The cost for maintenance	$\text{J} \cdot \text{gC}^{-1} \cdot \text{d}^{-1}$	2755.10	Estimated
β_p	Protein-specific structural requirement for maintenance	$\text{gC} \cdot \text{gC}^{-1}$	0.7269	Estimated
β_L	Lipid-specific structural requirement for maintenance	$\text{gC} \cdot \text{gC}^{-1}$	0.2363	Estimated
β_H	Carbohydrate-specific structural requirement for maintenance	$\text{gC} \cdot \text{gC}^{-1}$	0.0367	Estimated
δ_p	Protein-specific structural requirement for growth	$\text{gC} \cdot \text{gC}^{-1}$	0.5478	Estimated
δ_L	Lipid-specific structural requirement for growth	$\text{gC} \cdot \text{gC}^{-1}$	0.4404	Estimated
δ_H	Carbohydrate-specific structural requirement for growth	$\text{gC} \cdot \text{gC}^{-1}$	0.0117	Estimated
E_p	Energy content of protein	$\text{J} \cdot \text{gC}^{-1}$	32312.64	Estimated
E_L	Energy content of lipid	$\text{J} \cdot \text{gC}^{-1}$	51439.43	Estimated
E_H	Energy content of carbohydrate	$\text{J} \cdot \text{gC}^{-1}$	42922.90	Estimated
d	Energy cost for growth	$\text{J} \cdot \text{gC}^{-1}$	11228.77	Estimated

dl = dimensionless. * Values for determining food quality.

^bSee Table 3 for values used in the simulation

Furthermore, energy production by the modelled copepod is not dependent on fixed substrates. Rather, substances are respired based on the balance between their availability and the requirements (both energy and structural) of consumers. This is in keeping with findings demonstrating that zooplankton exhibit plastic preference for respiratory substrate (Roman, 1983). Consequently, the limiting impact of chemical elements on growth of copepods occurs via the different compound utilisation pathways (see also chapter 2 of this thesis).

3.3 MODEL DESCRIPTION

Overview

In nature, elemental compositions of organisms are a reflection of their compound constituents. This is true in both algae (Geider and Roche, 2002) and zooplankton (Ventura 2006). Therefore here, only compound constituents of organisms are considered. All substances are assumed to belong to one of three compound groups: proteins (P), lipids (L) and carbohydrates (H). There are very few experimental studies describing the effect of food C:N ratio on copepod production that yields data suitable for model parameterization and verification. As a result, earlier models investigating the impact of limiting nutrients on marine copepod growth and production (e.g. Anderson and Hessen 1995; Touratier 1999; Kuijper et al. 2004) have typically been fitted to the *Acartia tonsa* egg production data of Kiørboe (1989). For my results to be comparable, the model presented here would be subjected to the same test. I, therefore, use *Acartia tonsa* as my model organism and assume egg production to be the only form of growth. This assumption is consistent with the fact that *Acartia* adults do not undergo significant structural growth (Miller et al. 1977).

The model distinguishes between the biochemical compositions of adults and eggs in keeping with experimental data demonstrating that structural biochemical requirement of zooplankton varies with their stage of development (Evjemo et al., 2003; Bruce et al., 2005). In the model, the relative composition (by C) of individual chemical substances in adults, β_i , and eggs, δ_i , are assumed fixed at specific ambient conditions. This is because the data against which the model is to be tested comes from experiments conducted under constant ambient conditions that may not necessitate structural biochemical changes within animals. However, if required, both β_i and δ_i can be made a function of ambient conditions such as temperature (see chapter 4 of this

thesis). So for an animal requiring i to n different compounds, $\sum_{i=1}^n \beta_i = 1$ and $\sum_{i=1}^n \delta_i = 1$.

Table 2. Model variables.

Variable	Description	Unit
α_i	Compound-specific composition of prey carbon	gC.gC^{-1}
Z_{ing}	Slope or intensity of prey ingestion response to food quality	$\text{gC.gC}^{-1}.\text{d}^{-1}$
Z_{ceff}	Slope or intensity of prey capture efficiency response to food quality	L.gC^{-1}
I_{td}	Maximum food ingestion threshold	$\text{gC.gC}^{-1}.\text{d}^{-1}$
I_c	Actual food ingestion rate	$\text{gC.gC}^{-1}.\text{d}^{-1}$
η	Half saturation constant for substrate assimilation	dl
λ_i	Compound-specific assimilation efficiency	dl
ρ_i	Fraction of chemical-specific assimilate catabolised for energy to power maintenance	dl
γ_i	Fraction of chemical-specific assimilate catabolised for energy to power growth	dl
x_i	Excess fraction of chemical-specific assimilate	dl
F_c	Total carbon assimilation	$\text{gC.gC}^{-1}.\text{d}^{-1}$
F_c	Total carbon egestion	$\text{gC.gC}^{-1}.\text{d}^{-1}$
m_c	Total carbon catabolism for energy to power maintenance	$\text{gC.gC}^{-1}.\text{d}^{-1}$
g_c	Total carbon catabolism for energy to power growth	$\text{gC.gC}^{-1}.\text{d}^{-1}$
X_c	Total excess carbon	$\text{gC.gC}^{-1}.\text{d}^{-1}$
G	C-specific growth rate	$\text{gC.gC}^{-1}.\text{d}^{-1}$
K_c	C-specific gross growth efficiency	gC.gC^{-1}
U_i	Substrate-specific net utilisation efficiency	gC.gC^{-1}
L_i	Substrate-specific potential to limit growth	dl
Q	Food quality	dl

dl = dimensionless

For the simulations, two main metabolic processes are considered, these being maintenance and growth metabolism (Anderson et al., 2005). Energy requirements for these processes (b for maintenance, and d for producing an egg) are fixed, as expected based on the design (e.g., constant temperature, food supply) of the experiment against which the model is to be tested. Within the model, animals' requirements for maintenance are satisfied first, with subsequent growth possible

only when there are extra resources available for egg production. Thus growth in the model can be described using equation 1, where food ingestion minus voiding equals assimilation:

$$\text{Growth} = (\text{food ingestion} - \text{food voiding}) - \text{maintenance budget} \quad (1)$$

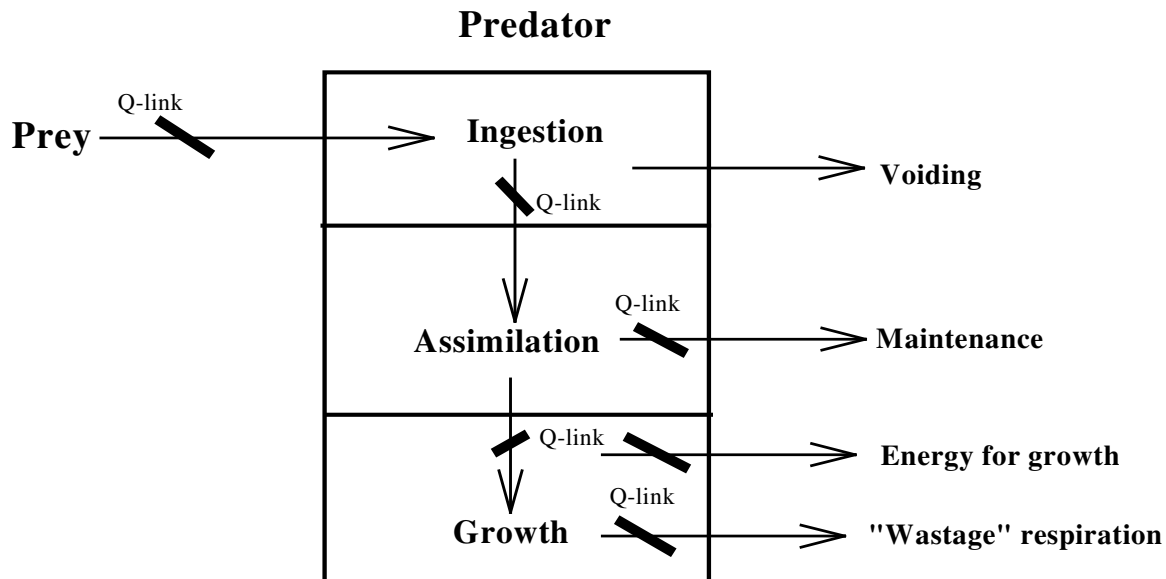


Figure 1. Conceptual scheme for the regulation of food uptake and utilization based on food quality (Q-link). Prey are ingested, followed by assimilation and growth with loss of substrates through voiding and respiration. The rate for food consumption and utilization becomes constant and independent of food quality when the link to food quality Q-link is disabled. Figure modified from Mitra (2006). Conceptual scheme for the metabolic fate of compounds ingested by consumers is the same as described within Figure 1 of chapter 1.

Figure 1 is a schematic representation of the model. Model parameters and variables are listed in tables 1 and 2 respectively. Copepods have been observed to discriminate and selectively ingest prey organisms based on their chemical composition (Roman, 1984; Cowles et al., 1988; Jones and Flynn, 2005). It has therefore been assumed that prior to grazing, zooplankton are able to evaluate the nutritional quality of their prey (i.e. food quality determination) by weighing their chemical requirements and instantaneous (maximum) capacity for metabolism against the constituents of the available prey. Via this approach, the model consumer determines the specific limiting potential of individual constituents of the prey and assigns a quality value (i.e. food quality) to it. This then becomes the basis upon which grazing rate (I_c), assimilation efficiency for different compounds (λ_i), and metabolism are regulated in order to satisfy both the energy (i.e., b

and d) and structural (i.e., β_i and δ_i) requirements of the consumer. Further arguments for the model structure are provided in the following sections.

3.3.1 Food quality

As discussed above, zooplankton can and do alter the rate of food ingestion, assimilation and metabolism in order to obtain the required balance of nutrients for optimal growth (Roman, 1983; Cruz-Rivera and Hay, 2000; Logan et al., 2004; Tirelli and Mayzaud, 2005). This capability is an emergent property of evolution and as a result is difficult, if not impossible, to fully parameterised. Currently in models for investigating biochemical control of zooplankton production (e.g. Mitra, 2006; Flynn and Mitra, 2009), the basis for feeding regulation by model animals is food quality (Q). Similar approach is employed in this study to simulate acclimation of feeding to prey biochemical composition. However, unlike previous models (e.g. Mitra 2006), food quality here does not describe elemental stoichiometric disparity between consumers and their prey. This is because of the significant shortfalls associated with the approach (these have been discussed within chapters 1 and 2 of this thesis). Rather here, food quality describes the potential growth consequence for feeding on specific prey items. It was determined using the new food quality model (FQM) proposed within chapter 2 of this thesis. For readers interested in only this section of the thesis, a brief description of FQM is provided below.

FQM uses the net efficiency at which a prey biomass is converted into that of a consumer as the yardstick for food quality. It distinguishes between stage-specific structural biochemical requirements of consumers in keeping with experimental observation (Evjemo et al. 2003; Brucet et al. 2005). It also does not treat consumers' structural demand for carbon as a "unitary requirement" but discriminate among the needed biochemical forms of carbon. This is critical for a realistic determination of carbon impact on copepod growth, because unlike nitrogen that dominantly occurs in only few compounds (mainly proteins – Geider and Roche 2002), carbon occurs in diverse and perhaps functionally different macromolecules. As a result of this assumption, energy production by consumers within FQM is not dependent on fixed substrates. Rather, substances are respiration based on the balance between their availability and the requirements (both energy and structural) of consumers. This is in keeping with experimental data demonstrating that zooplankton exhibit plastic preference for respiratory substrate (Roman 1983; Anderson 1992). FQM incorporates animals' biochemical budget for both maintenance (i.e., all processes not associated with new biomass production) and growth, and considers the fact that animals can derive fitness gain(s) from food resources occurring in excess of their structural requirement by preferentially catabolising such substances for energy (Anderson and Hessen 2008).

So for an animal accessing a prey containing i to n different macromolecules, FQM determines potential utilisation efficiency (U_i , dimensionless) for each chemical substance, their respective potential to limit the growth of the animal (L_i , dimensionless) and the quality (Q , dimensionless) of the food quality by using equation 2, 3 and 4 respectively:

$$U_i = \frac{G\delta_i}{I_{mg}\lambda_{mg}\alpha_i(1-\rho_i)(1-\gamma_i)} \quad (2)$$

$$L_i = \min(U_i, U_j, \dots, U_n, 1) \quad (3)$$

$$Q = \min(L_i, L_j, \dots, L_n, 1) \quad (4)$$

Where, α_i is chemical-specific composition (in terms of carbon) of a prey item, I_{mg} denotes maximum food ingestion rate required for both maintenance and maximum growth by a consumer, λ_{mg} is maximum food assimilation efficiency needed for satisfying the same requirements, ρ_i represents the fraction of each assimilate that could be used for maintenance, while γ_i specifies the fraction of each assimilate that could be respired for energy to power growth rate (G) without adversely affecting consumers' structural composition requirement (δ_i) for each chemical. The product of I_{mg} and λ_{mg} describes maximum feeding rate required for both maintenance and maximum growth by consumers. These are fixed parameters, independent of food composition as in previous numerical analyses of food quality (Anderson et al. 2005). This approach disables the animals' ability to modulate the cost-benefit for processing different food items via feeding regulation (e.g. DeMott et al. 1998), thus making the quality of different prey items comparable. The parameters ρ_i , γ_i and G are variables that depend on the balance between substrate supply and the requirement (both energy and structural) of consumers. So within FQM, only substances supplied above the structural biochemical needs of consumers are used for maintenance (with $0 < \rho_i < 1$) and/or respired (with $0 < \gamma_i < 1$) for energy to power growth. Conversely, substances occurring below the structural requirement of consumers are spared (with $\rho_i = 0$; $\gamma_i = 0$) for growth. Thus within FQM, food quality is dependent on the interplay between prey biochemical composition and the metabolic physiology of the consumers. Consequently, when $Q = 1$ (\equiv good quality food), a consumer could achieve maximum net use efficiency (of 100%, which is possible only theoretically) for each ingested chemical. On the other hand, when $Q = 0$ (\equiv bad quality food) then a prey has no nutritional value for growth of the consumer. This is because in the absence of alternative prey, the consumption of poor quality food does not translate into growth (see Jones and Flynn 2005), as acquired resources would be utilized for only maintenance to ensure

survival. After assigning food quality values to individual prey, the modelled consumer regulates food ingestion, assimilation, and metabolism in order to mitigate the impact of bad quality food or maximize gains from good quality food.

3.3.2 Food quality effect on food consumption and metabolic physiology

Food ingestion

There is a food concentration threshold below which grazing does not occur in zooplankton (Strom et al. 2000). Above this threshold, the functional form of zooplankton grazing is diverse (see review by Gentleman et al. 2003). Furthermore, zooplankton exhibit several types of grazing response to changes in the quality of food (Mitra and Flynn 2005). Observations however indicate that both prey capture efficiency (Poulet and Marsot 1978) and maximum food ingestion (Houde and Roman 1987; Tirelli and Mayzaud 2005) depend on prey nutritional status. Food ingestion rate (I_c , $\text{gC.gC}^{-1}.\text{d}^{-1}$) is therefore assumed to depend on the prey capture efficiency (w , L.gC^{-1}) and maximum food ingestion threshold (I_{td} , $\text{gC.gC}^{-1}.\text{d}^{-1}$) according to Ivlev (1955):

$$I_c = I_{td} * \left(1 - \exp^{-w*V}\right) \quad (5)$$

Where, V is prey concentration (gC.L^{-1}). I then incorporate feeding regulation by assuming that both w and I_{td} increases with the quality of food; this is akin to the optimum behaviour approach of Mariani and Visser (2010). So within the model, the threshold for food ingestion equals maximum food ingestion rate needed for both maintenance and maximum growth only when food quality is good (i.e. $I_{td} = I_{mg}$ when $Q = 1$); else, I_{td} varies with food quality as described by equation 6:

$$I_{td} = I_m + (I_{mg} - I_m) * Q^{Z_{ing}} \quad (6)$$

Where, I_m ($\text{gC.gC}^{-1}.\text{day}^{-1}$) is the threshold for food ingestion required for grazing to satisfy only maintenance, and Z_{ing} (dimensionless) defines the slope (or intensity) of the response to food quality (Figure 2A). Setting $I_m = I_{mg}$ makes grazing independent of food quality.

Similarly, prey capture efficiency, w , was configured to vary between a minimum value required for grazing to satisfy only maintenance, and a maximum needed for grazing to satisfy both maintenance and maximum growth requirements of the consumer using equation 7:

$$w = w_{mg} + (w_{mg} - w_m) * Q^{Z_{ce}} \quad (7)$$

where, w_m is the minimum prey capture efficiency required for grazing to satisfy only maintenance, and w_{mg} represents prey capture efficiency for maximum food ingestion to satisfy

both maintenance and maximum growth. Z_{ce} defines the intensity of prey capture response to food quality. Setting $w_m = w_{mg}$ makes prey capture efficiency independent of food quality.

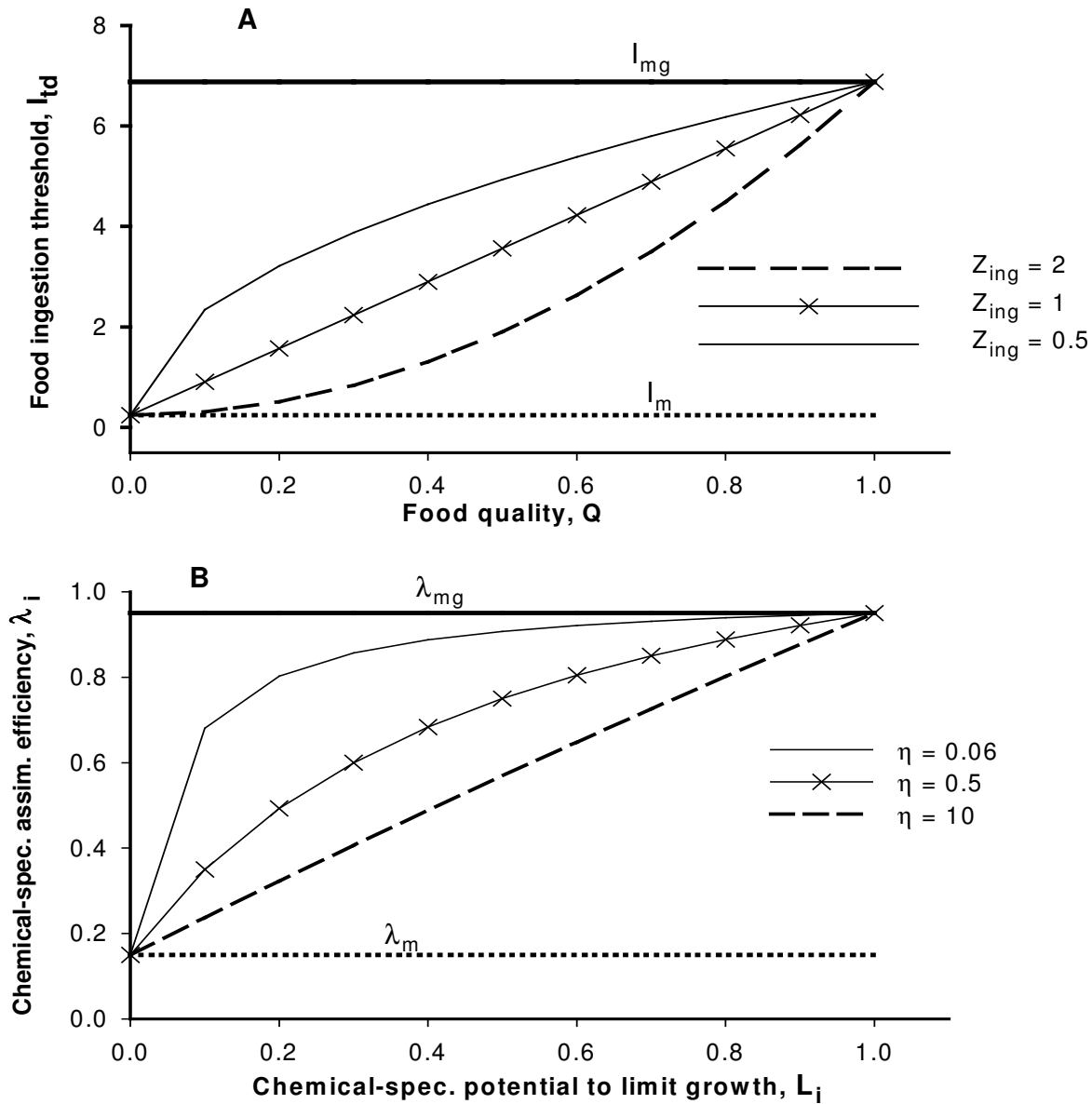


Figure 2. The effect of auxiliary variables (Z_{ing} and η) on the functional form of (A) grazing behaviour, and (B) substrate-specific assimilation efficiency. I_{mg} and λ_{mg} respectively represent food ingestion rate and assimilation efficiency needed for maintenance and maximum growth. Conversely, I_m and λ_m respectively represent food ingestion rate and assimilation efficiency required for only maintenance. Response is shown for prey capture efficiency because the nature of the response is similar to that of ingestion threshold. See text associated with equations 6 and 8 for further explanation

Food assimilation

There are two major approaches for modelling substrate assimilation by heterotrophic consumers. One is based on ecological stoichiometry (e.g. Mitra, 2006), and the other is based on regulatory physiology (e.g. Anderson et al., 2005). In terms of the former approach, it is assumed that assimilation of substrates from ingested matter proceeds according to animals' own structural requirements for chemical substances. This is supported by observation that animals can decrease the efficiency with which they assimilate C whereas keeping that of X constant, when prey C:X exceeds animals' structural composition requirement (see for example DeMott et al., 1998). This could be achieved by regulating the activity of digestive enzymes (Darchambeau, 2005; Landry et al., 1984; Hassett and Landry, 1983) and/or the gut retention time of food particles (Darchambeau, 2005; Santer and Van Den Bosch, 1994). This approach may enable animals to limit the cost for processing nutritionally poor diet by not assimilating excess/unwanted substances into their metabolic machinery.

The regulatory physiology approach on the other hand, assumes that animals rely on their metabolic physiology to maintain their structural composition requirement and thus assimilation of substances occur independent of animals' composition requirement. The approach assumes that animals excrete (as organic compounds or CO₂) substrates they assimilate in excess of their structural requirement (Anderson et al., 2005; Anderson and Hessen, 2005). This is also supported by several experimental observations (Jansen and Hessen, 2007; Jansen et al., 2006; Darchambeau et al., 2003). In addition, the approach provides consumers the options to either store excess assimilates or utilize them as substrates for biosynthesis, where they have the capacity to do so.

In this study, a blend of the two approaches has been used to model substrate assimilation. I assumed that the efficiency at which an ingested chemical is assimilated for metabolism varies with the potential for that chemical to limit egg production. This assumption is consistent with previous modelling studies (Mitra 2006). Here, the growth limiting potential (L_i) for a chemical was calculated using equation 3, based on the experimental observation that when food is nutritionally imbalanced, consumers alter their physiology to ensure efficient retention and utilisation of chemicals in short supply (Raubenheimer and Simpson 1998). Thus, $0 < L_i < 1$ when the supply of a chemical exceeds the consumers' requirement and a decrease in assimilation efficiency is enabled in the present model for such chemicals. $L_i = 1$ indicates low availability of a compound and/or the consumer is capable of utilizing all of it even when assimilated at a maximum efficiency (i.e., limiting substrates). For such compounds, maximum assimilation efficiency is maintained in order to satisfy consumers' requirements.

Here too, I assumed that an evolutionarily consistent behaviour is that which optimizes the growth of copepods, and accordingly configured assimilation efficiencies to maximize uptake of limiting compounds and moderate that of non-limiting compounds. Assimilation efficiency for each substrate (λ_i , dimensionless) was allowed to vary between a minimum (λ_m , dimensionless) required for only maintenance and a maximum (λ_{mg} , dimensionless) needed for both maintenance and maximum growth. For individual compounds, the functional form of λ_i between these two values may be diverse. Following Mitra (2006), λ_i was described as a hyperbolic function of a chemical's potential to limit the growth of consumers:

$$\lambda_i = \lambda_m + (\lambda_{mg} - \lambda_m) * (1 + \eta) * \frac{L_i}{L_i + \eta} \quad (8)$$

where, η defines the intensity of the response; the higher the η value, the stronger the intensity of the response and vice versa (Figure 2B). Setting $\lambda_m = \lambda_{mg}$ makes assimilation efficiency independent of L_i .

Assimilation rate for individual substrates (A_i , gC.gC⁻¹.day⁻¹) and total carbon (A_c , gC.gC⁻¹.day⁻¹) is then calculated as follows:

$$A_i = I_c \alpha_i \lambda_i \quad (9)$$

$$A_c = I_c \sum_{i=1}^n \alpha_i \lambda_i \quad (10)$$

Unassimilated fraction of each substance is egested as faecal pellets. The egestion rate for individual substrates (F_i , gC.gC⁻¹.day⁻¹) and total carbon assimilation rate (F_c , gC.gC⁻¹.day⁻¹) is calculated as follows:

$$F_i = I_c \alpha_i (1 - \lambda_i) \quad (11)$$

$$F_c = I_c \sum_{i=1}^n \alpha_i (1 - \lambda_i) \quad (12)$$

Dividing equations 10 and 12 with I_c gives the fraction of total ingested food carbon that is assimilated and those egested as pellet respectively.

Metabolism

I parameterised the metabolic physiology of the model consumer as described within chapter 2 of this thesis. The approach assumes that the mass of a consumer consists of “reserve biomass” from which resources are extracted for metabolism, and a “structural biomass” that is permanent and has an associated maintenance cost, which is met using the content of the reserve

biomass. Hence in this model, substrates assimilated by consumers are directed into a reserve biomass before being utilised for the needs of the consumer. For a consumer assimilating i to n different chemical substances, the carbon content of this reserve biomass is divided into molecule-specific fractions, β_i (gC.gC^{-1}) such that:

$$\sum_{i=1}^n \beta_i = 1 \quad (13)$$

Results from experimental studies suggest that zooplankton (including *Acartia tonsa*) can reproduce during inadequate food supply (Alonzo et al. 2000) and starvation (Ederington et al. 1995) by rely upon both their reserves and structural biomass as the source of the protein and phospholipids incorporated into eggs, as these compounds are not stored in large amount by copepods (Sargent & Falk-Petersen 1988; Ventura, 2006). Hence in this model, I do not distinguish between the biochemical composition of reserve and structural biomass. Rather, a balance in biochemical composition between consumers' reserve and structural biomass is assumed. Within the model, this is achieved by applying the phenomenon of substrate sparing (McGoogan and Gatlin 1999; Arnould et al. 2001) for any compound supplied below consumers' structural requirement.

For determining resource utilisation, I divided metabolism into two components, these being maintenance and growth metabolism, and assumed that substrates assimilated by consumers are used first for maintenance, with growth being possible only when the amount of assimilated food exceeds that required to cover the costs of maintenance. The justification for this approach has already been provided within chapter 2 of this thesis. So in this study, maintenance refers to all non-growth requirements and processes critical for the survival of organisms. This may include thermoregulation (Nanton and Castell 1999; Hasset and Crockett, 2009), ion transport (Milligan and McBride 1985), protein/biomass turnover (Mente et al. 2002) etc. For easy parameterisation of the model, I employed here an intermediate complexity approach to model parameterisation (e.g. Hannah et al., 2010) by separating maintenance into two components, these being energy, b ($\text{J.gC}^{-1}.\text{d}^{-1}$) and a budget for structural requirements (β_i). Thus, b covers all the costs associated with maintenance. On the other hand, β_i represents carbon-specific fraction of different chemicals in consumers' biomass. It is employed to ensure that consumers' biomass composition requirement for specific substances (such as lipids for thermo-regulation: Hasset and Crockett, 2009) is not compromised during catabolism of assimilates for maintenance energy.

After maintenance, remaining substrates are used for growth. Here too, an intermediate complexity approach has been taken by separating consumer's requirement for growth into energy (d) and structural components (δ_i). d is the energy costs for a unit C growth. Egg production is

assumed to be the only form of growth. Hence, δ_i represents the fraction of egg biomass constituted by a specific substance. It is also employed here to ensure that the need by eggs for specific substance for development and subsequent hatching are met during egg production.

The respiratory processes required for meeting the energy requirements for these processes were therefore termed maintenance and growth respiration respectively. Catabolism of compounds for metabolic energy was considered a mechanism for controlling chemical composition of the consumer. Thus, the use of compounds as substrates for respiration was regulated in order to satisfy the consumer's requirements for chemical substances. When food supply exceeds consumer's maintenance requirement (i.e. $I_c > b / \sum_i^n \alpha_i \lambda_i E_i$), maintenance and growth can concurrently occur.

Under such condition, energy as well as material requirements for maintenance (b and β_i) and growth (G , E_g and δ_i) would be met simultaneously. i.e.

$$\text{For maintenance} \left\{ \begin{array}{l} b = I_c \sum_{i=1}^n \alpha_i \lambda_i \rho_i E_i \quad (14) \\ \beta_i = \frac{I_c \alpha_i \lambda_i (1 - \rho_i)}{I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i)} = \frac{\alpha_i \lambda_i (1 - \rho_i)}{\sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i)} \quad (15) \end{array} \right.$$

$$\text{For growth} \left\{ \begin{array}{l} G = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i - x_i) \quad (16) \\ E_g = Gd = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) \gamma_i E_i \quad (17) \\ \delta_i = \frac{I_c \alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i - x_i)}{I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i - x_i)} = \frac{\alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i - x_i)}{\sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) (1 - \gamma_i - x_i)} \quad (18) \end{array} \right.$$

Where, ρ_i represents the fraction of each assimilate that could be respired for maintenance without adversely affecting the structural biochemical requirement of the consumers, E_i denotes energy content (J.gC^{-1}) of each chemical, G is total growth rate ($\text{gC.gC}^{-1}.\text{d}^{-1}$), E_g is total energy requirement ($\text{J.gC}^{-1}.\text{d}^{-1}$) for growth, γ_i represents the fraction of each chemical assimilate that could be respired to power growth without adversely affecting the required biochemical composition (i.e. δ_i) of the new biomass, and x_i denotes the fraction of assimilates that may be in excess of consumers' requirement for both maintenance and growth. Dividing equation 16 by I_c gives carbon-specific gross growth efficiency (K_c).

It is clear from equations 14 to 18 that the number of eggs that can be produced by copepods in my model is dependent on assimilates remaining after maintenance as well as on the energy and structural material requirements for egg production. Due to the non-linear nature of this problem, the amount of each substrate needed to be respired was calculated iteratively by determining the magnitudes of ρ_i , γ_i and x_i that meet consumer's requirements for maintenance and growth. Thus, the required energy budget, b , for maintenance is attained by conversion of a specific mass (m_i ; $\text{gC.gC}^{-1}.\text{d}^{-1}$) of each assimilated substance via the following equation:

$$m_i = I_c \alpha_i \lambda_i \rho_i \quad (19)$$

Total food catabolism for maintenance is then described by equation 20, as the sum of the solutions to equation 19 for different chemicals.

$$m_c = I_c \sum_{i=1}^n \alpha_i \lambda_i \rho_i \quad (20)$$

where, m_c is total rate ($\text{gC.gC}^{-1}.\text{d}^{-1}$) with which carbon is utilised for maintenance. Dividing the above with I_c gives the fraction of total ingested carbon that is used for maintenance.

Depending on the balance of the biochemical substances remaining after maintenance, growth could be limited by any substances, which could lead to excesses in others. To ensure growth optimisation, only part (γ_i , dimensionless) of substrates that are in excess of the specific structural needs of the animals are catabolised for energy to power growth. So for limiting substances, $\gamma_i = 0$, while $1 < \gamma_i > 0$ for non-limiting substances. For each chemical substance however, the actual magnitude of γ_i depends on the extent to which zooplankton can convert substrates remaining after maintenance into growth while satisfying both energy (d) and structural requirements (δ_i) for egg production. Egg production ceases when any of these conditions is not met. When this is reached, the value of γ_i could be used to calculate the quantity of individual substances and total C that could be respectively respired for growth:

$$g_i = I_c \alpha_i \lambda_i (1 - \rho_i) \gamma_i \quad (21)$$

$$g_c = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) \gamma_i \quad (22)$$

where g_i and g_c indicate the rates ($\text{gC.gC}^{-1}.\text{d}^{-1}$) with which each chemical and total carbon are respectively catabolised for energy to power growth. Dividing equation 21 and 22 respectively by $I_c \alpha_i$ and I_c gives the fraction of each ingested chemical and total food that is catabolised to power growth.

Given x_i as the proportion of individual substances that would remain when growth ceases due to imbalance(s) in the diet of the zooplankton, the quantity of individual substances and total C that may neither be used for maintenance nor growth could be calculated respectively as

$$X_i = I_c \alpha_i \lambda_i (1 - \rho_i) x_i \quad (23)$$

$$X_c = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) x_i \quad (24)$$

Where, X_i and X_c represent that rate (d^{-1}) at which each chemical and total C may be in excess of animals' requirement for both maintenance and growth. The fate of these excess chemicals acquired by zooplankton is several (see Anderson and Hessen, 2008). In this study, I assume that *Acartia* releases excess assimilates via respiration decoupled from maintenance and egg production. This assumption is based on the observation that that zooplankton with little capacity for storing resources can rid themselves of excess food via respiration decoupled from biochemical/mechanical work (Anderson et al., 2005). As a result, dividing equations 23 and 24 respectively by $I_c \alpha_i$ and I_c gives the fraction of each ingested chemical and total C that are voided to satisfy the structural biochemical requirement of the animals.

3.4 PARAMETER DETERMINATION AND MODEL IMPLEMENTATION

As justified above, the model here is calibrated to fit Kiørboe's experimental relationship between food C:N ratio and egg production by *Acartia tonsa* (i.e., Kiørboe 1989). During the experiments, *Acartia* females were supplied with saturating amount of the diatom *Thalassiosira weissflogii*. So an unlimited food concentration of $1E-3 \text{ gC L}^{-1}$ (Kiørboe et al. 1985) is assumed for this study. In the experiment, C:N-ratio of the diatoms was varied by manipulating the concentration of nutrient in the algal growth medium. Hence, the carbon content of the algae was divided into protein (P), lipid (L) and carbohydrate (H) specific fractions (i.e., α_i ; gC.gC^{-1}) using published relationships between diatom C:N ratio and macromolecular composition (Anderson, 1994; see also equation 39a-d of chapter 2 of this thesis). Typically in algae, carbohydrate occurs in structural forms that are bounded in cell walls, and storage forms that are mostly in solution in the cytoplasm of cells (Geider and Roche 2002). While storage carbohydrates are easily assimilated, structural carbohydrates are barely assimilated by planktonic herbivores (Reinfelder and Fisher 1991; Anderson 1994). I did not distinguish between reserve and structural carbohydrates, and so the carbohydrate composition of the algae is the sum of the two forms. The implication of this assumption will be discussed later.

Unlike other copepods (e.g. *Calanus hyperboreus*), *Acartia* adults have considerably less capacity for substrate storage (Sargent and Falk-Petersen 1988; Lee et al., 2006) and so could be assumed to maintain constant biochemical composition in this study. These adults also do not undergo significant structural (exoskeleton) growth (Miller et al., 1977), thus justifying my assumption of egg production as the only form of growth. Unless stated, parameters were estimated, and hence had the same value as described under model parameterisation section of chapter 2.

In order to ensure that the upper threshold for food uptake represent that of *Acartia tonsa*, I_{mg} , w_{mg} and λ_{mg} were determined from published experimental studies involving the species. Values for these parameters, unless stated, were estimated following methods described by Houde and Roman (1987). Parameters were chosen after comparing estimates from several studies conducted at similar laboratory conditions. In an experiment, Besiktepe and Dam (2002) investigated grazing and defecation as function of diet in *Acartia tonsa*. Their study involved species representing the main prey items for copepods (i.e. diatoms, flagellates, dinoflagellates and ciliates). The highest food ingestion rate they measured was $28.9 \mu\text{gC individual}^{-1}\text{day}^{-1}$. Per unit C of the copepod, this rate of food ingestion is equivalent to $6.88 \mu\text{g C } (\mu\text{gC})^{-1} \text{day}^{-1}$ (assuming the carbon weight of adult *Acartia* is $4.2\mu\text{g}$, Kiørboe, 1989). This, to my knowledge, is the highest ingestion rate ever reported for *Acartia tonsa*. So it was used for I_{mg} in my model. In the same experiment, the highest assimilation efficiency measured was approximately 95 %, recorded when *Acartia* was fed on the ciliates *Uronema sp.* This food assimilation efficiency is comparable with the maximum assimilation efficiency observed in other studies (e.g. Bämstedt et al., 1999). It was therefore taken to be the maximum assimilation efficiency that *Acartia* can achieve (i.e. $\lambda_{mg} = 0.95$). The upper limit for prey capture efficiency was extracted from the work of Støttrup and Jansen (1990). Using data from figure 2c of their report, highest capture efficiencies of 2500 L.gC^{-1} was estimated. This was taken as the value for w_{mg} .

However, parameters describing the lower boundary of feeding behaviour (i.e. w_m , I_m and λ_m) needed for satisfying animals' requirement for only maintenance could not be determined from laboratory studies. This is due to the fact empirical studies on the maintenance requirement of copepods are typically starvation experiments, with no measurement of feeding rate (e.g. Tsuda 1994; Thor 2003). Also making it impossible to determine minimum threshold for food uptake from laboratory measurement is the fact that, the quantity of substrates that could be used for only maintenance when prey has no nutritional value for growth (i.e., $Q = 0$), would depend on prey's chemical composition as biochemical substances differ in their energy densities. Similarly,

parameters describing the intensity of animals' feeding response to food quality (i.e. Z_{ce} , Z_{ing} and η) could not be derived from laboratory studies. This is because the potential effect of a prey and its constituents on the growth of predators is not directly measurable, as it is practically impossible to disable animals' ability to adjust feeding behaviour to their nutritional and functional needs.

So for this study, the parameters w_m , I_m , λ_m , Z_{ce} , Z_{ing} and η were determined by fitting the model to experimental data of Kiørboe (1989). Specifically, the growth response of the copepod to changes in algal C:N was simulated and compared with the observations in order to determine the parameter values that could best describe the dynamics of egg production under the experimental conditions. At each combination of w_m , I_m , λ_m , Z_{ceff} , Z_{ing} and η values, the sum of square error between model prediction, and the observed data was calculated using equation 23 (Haefner, 2005):

$$\varepsilon_i = \sum_i \left(f(x_i, w_m, I_m, \lambda_m, Z_{ceff}, Z_{ing}, \eta) - y_i \right)^2 \quad (25)$$

where ε_i represents the error between prediction and observation (y_i) when the model, f , was run with the i^{th} combination of w_m , I_m , λ_m , Z_{ceff} , Z_{ing} and η ; x_i is prey's chemical composition. The best parameter values were the combination that gave least ε_i value. These estimates were then evaluated by fitting the model to the egg production data of Checkley (1980; data extracted from table 7 of the paper).

Before initiating feeding, the model copepod first determines the quality (Q) of each prey, and growth limiting potential (L_i) of prey biochemical constituents based on its maximum capacity for food uptake (I_{mg} and λ_{mg}) as well as specific requirement for energy (b and d) and structural biochemical substances (β_i and δ_i). So during food quality determination, I_c was assigned the value of I_{mg} by setting $I_m = I_{mg}$ in equation 5. Similarly, λ_i was assigned the value of λ_{mg} during food quality determination by setting $\lambda_m = \lambda_{mg}$ in equation 8. In the model, the equation (4) describing food quality is not linear, as it can only be solved by simultaneously satisfying the conditions for maintenance (i.e. equations 14 to 15) and growth (16 to 18). As a result, an iterative approach was therefore used to estimate how acquired substrates could be used for maintenance (ρ_i), respired to power growth (γ_i) and/or voided (x_i) in order to satisfy the required structural biochemical composition of the copepod. Detailed description of the iteration procedure can be found within chapter 2 of this thesis. ρ_i , γ_i and x_i values predicted by the procedure were then

inserted in the appropriate equation above to calculate growth rate and the amount of each substrate that may be respired for maintenance and growth as well as those that may be voided.

In the model, food composition, consumers' biochemical demand for maintenance and growth, feeding and all physiological processes are characterised based on carbon. This is because carbon, unlike nitrogen, is closely related to energy content of food items (Sterner and Robinson 1994) and occurs in every organic compound. So food ingestion, assimilation, respiration and growth in terms of carbon were simply the sum of the respective rates for the individual compounds (given respectively by I_c , A_c , M_c or g_c , and G). For converting these rates into N-specific rates, I followed previous studies (Kuijper et al. 2004) and assume that N occurs only in proteins. So N-specific ingestion, assimilation, respiration and growth rates were determined by dividing the respective rates for protein by protein C:N mass ratio (assumed to 3.2; Vollenweider 1985).

3.5 RESULTS

Food composition and quality

Figure 3A shows carbon content of the diatom in Kiørboe's experiment (Kiørboe, 1989) divided into protein-, lipid- and carbohydrate-specific fractions. For most of the algae, the results show that the bulk of algal carbon is associated with carbohydrate. Carbohydrate composition of the algae increased with algal C:N, contributing over 80% of the algal carbon when algal C:N was highest. Conversely, protein composition of the algae decreased with increasing algal C:N, and exceeded that of carbohydrate only when algal C:N was lower than ~6.5. Lipid composition of the algae was generally low, varying from 12.1% in algae with lowest C:N to 3.6% when algal C:N was highest. This algal biochemical composition differs markedly from that required for the structure of the model copepod. Unlike the algae, protein constitutes the bulk of *Acartia* biomass, followed by lipid and carbohydrate (Table 1).

Accordingly, the results show that the potential at which most food biochemicals could limit *Acartia* egg production varies with food C:N (Figure 3B). The results show that growth-limiting potential of lipid was constant and the highest ($L_L = 1$) over the entire range of algal C:N ratios. This was because lipid content of the algae was relatively low (figure 3A). On the contrary, protein and carbohydrate composition of the algae was significantly higher than that required for the structure of the model copepod. As a result, protein and carbohydrate limiting potentials were less than that of lipid, with $L_P > L_H$ at all algal C:N ratios. L_P increased with algal C content, peaked at algal C:N of ~23, and thereafter declined. The potential for carbohydrate to limit *Acartia*'s growth was predicted to continuously decrease with increasing algal C:N.

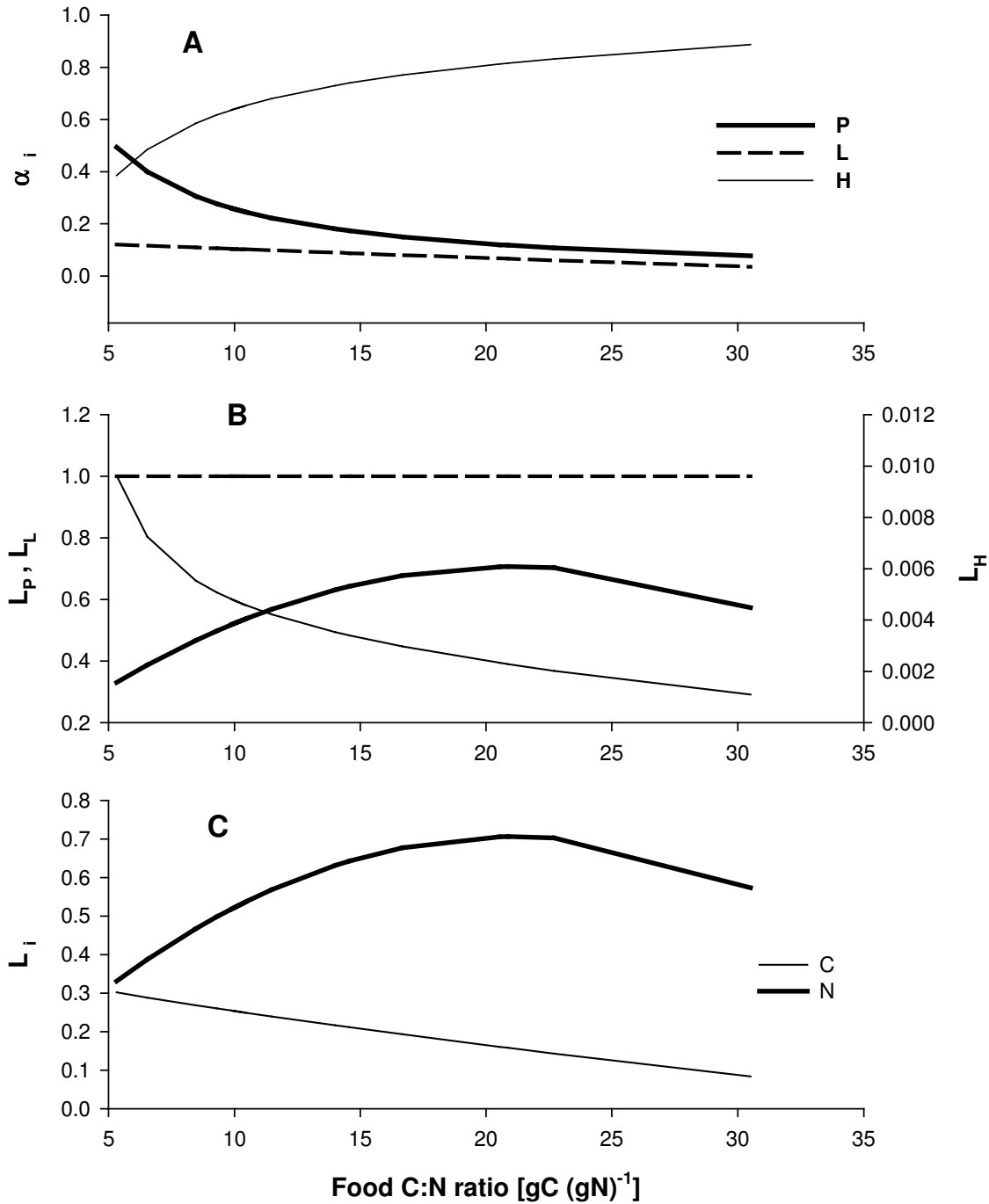


Figure 3. A: Macromolecular biochemical composition (α_i , gC.gC⁻¹) of algae at different algal C:N. See text associated with equation 39a-d for a detailed description of how α_i for each compound was calculated. B & C: Model predicted growth limiting potential (L_i , dimensionless) of individual substances. Subscript i represents the macromolecules of proteins (P), lipids (L) or carbohydrates (H). It represents carbon, C, or nitrogen, N in subplot C.

Although these results reflect the requirement differences the model set between biochemical substances for egg production (i.e. δ_i), the approach used here evaluates the growth-limiting potentials of individual compounds based on consumers' entire metabolic capacity.

Figure 3C shows the limiting potentials of algal C, L_C , and N, L_N . These were calculated based on the potential efficiencies (i.e. U_C and U_N) at which each ingested chemical element could be converted into growth. U_C and U_N were estimated by dividing their element-specific growth rates with the difference between the rate with which each was assimilated and used for standard respiration. Thereafter, U_C and U_N were used in equation 3 to determine L_C and L_N respectively. Growth, assimilation and respiration rates for C were simply the sum of the respective rates for the individual compounds (given respectively by G , A_c and $M_c + g_c$). N was assumed to occur solely in proteins as protein-N may constitute as much as 95% of total cellular nitrogen of algae (Thomas and Krauss 1955). So N-specific ingestion, assimilation, respiration and growth rates were determined by dividing the respective rates for protein by the assumed protein C:N ratio (by mass). The potential fate of ingested nitrogen was therefore dependent on that of protein. As a result, $L_N = L_P$ at all algal C:N ratios. On the other hand, L_C decreased linearly with increasing algal C:N ratio, due mainly to increasing availability of excess carbohydrate carbon. Results from the simulation indicate that the potential for both C and N to limit *Acartia* egg production is more comparable when algal C:N is low (Figure 3C). This was mainly because excess carbohydrate C composition of the food decreased with algal C:N (Figure 3A). The results here therefore indicate that a threshold C:N ratio for algae could be reached, at which L_C would equal L_N , and below which L_C would exceed L_N (i.e. threshold elemental ratios, TER: Urabe and Watanabe, 1992). It is obvious from Figure 3C that TER could occur at algal C:N ratios below the range of the experimental data. Unfortunately, L_C and L_N could not be calculated for food C:N < 5 as the function for determining the composition of individual compounds (α_i) from algal C:N ratios breaks down for food C:N < 5. As a result, TER was determined by extrapolating the lines L_C and L_N in Figure 3C to algal C:N ratios below 5 using a curve fitting method (Sigmaplot 10.0). The results (not shown) indicated TER of 4.8, which is comparable with the threshold C:N ratios estimated by others for *Acartia tonsa* (e.g. Touratier et al. 1999; chapter 2 of this thesis).

This results suggest that biochemical imbalance between *Acartia tonsa* and its food could be exacerbated when the copepod feeds on high C:N algae, as an increase in C:N shifts algal biochemical composition away the structural needs of the copepod. Accordingly, results here show that food quality decreases with increasing algal C:N (Figure 4), meaning that egg production by

Acartia may decrease with increasing algal C:N. This is consistent with observed effect of algal C:N on copepod egg production (Checkley 1980; Kiørboe 1989). Contrary to previous results (cf. Figure 7C of chapter 2), a unimodal relationship between food quality and algal C:N ratios did not come into play here. This is because the threshold elemental ratio, at which growth-limitation switches from one element to the other, lies below the range of algal C:N ratios in this study.

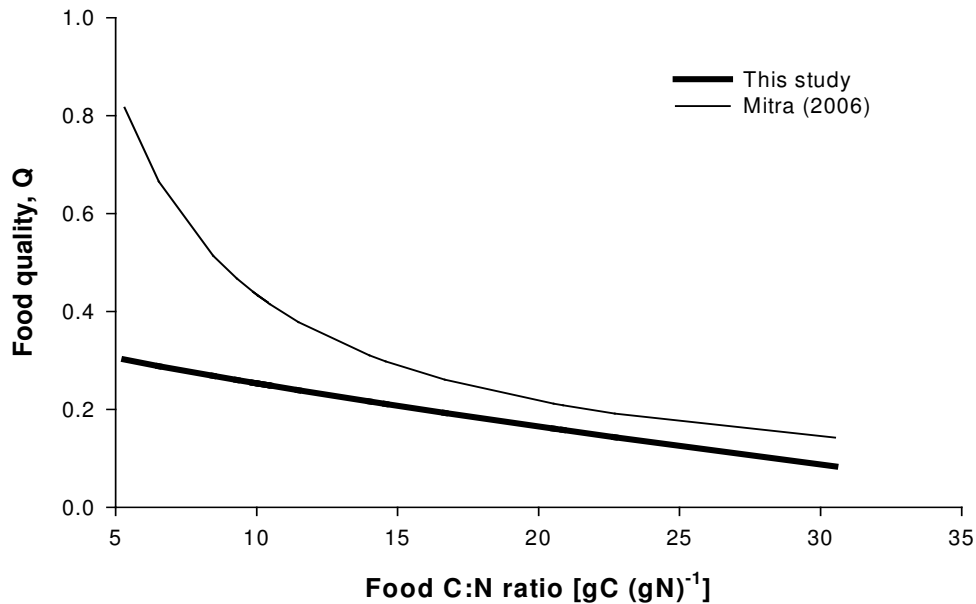


Figure 4. Comparison of this model with the stoichiometric approach to defining food quality exemplified by Mitra's model [i.e., equation 3 of Mitra 2006; where $Q = \min(ZNC / SNC, 1)$ with ZNC and SNC being zooplankton and seston N:C ratios respectively]. For implementing Mitra's model, C:N ratio of 0.23 was used based on the assumed biochemical composition of adult *Acartia* (Table 1). This N:C ratio for *Acartia* tissues is comparable with what has been used in other studies (e.g. Touratier et al. 1999; Kuyjper et al. 2004a; Mitra 2006)

Usually in aquatic ecology, stoichiometric disparity between elemental composition of consumers and their prey is used to define food quality, typically with no regard for consumers' metabolic physiology (e.g. Mitra et al. 2003; Mitra 2006). Figure 4 shows the comparison of the approach of this study (equation 4) and the stoichiometric approach to defining food quality. Both models predict food quality to decrease with increasing algal C:N. However, food quality values predicted by the model presented here are significantly lower than those predicted by the stoichiometric model. This emphasizes the distinction between this study and previous models.

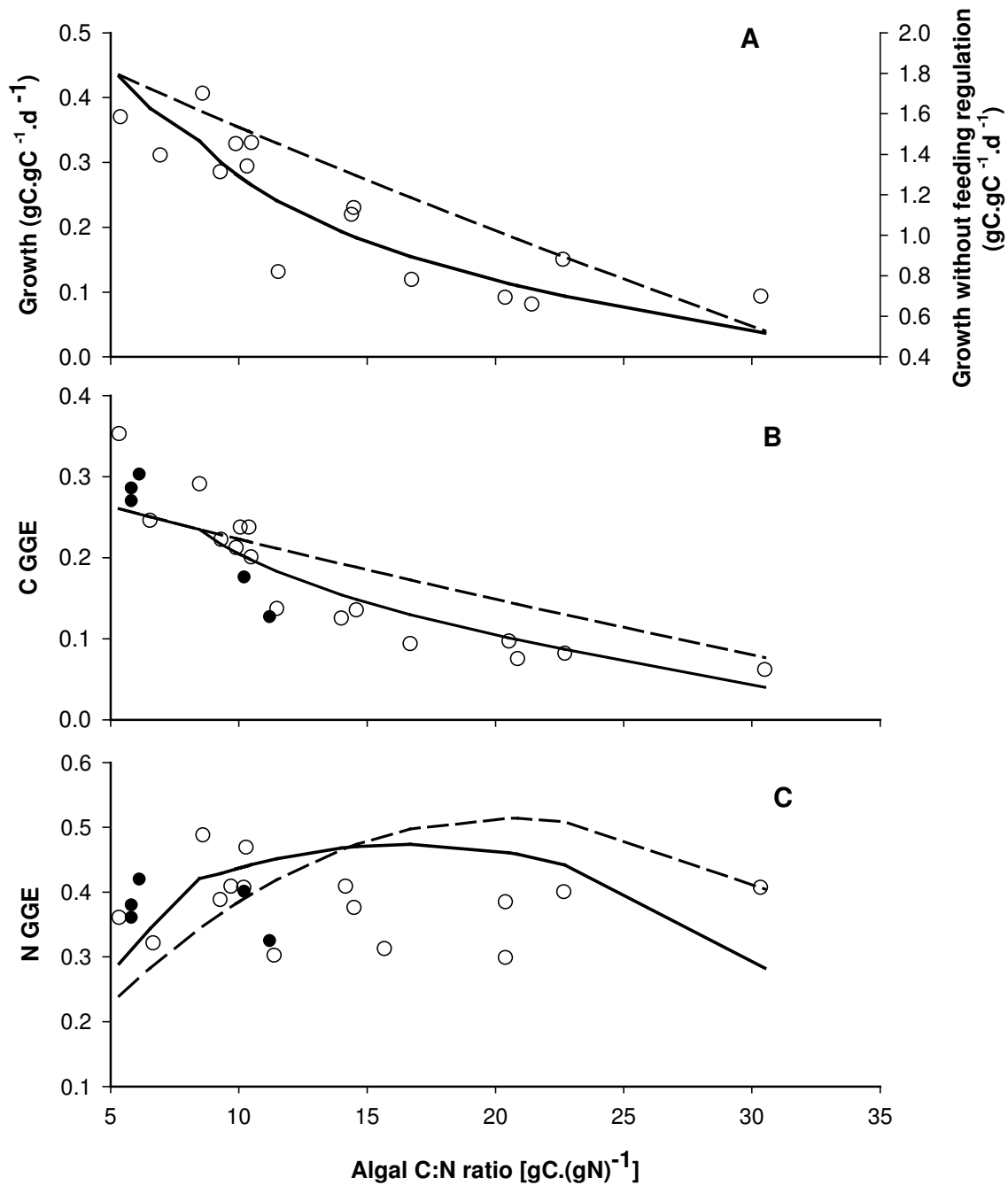


Figure 5. Model fit (lines) to *Acartia*'s egg production data (open circles) from Kiørboe (1989). (A) Growth rate, (B) carbon-specific gross growth efficiencies, C GGE, and (C) nitrogen-specific gross growth efficiencies, N GGE. Solid lines represent model output when the rate for food ingestion and assimilation is dependent of food quality. Broken lines represent when the rate for food ingestion and assimilation is constant and independent of food quality. Shown also are the egg production data of *Paracalanus parvus* (closed circles; Checkley, 1980). See Table 3 for fitted parameters.

Food consumption and growth

In this study, food quality represents potential growth consequences for feeding on specific prey items. This is because food quality was determined by negating animals' ability to mitigate or avoid dietary biochemical limitation via feeding regulation. This approach is comparable with previous numerical analysis of food quality (cf. Anderson and Pond 2000; Anderson and Hessen 2005). So after assigning food quality value to each diatom based, the model consumer was allowed to regulate the rate of food ingestion, assimilation, and respiration in order to maximise growth under each feeding condition. For the simulations, parameters describing the lower boundary of feeding behaviour (i.e., w_m , I_m and λ_m), as well as those describing the intensity of animals' feeding response to food quality (i.e., Z_{ce} , Z_{ing} and η) were tuned (values given in Table 3) in order to fit the model to experimental data (Kiørboe 1989). The justification for the approach is provided above (see section 3.4).

Table 3. Tuned values used for fitting model to observation (Figure 5). See Tables 1 and 2 for definitions

Constant	Unit	Value
w_m	L.gC ⁻¹	500
I_m	gC.gC ⁻¹ .d ⁻¹	0.24
λ_m	–	0.01
η	–	0.032
Z_{ceff}	–	0.30
Z_{ing}	–	0.22

Figure 5 shows the agreement between the model present here and experimental observation. There was a close fit between my model and observed growth rate (Figure 5A), with ~72% of the observed variation in egg production rate being explained by the simulation (regression of predicted verses observed growth rates: gradient = 0.94 ± 0.16 , $r^2 = 0.7213$, $p < 0.0001$). Similarly, the model realistically reproduced C-specific gross growth efficiencies (C GGE) observed in the experiments. It predicts an average N-specific gross growth efficiency (N GGE) of ~0.43, which is similar to the average of 0.4 observed in calanoid copepods (Checkley 1980; Kiørboe 1989). N GGE was however predicted to decline when algal C:N is high (above ~23) because part of the assimilated N was catabolised for energy to power maintenance (see Figure 6B). These predictions compare with results from previous modelling studies (Kuijper et al. 2004). In addition, the C:N ratio of most of the algae naturally available to *Acartia tonsa* is mostly below 10

(Geider and Roche 2002). Hence, I conclude that my macromolecular approach has demonstrated the ability to realistically capture nutrient uptake and utilization by the *Acartia*.

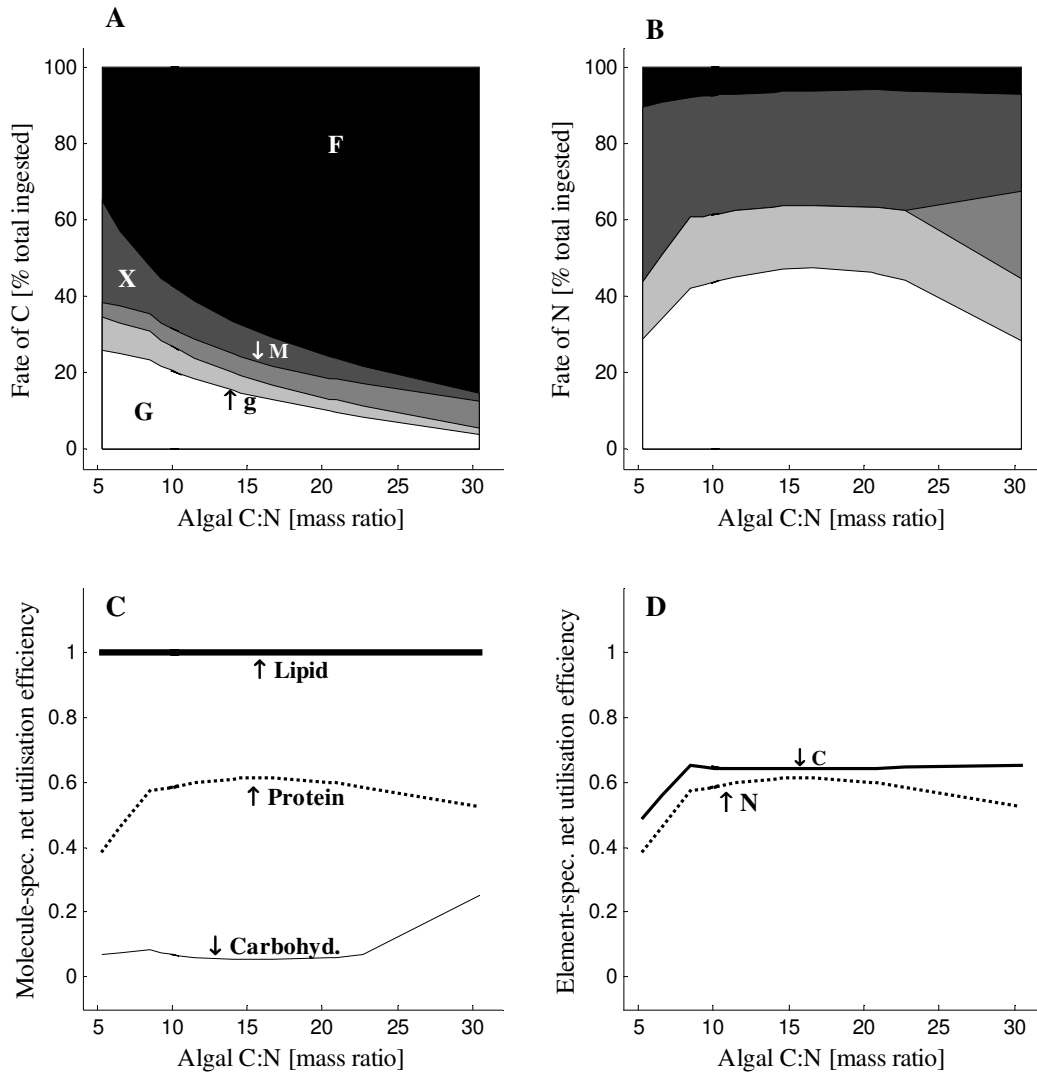


Figure 6. A&B: Predicted fate of ingested carbon (C) and nitrogen (N) at different algal C:N ratios. Respectively, F, M, g, X and G represent the fraction of total ingested element released as faecal pellets, used for biomass maintenance, respired for energy to power growth, voided to satisfy consumer's composition requirement, and those converted into eggs.

C&D: Net utilization efficiency (U_i) of assimilated chemicals assimilated. At each algal C:N, growth-limiting chemical(s) is/are those with highest U_i values (Anderson and Pond 2000).

The above results were obtained by configuring the model to enhance both the ingestion of good quality prey, as well as the assimilation of growth-limiting compounds. The results show that food quality declined with increasing algal C:N (Figure 4). Thus, the fits between model and data were obtained with an ingestion rate that decreased with increasing algal C:N. C ingestion was experimentally observed to be independent of algal C:N (Kiørboe 1989). However, the predictions of the model were not significantly different from the experimental average (t-test, $\alpha = 0.05$, $df = 33$, $t = -0.7655$, $p = 0.4494$. Model: $0.9 \geq I_c \leq 1.7$ with average $I_c = 1.29 \text{ day}^{-1}$; Observation: $0.9 \geq I_c \leq 1.7$ with average $I_c = 1.35 \text{ day}^{-1}$). The model can therefore be said to have simulated the observed grazing rates.

Figure 6 shows the fraction of ingested C and N released as faecal pellet as well as those allocated for the production of energy and eggs. In my approach, assimilation and subsequent utilization of chemical elements depends on that of protein, lipid and carbohydrate macromolecules. So for determining the fate of each chemical element ingested by copepods, a fixed molecular configuration for protein ($\text{C}_{59}\text{N}_{16}\text{H}_{94}\text{O}_{19}\text{S}_{0.5}$, 1346g, Vollenweider, 1985), lipid ($\text{C}_{18}\text{H}_{36}\text{O}_2$, 284g) and carbohydrates (CH_2O , 30g) (Anderson, 1992) was assumed. Hence in my analysis, the fate of algal nitrogen is dictated by that of protein. Conversely, the fate of algal carbon depends on all three macromolecules. The approach here is consistent that of published studies on the effect of macromolecules on cycling of chemical elements (Anderson 1992; Kuijper et al. 2004).

The results show that total lipid composition of the algae (α_L) is below the structural requirement (β_L and δ_L) of *Acartia* (Figure 3B). Consequently, lipid assimilation efficiencies was predicted to be highest and constant at ~ 0.95 . Also, protein assimilation efficiency was predicted to be high, increasing slightly from 0.90 to 0.93 with algal C:N. These results are consistent with experimental data on calanoid copepods (Head 1992; Harvey et al. 1987) and reflect *Acartia*'s high structural demand for proteins and lipids. Only carbohydrate assimilation efficiency was predicted to be low, decreasing from 0.25 to 0.05 with increasing algal C:N.

Consequently, the results here show that the proportion of ingested C that is assimilated by the copepod decreased from approximately 66 to 16% with increasing algal C:N. These predictions compare with experimental data demonstrating that the fraction of ingested C that is not assimilated, and hence released as faecal pellet by zooplankton increases with algal C content (DeMott et al., 1998; Tang and Dam, 1999). Here, the fraction of ingested C released as faecal pellet increase with algal C:N, reaching $\sim 84\%$ when algal C:N was highest. Conversely, N assimilation efficiency was not markedly influenced by algal C:N status. It stayed between ~ 90 to 93%. This reflects high protein assimilation efficiency and compares with experimental observation

of N assimilation by calanoid copepods (Landry et al., 1984; Hassett and Landry, 1988). As a result of the high N assimilation efficiency, the fraction of ingested N released as faecal pellet was very low, ranging between ~7 -10%.

Over the entire range of algal C:N ratios, just 8 – 12% of the total ingested C was predicted to be catabolised for energy to power both maintenance and growth. On the other hand, N catabolism was relatively high, constituting between 14 – 39% of all ingested N. These predictions compare with the results of other studies (Kjørboe et al. 1985; Kuijper et al. 2004). My results show that N is not respired for maintenance but spared for growth when C assimilation efficiency, and hence metabolic availability is also high. This occurred when algal C:N was low. The simulation show that N was not respired for maintenance until algal C:N ratio had exceeded ~23. These results are consistent with that of previous models (e.g. Kuijper et al. 2004). It is also consistent with experimental data on the use of non-carbon nutrients as substrates for respiration by zooplankton (Blazka 1966; DeMott et al. 1998). Respectively, the fractions of C and N allocated to egg production correspond to C GGE and N GGE as shown in Figure 5. These fractions are consistent with experimental observations (Checkley 1980; Kjørboe 1989). Generally, egg production by *A. tonsa* decreases when the diet of the copepod contains either excess C (Kjørboe 1989) or excess N (Augustin and Boersma 2006). This study predicts a decline in N GGE, at the extremes of N-replete and N-deplete prey, with an N assimilation efficiency that was independent of algal N content (Figure 5C). This is consistent with current understanding of how animals respond to changes in non-carbon elemental components of their food (Boersma and Elser 2006 and references therein). Conversely, no decline in C GGE was predicted when *Acartia* was fed on N-replete prey because the algal C:N ratio at which egg production could have been hampered by excess N was not encountered in this study (Figures 3C and 5B).

Excess C, and the fraction of ingested C voided without any production by the copepod was predicted to be highest at ~27% when algal C:N ratio was lowest. This was mostly carbon associated with excess protein, as N-rich algae contain relatively more protein (Figure 3) whereas the compound is easily assimilated by copepods. As algal C:N increased, carbohydrate metabolic availability declined due to the low efficiency at which it was assimilated, thus forcing the model copepod to efficiently utilise the compound for metabolism. Consequently, the fraction of ingested C voided by the copepod was lowest at ~2% when algal C:N was highest. On the contrary, high protein requirement for egg production “fooled” the model copepod into maintaining high assimilation efficiency for protein, and by extension N. However, most of the N acquired thereof was not utilized for egg production due to lipid limitation (Figure 6). As a result, the amount of excess N and hence the fraction voided without any production was very high, being ~25 – 45% of

the ingested N. So as experimentally observed (e.g. Støttrup and Jansen 1990; Jónasdóttir 1994; Anderson and Pond 2000; Klein Breteler et al. 2005), the results here indicate that inadequate dietary supply of lipid represent a significant biochemical constrain on the utilisation of chemical elements by marine copepods.

Does carbon or nitrogen limits marine copepods?

After evaluating the metabolic fate of chemical elements ingested by the copepod, the limiting roles of C and N in *Acartia* egg production was determined. Here, I followed previous studies (e.g. Anderson and Pond 2000) by assuming that limiting chemicals are used more efficiently than non-limiting ones. This assumption is based on experimental data demonstrating that animals alter feeding behaviour and metabolic physiology during poor feeding conditions in order to ensure efficient retention and utilisation of chemicals in short supply (Roman 1983; Raubenheimer and Simpson 1998; Arnould et al. 2001). As a consequence of this acclimative capability, an animal can utilize different chemicals at comparable gross efficiencies even when it feeds on nutritionally distinct food items (Simpson and Raubenheimer 1995). So in this study, the limiting role of C and N in *Acartia* egg production was investigated by comparing their net utilization efficiencies. Chemical-specific net utilization efficiencies were determined using equation 2 (cf. Anderson and Hessen 2005), but after the rates for food ingestion, assimilation, catabolism and growth have been adjusted to food quality (i.e. after model has been fitted to observation).

Figure 6D shows the relationship between algal C:N and the net efficiencies at which each chemical element was utilised by the copepod. The results show that over the entire range of algal C:N ratios, the net efficiency at which C was utilized for egg production by *Acartia* is higher than that of N. This high utilization efficiency for carbon was mainly due to the low metabolic availability of the element, as indicated by its high composition in the animals' faeces. Contrary, the fraction of ingested N that is assimilated and hence exposed to the metabolic machinery of the model copepod was determined to be always high. The resultant superfluous supply of N reduced the utilization efficiency of the element below that of C. Based on these results and the assumption that limiting chemicals are used more efficiently than non-limiting ones, I conclude that C, and not N, mainly limits *Acartia* egg production. Though the model approach here is physiologically superior, these predictions are consistent with what has earlier been suggested by Anderson and Hessen (1995), even though their model, unlike the one presented here, was unable to predict the growth dynamics observed by Kiørboe (1989). Other models tuned/validated against the same data as done here could not predict this because, among others reasons, they determine substrates'

metabolic availability by relying on fixed food ingestion rate and/or constant assimilation efficiency (Anderson and Hessen 1995; Touratier et al. 1999; Kuijper et al. 2004). Here, a main reason for C-limitation was that a substantial portion of the algal C was held in a compound (carbohydrate) that could not be assimilated, thus causing C to be in short supply relative to demand.

To determine the extent to which the result here is dependent on the model parameters, the sensitivity of chemical utilization efficiencies to changes in model parameter values was analysed. The results are presented within Table 4. They show that within the range of algal C:N naturally encountered by *Acartia*, the prediction that C utilization efficiency is higher than that of N is robust to variations in all model parameters. N utilisation efficiency exceeded that of carbon only when protein and lipid requirements for egg production were reduced at algal C:N ≥ 17 . Naturally, algal C:N does not exceed 17 (Geider and Roche, 2002). Furthermore, *Acartia* inhabits epipelagic waters, where the general C:N of particulate organic matter is relatively low (<10 ; Copin-Montegut and Copin-Montegut, 1983; Chester and Stoner, 1974). The results here therefore suggest that it may be more likely for carbon than nitrogen to limit egg production by *Acartia tonsa*. This contradicts the general assertion that *Acartia* production is limited by nitrogen (e.g. Kuijper et al., 2004a; Jones et al., 2002; Touratier et al., 1999; Kiørboe, 1989).

3.6 DISCUSSION

Copepods are heterotrophic and hence depend on complex organic molecules for nutrition. At the same time, dietary supply of chemical compounds vary substantially among different prey items, or within a single prey species due to changes in the oceanic environment (Kattner et al. 1983; Mayzaud et al. 1989). Consequently, marine copepods, like most animals, must always balance food consumption with their biochemical nutritional requirement for growth and successful reproduction. Understanding and predicting the effect of chemical elements on copepod production therefore requires a perspective that views copepods as individuals capable of adapting their behaviour, both pre and post-food acquisition, to their specific biochemical needs (Mitra and Flynn 2005). Such an individual-based perspective forces some rethinking about the impact of chemical elements on copepod production. This is because the effect of chemical elements may be contingent on consumers' own feeding behaviour and/or metabolic physiology (Anderson and Hessen 2005; chapter 2 of this thesis). In this study, a model that incorporates the capability of animals to regulate food uptake and utilization based on the macromolecular biochemical composition of prey organisms was developed, and employed to investigate the limiting role of food C and N in egg production by marine copepods. The model is able to reproduce experimental

observations in which copepod egg production rate and carbon gross growth efficiency decreases with increasing algal C:N ratio (Checkley 1980; Kiørboe 1989). It also realistically captures the low (~0.4) and constant nitrogen gross growth efficiency observed in these experiments (Figure 5). However, contrary to popular opinion (White 1983; Elser and Hasset 1994; Touratier et al. 1999; Kuyper et al. 2004), results from my simulation suggest that carbon, and not nitrogen, may be the primary limiting nutrient for the production of marine copepods.

Before discussing the reasons and implication of my results, I want to mention that the basis for my model is the generic zooplankton model of Mitra (2006). However, it has important differences. First, it accounts for the biochemical characteristics of chemical substances, which makes this study more realistic as the fate of bio-elements are dictated by the compounds in which they occur and not vice versa (Tang and Dam 1999). Second, it distinguishes between chemical composition requirements of copepod adults and eggs. The justification is that eggs need specific compounds, such as polyunsaturated lipids (Xu et al. 1994, 1993) for processes like embryogenesis and hatching that do not occur in adults and as consequence may differ from adults in terms of their biochemical constituents (see Koski 1999). Grazing is a function of gut size, digestive enzyme activity and assimilation capability of copepods (Tirelli and Mayzaud 2005). Accordingly, the effect of a prey's chemical composition on food consumption can be constrained by consumers' intake as well as metabolic capabilities. So the final distinction between this study and that of Mitra is that this study does, but Mitra (2006) does not consider consumer's metabolic capabilities when evaluating potential prey organisms for consumption. Furthermore, my approach is consistent with results from earlier studies suggesting that food quality is contingent on the maximum rate of food ingestion and assimilation, as well as the respiratory physiology of animals (Hessen and Anderson 2005).

Assuming copepods evaluate potential prey organisms based on their elemental constituents, Figure 4 shows the comparison between the food quality function employed here and that of Mitra (Mitra 2006). Both models predict that food quality decreases with increasing algal C:N content. However, at all algal C:N ratios, food quality predictions of this model are significantly lower than those of Mitra. Feeding response of the consumer to food quality therefore differs between the two studies. This can be seen by comparing the results of this study (Figure 5) with that of Mitra. Figures 4 of Mitra (2006; not shown here) provide all the information needed for the comparison. In Mitra's study, no significant change in copepod egg production was observed whether model copepods engage in food quality dependent regulation of food uptake (ingestion and assimilation) or not (Mitra 2006). This is unrealistic, as feeding regulation cannot be said to be irrelevant to egg production (see Cruz-Rivera and Hay 2000; Jones and Flynn 2005). In this study,

realistic agreement between model and observation could only be achieved through food quality dependent regulation of food ingestion and assimilation (Figure 5). It can therefore be argued that the model presented here represents a significant improvement over existing approach to modelling secondary production.

Table 4. Sensitivity (S_i) of element-specific net utilization efficiency (U_i) to model parameters.

$S_i = [(R_a - R_n) / R_n] / [(P_a - P_n) / P_n]$, where R_n is U_i for a given element for the standard case with parameter value P_n , and R_a is U_i for the case when the parameter is given a new value P_a . Subscript i refers to C or N. See Tables 2 for definition of parameter. Base indicates all parameter values were same as given in Tables 1 and 2. Parameters were increased (P1) and decreased (P2) by 10%. For λ_{mg} , P1 was not done since that gives an unrealistic $\lambda_{mg} > 1$, rather λ_{mg} was decreased 30% to reflect the greater uncertainty in the parameter. For β_i and δ_i whose sum must equal 1, when the value for one compound was changed, the difference was shared equally between the others. $U_N : U_C > 1$ means N utilisation efficiency exceeds that of C (in bold), $U_N : U_C < 1$ when C utilisation efficiency exceeds that of N.

Param	Values	Response								
		Algal C:N = 7			Algal C:N = 17			Algal C:N = 30		
		S_N	S_C	$U_N : U_C$	S_N	S_C	$U_N : U_C$	S_N	S_C	$U_N : U_C$
	Base	1.00	1.00	0.83	1.00	1.00	0.96	1.00	1.00	0.80
<i>b</i>	P1	0.00	-0.02	0.83	0.02	0.00	0.96	0.00	0.00	0.80
	P2	0.00	-0.02	0.83	0.02	0.00	0.95	0.00	0.00	0.80
<i>d</i>	P1	-0.02	-0.03	0.83	0.01	0.00	0.96	0.00	0.00	0.80
	P2	-0.02	-0.03	0.83	0.01	0.00	0.95	0.00	0.00	0.80
I_{mg}	P1	-0.03	-0.07	0.83	0.11	0.01	0.98	0.00	0.00	0.80
	P2	-0.04	-0.08	0.82	0.14	0.01	0.94	0.00	0.00	0.80
I_m	P1	0.00	-0.01	0.83	0.01	0.00	0.96	0.00	0.00	0.80
	P2	0.00	-0.01	0.83	0.01	0.00	0.95	0.00	0.00	0.80
w_{mg}	P1	-0.01	-0.03	0.83	0.04	0.00	0.96	0.00	0.00	0.80
	P2	-0.01	-0.03	0.83	0.05	-0.01	0.95	0.00	0.00	0.80
w_m	P1	-0.01	-0.02	0.83	0.04	0.00	0.96	0.00	0.00	0.80
	P2	-0.01	-0.02	0.83	0.04	0.00	0.95	0.00	0.00	0.80
λ_{mg}	P2	-0.04	-0.10	0.81	0.17	-0.02	0.90	0.00	0.00	0.80
λ_m	P1	0.00	-0.01	0.83	0.02	0.00	0.96	0.00	0.00	0.80
	P2	0.00	-0.01	0.83	0.02	0.00	0.95	0.00	0.00	0.80
Z_{ceff}	P1	0.01	0.03	0.83	-0.06	0.01	0.95	0.00	0.00	0.80
	P2	0.01	0.03	0.83	-0.06	0.01	0.96	0.00	0.00	0.80
Z_{ing}	P1	0.03	0.08	0.82	-0.16	0.02	0.94	0.00	0.00	0.80
	P2	0.03	0.08	0.83	-0.15	0.01	0.97	0.00	0.00	0.80

Table 4 continued.

Param	Values	Response								
		Algal C:N = 7			Algal C:N = 17			Algal C:N = 30		
		S_N	S_C	$U_N:U_C$	S_N	S_C	$U_N:U_C$	S_N	S_C	$U_N:U_C$
η	P1	0.12	0.20	0.82	-0.15	0.02	0.94	0.00	0.00	0.80
	P2	-0.15	0.02	0.83	-0.19	0.02	0.97	0.00	0.00	0.80
β_P	P1	-0.54	-0.51	0.82	-1.79	-1.80	0.96	-9.60	-9.53	0.67
	P2	-0.49	-0.08	0.86	-1.98	-1.92	0.96	-3.21	-1.84	0.90
β_L	P1	0.00	0.00	0.83	1.26	1.23	0.96	1.72	1.27	0.84
	P2	0.00	0.00	0.83	1.18	1.18	0.96	1.50	1.21	0.78
β_H	P1	0.00	0.00	0.83	-0.10	-0.10	0.96	-0.10	-0.10	0.80
	P2	0.00	0.00	0.83	0.10	0.10	0.96	0.17	0.09	0.80
δ_P	P1	1.29	1.19	0.83	1.64	1.65	0.95	1.87	1.78	0.81
	P2	1.14	2.63	0.99	-1.56	1.90	1.36	-2.12	1.50	1.15
δ_L	P1	-0.83	-0.75	0.82	-1.15	-1.04	0.94	-1.14	-1.09	0.80
	P2	-0.29	1.33	0.98	-3.01	0.44	1.30	-3.79	-0.02	1.11
δ_H	P1	-0.06	-0.18	0.83	0.22	0.00	0.98	0.02	0.02	0.81
	P2	-0.07	-0.20	0.82	0.24	-0.01	0.93	0.02	0.02	0.80
E_P	P1	-0.15	-0.12	0.82	-0.11	0.10	0.95	-0.25	-0.18	0.80
	P2	-0.18	-0.14	0.83	-0.13	0.12	0.96	-0.32	-0.23	0.81
E_L	P1	0.00	0.00	0.83	0.06	-0.01	0.96	0.00	0.00	0.80
	P2	0.00	0.00	0.83	0.07	-0.01	0.95	0.00	0.00	0.80
E_H	P1	-0.07	-0.10	0.83	-0.04	-0.09	0.96	-0.03	-0.02	0.80
	P2	-0.08	-0.11	0.82	-0.04	-0.10	0.95	-0.03	-0.02	0.81

It is well known that the growth and reproduction by copepods decreases with increasing algal C:N ratio (Checkley 1980; Kiørboe 1989; Jones and Flynn 2005; but see Augustin and Boersma 2006). Identifying the mechanisms influencing such a response is therefore important for understanding and modelling the dynamics of marine ecosystems and biogeochemical cycling of chemical elements. For example, feeding regulation to optimize the uptake and retention of growth limiting chemicals may introduce elemental imbalance in nutrients available for phytoplankton (Elser et al. 1988), and/or increasing predation pressure on some algal species (Schmidt 2008). Such processes could alter algal community structure and hence the chemical composition of detritus to be remineralised in the upper ocean (Schmidt 2008). Furthermore, ecological stressors such as pollution and aquaculture significantly increase nutrient levels in water bodies, which impact the nutritional status of microalgae (Guo et al. 2009; Savage et al. 2002), and arguably modifies the dynamics of aquatic herbivory and production (Boersma and Elser 2006). So understanding the effect of algal nutritional status on the growth herbivores is also critical for our

ability to describe and mitigate the ecological consequences for our exploitation of the marine environment.

Contrary to popular understanding, the results of this study suggest that N may not be the primary nutrient limiting egg production by calanoid copepods. This is based on two major predictions of the model. First, the model predicts that copepods are more efficient at assimilating N from the food they ingest. On average, N assimilation efficiency was predicted to be ~90% (Figure 6), as shown by experimental studies (Landry et al. 1984; Hassett and Landry 1988). As a result, N supply for new biomass formation exceeded the requirement of the copepod. This occurred independent of the biochemical composition of the ingested food. Secondly, the model predicts that copepods are less efficient at assimilating C from the food they ingest. Highest C assimilation efficiency predicted by the model was approximately 66%, as observed in other studies (Tang and Dam, 1999). So compared with N, C supply for metabolism was low, thus making it more likely for carbon than nitrogen to limit egg production by the model copepod. As determined here, measured assimilation efficiencies for N are typically greater than that of C (Landry et al. 1984; Hassett and Landry 1988; Tang and Dam 1999). Here, a major constraint on C uptake for metabolism was the refractory effect of algal carbohydrates on carbon assimilation. Typically in algae, carbohydrate occurs in structural and so-called “storage” forms, which are assimilated at different efficiencies by copepods (Anderson 1994) thereby impacting the metabolic availability of the compound. In my approach prey structural or storage components were not considered. Rather, the two forms of carbohydrate were added together to represent composition of the algae. Therefore, the predicted impact of algal carbohydrate on C assimilation efficiency may be likely when copepods feed on algae with high indigestible components such as cellulose, mucus (mainly in phaeocystis; Adler et al. 2007), and frustules in diatoms. These substances can decrease substrate uptake by causing prey rejection, decreasing food ingestion via physical obstruction (e.g. by mucus), or decreasing the reactivity of digestive enzymes as argued by previous authors (Van Donk and Hessen 1995; Van Donk et al. 1997). The results of this study are therefore realistic and justified, and have implications for the role of copepods in C and N cycling within marine systems.

Two different pathways for C and N cycling emerge from the results presented here. The fraction of ingested N lost as part of faecal pellet was predicted to be lower and largely independent of prey nutritional status (Figure 6B). Thus, most of the N fixed by algae during primary production may be (i) incorporated into biomass of herbivores (here via egg production) that could be transferred to higher trophic levels via secondary consumption, or (ii) released as metabolic by-product such as ammonia (here via X_N , M_N , and g_N) that may be re-assimilated by phytoplankton for autotrophic production. However, this may not be likely for C because its

metabolic availability was significantly influenced by food quality. The model predicts C lost as pellets to increase, and C integration into zooplankton biomass to decrease, with increasing algal C:N (Figures 5B and 6C). As a result, more phytoplankton C may be exported into deeper waters, possibly as part of marine snow if faecal pellets are not consumed or broken up by other organisms (see for example Smetacek 1980).

Given the substantial portion of phytoplankton production ingested by copepods (see section 1.3 of chapter 1), it can be argued based on the results here that copepods do not only package algal material into rapidly sinking aggregates via grazing (Ebersbach and Trull 2008), they may also determine the C:N characteristics of the aggregated algal cells via differential assimilation of macromolecules. By depleting faecal pellets of nitrogen, as the results here (Figure 6) suggest, copepods may increase the stoichiometric ratio between C and N within the organic matter transported to the deep ocean. The impact of this on ocean – atmosphere carbon exchange could be significant since the strength of the biological carbon pump, which transfers ~ 10 GT of planktonic photosynthetic C (year)⁻¹ into the oceans interior (Boyd and Trull 2007) and without which atmospheric CO₂ levels would be 30% higher than in a pre-industrial world (Siegenthaler and Sarmiento 1993), hinges on the C:N ratio of seston (Omta et al. 2009). A more detailed, ecosystem-based, investigation is therefore required to fully ascertain how algal macromolecules and their effect on food ingestion, assimilation and metabolism by planktonic herbivores may impact the stoichiometric characteristics of particulate organic matter. A more detailed, ecosystem-based, investigation is therefore required to fully ascertain how food-quality-mediated trophic processes may impact the stoichiometric characteristics of particulate organic matter. Here, a heuristic value of the food quality model present under chapter 2 has been demonstrated, and hence could serve as a realistic basis for representing algal macromolecules and their effect on food ingestion, assimilation and metabolism by planktonic herbivores within theoretical models for investigating C and nutrient within aquatic ecosystems.

Chapter 4

Zooplankton growth: The interplay between temperature and prey biochemical composition

4.1 ABSTRACT

Temperature and prey biochemical composition are major controlling factors in zooplankton production. These two factors can interact to influence the dynamics of zooplankton production. While this has been shown by several experimental studies, it has been ignored or poorly captured in previous models for marine ecosystems in part because a realistic framework, for modelling the dependence of zooplankton production on the interaction between ambient temperature and prey biochemical composition is lacking. Here, I address this shortfall by proposing a new modelling framework. In the model, animals first evaluate the nutritional value of prey organisms based on their temperature-specific demand for energy and structural biochemical substances. They then regulate their feeding behaviour as well as metabolic physiology in order to satisfy their temperature specific biochemical requirements for maintenance and growth. The model realistically simulates growth rate, as well as gross growth efficiency of calanoid copepods, under variable temperature and food conditions. Optimum temperatures for egg production by two copepod species with different ranges of temperature tolerance were also realistically predicted. The results show that zooplankton growth can be maximized when copepods consume nitrogen-rich food, with the benefits being greatest at higher temperatures. This suggests that copepods may require nitrogen-rich food for successful reproduction at higher temperatures. The model indicates that the growth response of copepods to ambient temperature changes is driven mainly by temperature-induced changes in animals' demand for maintenance. Ecologically, this means that the minimum food concentration threshold required by copepods for reproduction may have a temperature dependence, which may be related to the cost of maintenance. Considering temperature effect on animals' biochemical requirement for maintenance may be therefore vital for understanding temperature constraints on zooplankton production.

4.2 INTRODUCTION

Although, a variety of environmental factors affect animal production, recent research has mainly focused on prey biochemical composition (Österblom et al. 2008; Müller-Navarra 2008) and temperature (Harley et al. 2006) as both affect ecosystem function via their impact on the rates of biochemical and physiological processes that influence individual and

population-level traits of organisms (Hochachka and Somer 1984; Johnston and Bennett 1996).

In zooplankton, the influence of temperature on population dynamics is well documented, especially in temperate seas (McLaren et al. 1989 and references therein). It has even been suggested that temperature alone can explain all the variability within *in situ* zooplankton production (Huntley and Lopez 1992). Similarly, the impact of prey biochemical composition on zooplankton production is well known (Kiørboe 1989; Jónasdóttir 1994; Müller-Navarra et al. 2000; Broglio et al. 2003). It has therefore been suggested that the dynamics of zooplankton production may be determined by an interaction between ambient temperature and prey biochemical composition (Cole et al. 2002). This has been confirmed by recent experimental studies (Masclaux et al., 2009; Isla et al., 2008). It has however been ignored or poorly captured in previous models for marine ecosystems in part because a realistic framework, for modelling the dependence of zooplankton production on the interaction between ambient temperature and prey biochemical composition is lacking. Typically, models for determining the effect of temperature on zooplankton production are based on functions that describe direct correlation between the growth of zooplankton and ambient temperature (e.g. Huntley and Lopez 1992; Kleppel et al. 1996; Hirst and Shearer 1997; Rinke and Petzoldt 2003; Dzierzbicka-Glowacka et al. 2009). These models are commonly developed based on laboratory or/and field observations of growth under different temperatures. Heuristically, such models are useful for studying general temperature effect on zooplankton production. However, they do not show the direct causal links between zooplankton production and temperature. Hence they have very little capacity for improving our understanding of how changes in ambient temperature might impact the crucial role of zooplankton in mediating carbon transfer between autotrophs and higher trophic level organisms.

Alternatively, others have modelled zooplankton production by linking temperature to processes such as grazing rate and metabolism (e.g. Ikeda and Motoda 1975; Stegert et al. 2007), or to the reaction kinetics of metabolic enzymes (Sharpe and DeMichele 1977). These models do not consider prey biochemical composition. Some (Stegert et al. 2007; Ikeda and Motoda 1975) also employ assumptions such as constant efficiencies for food assimilation and/or growth that are contrary to experimental observation (Iguchi and Ikeda, 2005). Clearly, a new approach for describing temperature effect on the rate of processes vital for the survival and production of zooplankton is needed.

This is particularly crucial, since both temperature and prey biochemical composition co-vary in many oceanic regions of the world, as changes in temperature also affect the biochemical content of microalgae (Thompson et al. 1992). Furthermore, the level of carbon dioxide (CO₂) in our atmosphere is expected to increase by about a factor of three, relative to the present value (of 380 µatm), by the middle of the next century (Houghton et al. 2001), with profound consequences for pelagic marine ecosystems and their functions (Harley et al. 2006). Among others, temperatures will increase (Wigley and Raper 2001) and water pH will decrease (Caldeira and Wickett 2003). Consequently, algal biochemical composition would change, which could shift algal C:N stoichiometry above today's Redfield C:N of 6.6 (Riebesell et al. 2007), and modify zooplankton traits such as trophic behaviour and physiology (Darchambeau 2005), population growth (Jones and Flynn 2005) and community structure (Guisande 2006).

The aim of this article is to present a new approach for incorporating temperature and prey biochemical into secondary production models. The approach is based on the assumption that temperature influences the biochemical requirements (i.e. energy and structural demand) of organisms and that the behavioural/physiological response of animals to temperature is driven by the need for them to satisfy their specific nutritional requirements for maintenance and growth (cf. Raubenheimer et al. 2009). Hence in my model, zooplankton organisms first evaluate the temperature-specific nutritional value of prey items based on their specific demand for energy and structural biochemical substances. They then regulate their grazing behaviour, food assimilation efficiency and metabolism in order to ensure continuous survival and growth. The impact of ambient temperature on zooplankton growth thus manifests itself via its effect on the animals' biochemical requirements for metabolic growth. To demonstrate the added value of this new approach, the model has been applied to simulate the combined effect of temperature and prey biochemical status on the dynamics of zooplankton growth. Furthermore, the model has been applied to investigate the effect of temperature on the rate for egg production by two Baltic Sea copepods: *Acartia tonsa* and *Temora longicornis*.

The abundance of these two copepods strongly varies with Baltic Sea temperature conditions (Möllmann et al. 2000). However, the two copepods prefer different temperatures for maximum growth (Holste and Peck 2006; Holste et al. 2009). Furthermore, adults of *Acartia* and *Temora* differ in their distributions within the Baltic Sea water column. While both copepods preferentially inhabit the upper 30 m (Hansen et al. 2006), *Temora* sometimes migrates below the permanent halocline (i.e. below 50 – 70 m) of the Baltic Sea (Schmidt 2006) where temperature and food conditions may be low. Poorly understood however are the

physiological mechanisms underlying the temperature tolerance differences between the two copepods, even though the Baltic Sea system is characterized by strong variability in temperature (Kahru et al. 1993; Matthäus and Schinke 1994; Lehmann et al. 2002; MacKenzie and Schiedek 2007) that could present organisms with considerable physiological challenge. Hence, my aim here is to elucidate potential physiological reasons for the temperature tolerance differences between *Acartia* and *Temora* by applying the proposed framework.

4.3 MODEL DESCRIPTION

Overview

Model parameters and variables are listed in Tables 1 and 2 respectively. Figure 1 is a schematic representation of the model. Copepods are defined by their structural chemical composition (β_i in adults, and δ_i for egg production, where i is the fraction of any biochemical substance in terms of C individual organisms), and by their requirement for energy (b for maintenance, and d for a unit egg production). These dictate the consumer's demand for metabolic substrates, which are met solely from dietary sources (α_i) via food ingestion (I_c) and assimilation (λ_i).

Temperature influences consumer's demand for energy and chemical substance, and thus the demand for metabolic substrates. Hence, at any temperature, the extent of food acquisition and processing is driven by the need to satisfy the specific requirement of the consumer. Food ingestion and assimilation are therefore variable, being indirectly determined by ambient temperature conditions. Similarly, the proportions of acquired substances that could be respired for energy to power maintenance (ρ_i) and growth (γ_i) are variable, dependent also on the temperature-specific requirements of the consumer. Zero reserve biomass for animals has been assumed, which in the case of some consumers, e.g. *Acartia tonsa*, is a reasonable assumption (Kuijper et al. 2004a). Thus in this model, nutritional excesses are not stored but released via "wastage respiration" (*sensu* Anderson et al., 2005) in accordance with the requirements of the animal. All substances are assumed to belong to one of three compound groups: proteins (P), lipids (L) and carbohydrates (H).

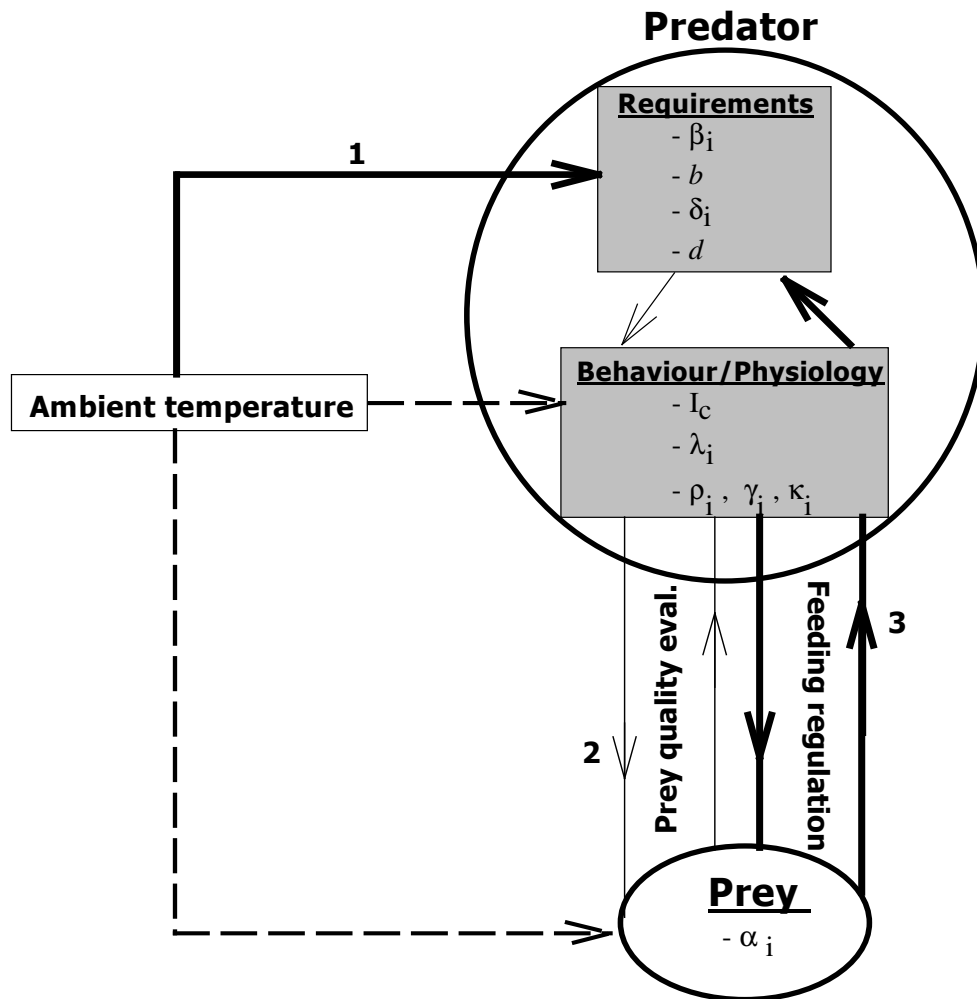


Figure 1. Model flow diagram, with numbers identifying the order of events in the model. Zooplankton organisms evaluate the nutritional value of prey items based on their temperature-specific demand for energy (b , d), and structural biochemical requirement (β_i , δ_i) for maintenance and growth. They then regulate food ingestion rate (I_c), assimilation efficiency (λ_i), and catabolism during maintenance (ρ_i) and growth (γ_i , x_i) in order to satisfy their specific biochemical requirement for metabolic growth. Subscript i represents proteins, lipids or carbohydrates; whereas α_i is the fraction of prey biomass made up of each macromolecule. The broken lines represent processes the model does not currently consider, such as temperature effect on: (1) prey biochemical composition (α_i) (Thompson et al. 1992), and (2) the reactivity of digestive and metabolic enzymes (e.g. Dutta et al. 2006). See text for further explanation. Symbols have been defined in Tables 1 and 2, as well as in the text.

Table 1. Model Parameters. See section 4.4 for details on how parameter values were calculated. dl = dimensionless. * = default values for determining nutritional quality of the prey.

Param.	Description	Unit	Value	Source	
Applies to both <i>Acartia tonsa</i> and <i>Temora longicornis</i>					
∂	Activation energy for metabolism	J	1.16E-19	Estimated	
I_{mg}	Maximum food ingestion rate	$\text{gC.gC}^{-1}.\text{d}^{-1}$	6.88*	Besiktepe & Dam 2002	
I_m	Minimum food ingestion rate	$\text{gC.gC}^{-1}.\text{d}^{-1}$	s	Fitted	
Z_{ing}	Food ingestion control parameter	dl	s	Fitted	
w_{mg}	Maximum prey capture efficiency	L.gC^{-1}	2500	Støttrup & Jansen 1990	
w_m	Minimum prey capture efficiency	L.gC^{-1}	s	Fitted	
Z_{ceff}	Prey capture control parameter	dl	s	Fitted	
λ_{mg}	Maximum food assimilation efficiency	dl	0.95*	Besiktepe & Dam 2002	
λ_m	Minimum food assimilation efficiency	dl	s	Fitted	
E_p	Energy content of protein	J.gC^{-1}	32312.64	Estimated	
E_L	Energy content of lipid	J.gC^{-1}	51439.43	Estimated	
E_H	Energy content of carbohydrate	J.gC^{-1}	42922.90	Estimated	
α_P	Protein fraction of total prey carbon	gC.gC^{-1}	0.26	Estimated	
α_L	Lipid fraction of total prey carbon	gC.gC^{-1}	0.69	Estimated	
α_H	Carbohydrate fraction of total prey carbon	gC.gC^{-1}	0.05	Estimated	
Applies to only <i>Acartia tonsa</i> when temperature does not affects lipid content of the animal					
d_0		JgC^{-1}	1377.96	Estimated	
Adult	Z_P	Required protein content of adult copepods	g.animal^{-1}	4.79E-6	Estimated
	Z_L	Required lipid composition of adult copepods	g.animal^{-1}	1.09E-6	Estimated
	Z_H	Required carbohydrate content of adult copepods	g.animal^{-1}	0.321E-6	Estimated

^sSee Table 4 for values used for simulations presented in Figures 3 to 8.

Table 1 continued

Param.	Description	Unit	Value	Source	
Applies to only <i>Acartia tonsa</i> when temperature does not affects lipid content of the animal					
Egg	Z_P	Required protein content of copepod eggs	g.animal^{-1}	7.52E-8	Estimated
	Z_L	Required lipid content of copepod eggs	g.animal^{-1}	4.21E-8	Estimated
	Z_H	Required carbohydrate content of copepod eggs	g.animal^{-1}	2.69E-9	Estimated
Applies to only <i>Temora longicornis</i> when temperature does not affects lipid content of the animal					
d_0		Energy cost for egg production at standard temperature	J gC^{-1}	1385.37	Estimated
Adult	Z_P	Required protein content of adult copepods	g.animal^{-1}	1.44E-5	Estimated
	Z_L	Required lipid composition of adult copepods	g.animal^{-1}	0.44E-5	Estimated
	Z_H	Required carbohydrate content of adult copepods	g.animal^{-1}	0.13E-5	Estimated
Egg	Z_P	Required protein content of copepod eggs	g.animal^{-1}	6.31E-8	Estimated
	Z_L	Required lipid content of copepod eggs	g.animal^{-1}	3.47E-8	Estimated
	Z_H	Required carbohydrate content of copepod eggs	g.animal^{-1}	2.23E-9	Estimated

4.3.1 Model formulation

Quantitative investigations of the exchange of biochemical substances between individual organisms and their environment involve, implicitly or explicitly, the derivation of biochemical budgets. At their simplest, biochemical budgets comprise an equation that relates intake to the post-ingestive fate of the ingesta (see for example Simpson and Raubenheimer 1995). That is, intake of a chemical substance is driven by the sum of the different requirements for that substance. i.e.

$$I_c \alpha_i \lambda_i = r_1 + r_2 + r_3 + \dots + r_N \quad (1)$$

Where, I_c is food ingestion rate, α_i is fraction of food constituted by a chemical i , λ_i is the assimilation efficiency for that compound, and r_1 to r_N represents the various uses for which a substance is required. Following established practice (e.g. Anderson 1992), chemical substances are assumed to be acquired for two main purposes, these being maintenance and growth.

Table 2. Model variables. dl = dimensionless; * indicates the quantity is a variable only when animals' structural requirement for lipid is influenced by temperature.

Variable	Description	Unit
T	Ambient temperature	K
b	Energy cost for maintenance	J.gC ⁻¹ .d ⁻¹
Z_L	Lipid content of zooplankton	g.animal ⁻¹
* β_i	Structural protein, lipid, or carbohydrate requirement of zooplankton for maintenance	gC.gC ⁻¹
d	Energy cost for growth	J.gC ⁻¹
* δ_i	Structural protein, lipid, or carbohydrate requirement of zooplankton for growth	gC.gC ⁻¹
V	Food concentration	gC.L ⁻¹
Q	Food quality	dl
I_{td}	Maximum threshold for C ingestion by zooplankton	gC.gC ⁻¹ .d ⁻¹
w	Prey capture efficiency	L.gC ⁻¹
I_c	C ingestion rate	gC.gC ⁻¹ .d ⁻¹
α_i	Protein-, lipid-, or carbohydrate-specific composition of total prey carbon	gC.gC ⁻¹
λ_i	Chemical-specific assimilation efficiency	dl
ρ_i	Fraction of assimilate catabolised for maintenance	dl
γ_i	Fraction of assimilate catabolised to power growth	dl
x_i	Fraction of assimilate catabolised for neither maintenance nor growth	dl
F_c or F_i	C or compound-specific defecation rate	gC.gC ⁻¹ .d ⁻¹
A_c or A_i	C or compound-specific assimilation rate	gC.gC ⁻¹ .d ⁻¹
M_c or M_i	C or compound-specific catabolism for maintenance	gC.gC ⁻¹ .d ⁻¹

Table 2 continued

Variable	Description	Unit
g_c or g_i	C or compound-specific catabolism to power growth	$\text{gC.gC}^{-1}.\text{d}^{-1}$
X_c or X_i	C or compound-specific catabolism for neither maintenance nor growth	$\text{gC.gC}^{-1}.\text{d}^{-1}$
G or G_i	C or compound-specific growth rate of zooplankton	$\text{gC.gC}^{-1}.\text{d}^{-1}$
K_c or K_i	C or compound-specific gross growth efficiency	gC.gC^{-1}
U_i	Maximum utilisation efficiency for each food chemical constituent	dl
L_i	Compound-specific potential to limit zooplankton growth	dl

Maintenance encompasses consumer's requirements for all non-growth processes such as thermoregulation (Bennett and Ruben 1979), ion transport across membranes (Milligan and McBride 1985), protein/biomass turnover (Mente et al. 2002) etc. These must be met before remaining substrates could be used for growth. Changes in any of these processes could influence demand for substrates and thus affect the overall compound budget of the consumer. However, here I employed an intermediate complexity approach to model parameterisation (e.g. Hannah et al. 2010) by separating maintenance requirement into two components: energy (b ; $\text{J.gC}^{-1}.\text{d}^{-1}$), and chemical-specific requirement (β_i ; gC.gC^{-1}) for the structure of zooplankton; this is akin to the dynamic energy budget (DEB) approach (Kooijman 1998) as described within chapters 2 and 3 of this thesis. Thus, b covers all the energy costs associated with maintenance (such as ion transport, locomotion). Conversely, β_i represents the structural requirement of zooplankton for specific substances (such as lipids for thermo-regulation; Farkas 1979; Nanton and Castell 1999; Hasset and Crockett 2009) and therefore, it ensures that the need by animals for specific substances is not compromised during catabolism. Given ρ_i as the fraction of assimilate that could be catabolised for maintenance energy, maintenance requirement of the consumer can be represented as:

$$b = \sum_{i=1}^n b_i = I_c \sum_i^n [\alpha_i \lambda_i \rho_i E_i] \quad (2)$$

$$\beta_i = \frac{I_c \alpha_i \lambda_i (1 - \rho_i)}{I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i)} = \frac{\alpha_i \lambda_i (1 - \rho_i)}{\sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i)} \quad (3)$$

$$\sum_{i=1}^n \beta_i = 1 \quad (4)$$

where E_i is the energy content (J.gC^{-1}) of each assimilated chemical.

After maintenance, the remaining substrates ($I_c \alpha_i \lambda_i (1 - \rho_i)$) are used for growth. As for maintenance, I have applied the DEB approach for the description of growth. Thus, growth budget comprises of temperature-specific budget for energy (d ; J.gC^{-1}) and structural requirements (δ_i ; gC.gC^{-1}) of the zooplankton. d represents the energy costs for producing a new zooplankton biomass. Conversely, δ_i defines the fraction of the new biomass that must be derived from individual compounds (hence, $\sum_{i=1}^n \delta_i = 1$). It is also employed here to ensure that specific structural biochemical requirement for new biomass formation is met during growth. Given γ_i as the fraction of assimilate that could be catabolised for energy to power growth without compromising δ_i , the process of growth is described using the following equations:

$$Gd = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) \gamma_i E_i \quad (5)$$

$$G = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) (1 - \phi_i) \quad (6)$$

$$\delta_i = \frac{I_c \alpha_i \lambda_i (1 - \rho_i) (1 - \phi_i)}{I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) (1 - \phi_i)} = \frac{\alpha_i \lambda_i (1 - \rho_i) (1 - \phi_i)}{\sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) (1 - \phi_i)} \quad (7)$$

where $\sum_i \delta_i = 1$, G represents total growth rate ($\text{gC.gC}^{-1} \cdot \text{d}^{-1}$) and $\phi_i = \gamma_i + x_i$ with x_i being the fraction of individual assimilates that would remain when growth ceases due to imbalance(s) in the diet of the zooplankton.

Equations 2 to 8 demonstrate the interdependencies between the requirements (structural or otherwise) of zooplankton and the processes of food ingestion, assimilation, and respiration. For example, as growth represents the balance between carbon intake and losses, intake and/or loss of carbon can as well be controlled by the consumers' demand for growth. Here, these interdependencies have been exploited to quantify the effect of temperature on individual life processes. Temperature has been assumed to influence only consumers' requirement for energy and lipids.

4.3.1.1 Temperature effect on biochemical requirements

Energy demand

In the model, animals expend energy only on maintenance and growth. Therefore any influence of ambient temperature on energy expenditure by the consumer is assumed to occur via b (the cost for maintenance) and d (the cost for producing a gram of egg carbon). Results from experimental studies however suggest that new biomass production is the dominant energy consuming process in zooplankton (Kiørboe et al. 1985; Thor 2003). Hence in my model, the influence of ambient temperature on energy requirement is determined via d . The magnitude of d at any temperature T (K) is described using equation 8, adapted from the universal temperature dependence function of Gillooly et al. (2001):

$$d = d_0 * \exp\left(\frac{\partial(T - T_0)}{kTT_0}\right) \quad (8)$$

where ∂ is process activation energy (J), and k is Boltzmann's constant ($J.K^{-1}$), whereas d_0 denotes the cost ($J.gC^{-1}$) for growth at standard temperature T_0 (assumed here to be 273.15 K).

The cost for the daily maintenance of animals in the model is calculated as one-third of that required for growth (i.e., $b = \frac{1}{3}d$; $J.gC^{-1}.d^{-1}$). This approach is based on results from several studies identifying that the oxygen consumption ratio between starved (not growing) and fed (growing) zooplankton is approximately 1:3 (Kiørboe et al. 1985; Tsuda 1994; Thor 2000, 2003) and an assumption that a fixed amount of energy is gained per every volume of oxygen respired ($\sim 20 J (ml O_2)^{-1}$; Makarieva et al. 2008).

Structural requirement

Based on experimental observations (Hassett and Crockett 2009; Sperfeld and Wacker 2009), I assumed that changes in ambient temperature influence structural demand for lipid by zooplankton, whereas animals' structural demand for protein and carbohydrates is independent of ambient temperature. The justification is that lipid is needed for maintaining membrane fluidity at colder temperatures (Farkas 1979; Kattner et al. 2007) and structural stability at warmer temperatures (Hassett and Crockett 2009). Here, the model is to be tested against egg production data on *Temora longicornis* and *Acartia tonsa* (Holste and Peck 2006; Holste et al. 2009). These copepods are not known to utilize lipid for warm-temperature adaptation (Hassett and Crockett 2009). Hence, only cold-temperature adaptation would be considered here.

The functional role of lipids in cold-temperature adaptation among zooplankton has been attributed mainly to phospholipids such as the polyunsaturated eicosapentaenoic acid

(EPA) and docohexanoic acid (DHA) (e.g. Farkas 1979). Here a simplifying assumption could be made that ambient temperature affects the total lipid requirement and not the specific lipid content of zooplankton. The justification is that the temperature for the solid–liquid phase transition of polyunsaturated phospholipids is not markedly lower than that of other lipids (Kattner et al. 2007 and references therein).

The functional nature of animals' structural lipid composition response to changes in ambient temperature is not known. Observations however suggest that zooplankton living at cold temperatures generally contain more lipids than their counterparts at relatively warmer waters (Farkas 1984; Nanton and Castell 1999; Lee et al. 2006). Hence, structural demand for lipid by animals can be inversely related to ambient temperature. This is described in my model using equation 9:

$$Z_L = Z_{Lmn} + (Z_{Lmx} - Z_{Lmn}) * \exp(-hT) \quad (9)$$

Where, Z_L represents structural demand ($\text{g}\cdot\text{animal}^{-1}$) for lipid at temperature T (K), Z_{Lmn} is minimum lipid requirement ($\text{g}\cdot\text{animal}^{-1}$) for ensuring optimum fitness (e.g. growth, reproductive success) at the warmest temperature the zooplankton can survive, whereas Z_{Lmx} is the maximum lipid requirement ($\text{g}\cdot\text{animal}^{-1}$) at the coldest temperature the animal can survive. The coefficient h (K^{-1}) defines the intensity (or slope) of the lipid demand in relation to changes in ambient temperature (Figure 2). Setting $Z_{Lmn} = Z_{Lmx}$ within equation 9 negates temperature effect on structural demand for lipid by animals. So for determining animals' structural requirement for proteins (Z_P ; $\text{g}\cdot\text{animal}^{-1}$), equation 9 could be used by assuming no difference between protein requirement at warm (Z_{Pmn} ; $\text{g}\cdot\text{animal}^{-1}$) and cold temperatures (Z_{Pmx} ; $\text{g}\cdot\text{animal}^{-1}$). Similarly, structural requirement for carbohydrates (Z_H ; $\text{g}\cdot\text{animal}^{-1}$) by zooplankton is determined by assuming no difference between warm (Z_{Hmn} ; $\text{g}\cdot\text{animal}^{-1}$) and cold temperature requirements (Z_{Hmx} ; $\text{g}\cdot\text{animal}^{-1}$).

Following this approach, temperature effect on the body weight of zooplankton (e.g. Corkett and McLaren 1978) could occur in my model via changes in the lipid content of the animals. Hence at every temperature, the relative contribution of each chemical substance to total carbon weight of the zooplankton during maintenance (β_i ; $\text{gC}\cdot\text{gC}^{-1}$) and growth (δ_i ; $\text{gC}\cdot\text{gC}^{-1}$) is calculated, assuming carbon constitutes 76% of lipids, 53% of proteins and 40% of carbohydrates (Ventura 2006). Here, I differentiate between biochemical composition requirement for maintenance and growth because biochemical demand for the two processes can differ. For example, growth among adults of some copepods (e.g. *Acartia*) is mainly the

production of eggs (Miller et al. 1977), which differ from adults in terms of their chemical composition (Koski 1999; Bruce et al. 2005).

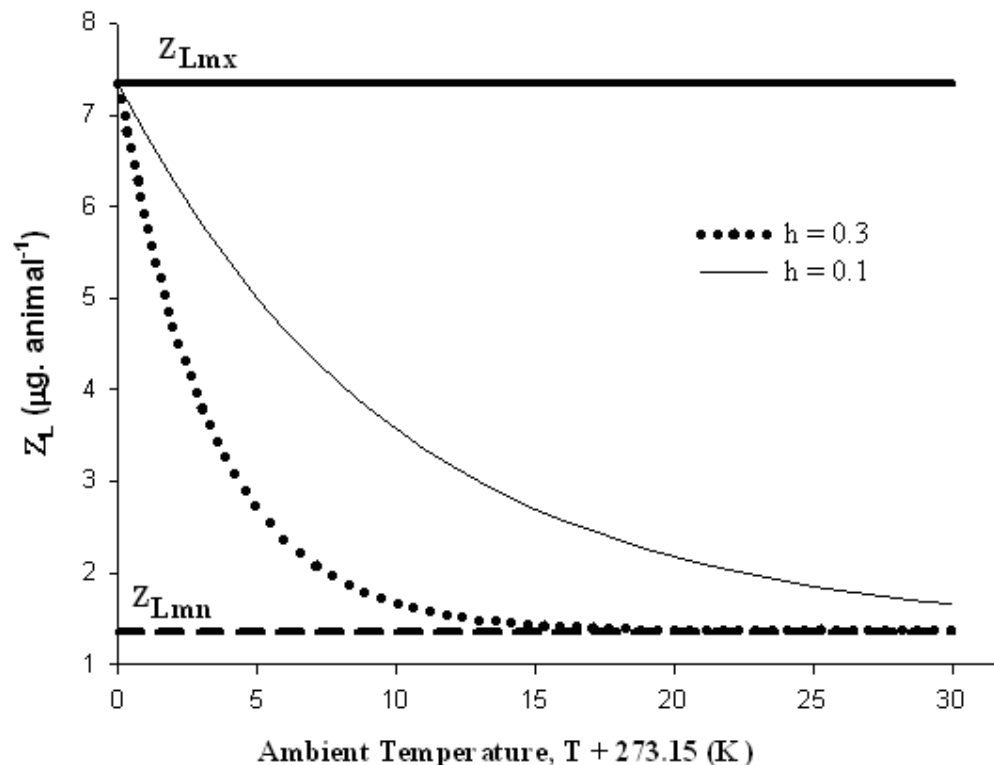


Figure 2. The effect of the auxiliary variable (h , K^{-1}) on the relationship between ambient temperature and structural lipid content (Z_L , $\mu\text{g.animal}^{-1}$) of zooplankton. Z_{Lmn} and Z_{Lmx} represent structural lipid requirements ($\mu\text{g.animal}^{-1}$) for surviving at warm and cold temperatures respectively. See text associated with equation 9 for further details

4.3.1.2 Temperature effect on feeding and metabolism

Temperature induced changes in the demand for energy and structural lipids, could be expected to affect animals' feeding behaviour and/or metabolic physiology. This is obvious from equations 2 to 7, and has been experimentally observed (Isla et al. 2008; Sperfeld and Wacker 2009). So food ingestion rate (I_c), assimilation (λ_i) and respiration (ρ_i , γ_i and x_i) of individual substances were regulated in order to satisfy temperature-specific requirements of the consumer. This was done as described under chapter 3 of this thesis. Here, only a summary of the approach has been provided for the benefit of readers interested in only this section of the thesis.

The manifestation of feeding and physiological processes are determined by the growth limiting potential of the available prey, which is dependent on intake as well as metabolic capacity of consumer at any temperature. In the model, b , d , β_i and δ_i set the metabolic capacity of the consumer, which together with the maximum food intake capacity ($\equiv I_{mg} \lambda_{mg}$, with I_{mg} and λ_{mg} being respectively maximum ingestion rate and assimilation efficiency) of the consumer define the potential efficiency ($0 \leq U_i \leq 1$) with which individual diet constituents can be used for growth (see text associated with equation 2 of Chapter 3). $U_i = 0$ when an assimilate does not form part of the animal structure and is entirely used for only energy production. This is however not expected in this study. Conversely, $U_i = 1$ when a substrate is provided below the consumer's structural requirement and so is not respired but spared for growth. $0 < U_i < 1$ means the availability of a compound exceeds consumer's requirement. Consumers can use or discard these chemical excesses, the consequence of which will adversely impact the efficiency with which substrates could be used for growth.

Therefore, the growth-limiting potential (L_i) of each diet constituent was calculated as the difference between its current U_i value and 1 using equation 10 (cf. Equation 3 of Anderson and Pond 2000).

$$L_i = \min(U_i, 1) \quad (10)$$

L_i values for individual substances were then used to define food quality (Q), which for a prey containing chemicals i to n was determined as

$$Q = \min(U_i, U_j, \dots, U_n, 1) \quad (11)$$

Consequently, $Q = 1$ (\equiv good quality food) when there is a perfect balance (of 1, which is possible only theoretically) between prey biochemical composition and temperature-specific requirements of zooplankton. Conversely, $Q = 0$ (\equiv bad quality food) when a prey has no nutritional value for the growth of the zooplankton at the temperature under consideration.

It was expected that any temperature induced changes in b , d , β_i and δ_i would affect Q . In other words, the energy and chemical consumption capacity of the consumer changes with temperature, which then influences how the consumer anticipates the utility of prey constituents. Therefore during grazing, prey capture efficiency (w , L.gC^{-1}) and the maximum threshold for food ingestion (I_{td} , $\text{gC.gC}^{-1}.\text{d}^{-1}$) are determined by the potential quality of the prey. Both parameters vary between a minimum value (I_m for I_{td} ; w_m for w) needed for food consumption to ensure survival when the available prey has no nutritional

value for growth (i.e. $Q = 0$), and a maximum value (I_{mg} for I_{td} ; w_{mg} for w) for grazing in the presence of an “ideal” prey (i.e. $Q = 1$). According to Mariani and Visser (2010), an evolutionary consistent behaviour is that which optimises the fitness (e.g. growth, reproductive success) of consumers. Hence in the model, a function configured to enhance ingestion of high quality prey was used, with the intensity of grazing response to food quality being determined by a parameter Z_{ing} . Similarly, prey capture efficiency was configured to enhance the capture of a good quality prey using the parameter Z_{ce} . Finally, total food ingestion rate (I_c) was calculated using an Ivlev (1955) function for a single prey system (see text associated with equations 5, 6 and 7 of chapter 3).

After the zooplankton ingests the prey, assimilation of the food takes place. It is assumed that the efficiency (λ_i , dimensionless) at which an ingested chemical is assimilated for metabolism depended on the potential (L_i , dimensionless) for that chemical to limit the growth of zooplankton and a parameter η (dimensionless) that defined the extent of the response; this assumption is consistent with previous studies (Mitra 2006). In the model, λ_i varied between a minimum efficiency (λ_m , dimensionless) required for only maintenance when $Q = 0$ and a maximum efficiency (λ_{mg}) needed for maximum growth when $Q = 1$. The actual value of λ_i was determined using a function configured to maximize the uptake of limiting substances and to moderate that of non-limiting ones (see text associated with equation 8 of chapter 3). After this, the respective assimilation efficiencies for individual substances were used in equation 12 to calculate the total amount of assimilated carbon (A_c). Unassimilated carbon was egested as faecal pellets, F_c (equation 13).

$$A_c = I_c \sum_{i=1}^n \alpha_i \lambda_i \quad (12)$$

$$F_c = I_c \sum_{i=1}^n \alpha_i (1 - \lambda_i) \quad (13)$$

Respiration of substrates for energy is considered a mechanism for removing excess assimilated substrates. Catabolism of substances for energy was therefore regulated in order to satisfy consumer’s temperature specific requirements for chemical substances. When food supply exceeds maintenance requirement, maintenance and growth can occur concurrently. When this happens, energy as well as material requirements for maintenance (equations 2 and 3) and growth (equations 5, 6 and 7) would be met simultaneously. Due to the non-linear

nature of this problem, the fraction of each substrate respired was calculated iteratively by determining ρ_i , γ_i and x_i that meet consumer's temperature requirements for maintenance and growth (remember $\phi_i = \gamma_i + x_i$). Thus, energy demand, b , for maintenance at any temperature is attained by catabolising a specific mass (m_i ; $\text{gC}\cdot\text{gC}^{-1}\cdot\text{d}^{-1}$) of each substance assimilated via the following equation:

$$m_i = I_c \alpha_i \lambda_i \rho_i \quad (14)$$

Total food catabolism for maintenance is then described by equation 15, as the sum of the solutions to equation 14 for different chemicals.

$$m_c = I_c \sum_{i=1}^n \alpha_i \lambda_i \rho_i \quad (15)$$

Dividing equation 15 with I_c gives the total fraction of ingested food that is catabolised for maintenance. Similarly, total energy demand, Gd , for total growth at any temperature is attained by catabolising a specific mass (g_i ; $\text{gC}\cdot\text{gC}^{-1}\cdot\text{d}^{-1}$) of each substance assimilated via the following equation:

$$g_c = I_c \alpha_i \lambda_i (1 - \rho_i) \gamma_i \quad (16)$$

Total food catabolism for growth is then described by equation 17, as the sum of the solutions to equation 16 for different chemicals.

$$g_c = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) \gamma_i \quad (17)$$

Dividing equation 17 with I_c gives the total fraction of ingested food that is catabolised for growth.

Given x_i as the proportion of individual substances that would remain when growth ceases due to imbalance(s) in the diet of the zooplankton, the quantity of individual substances and total C that may neither be used for maintenance nor growth could be calculated respectively as

$$X_i = I_c \alpha_i \lambda_i (1 - \rho_i) x_i \quad (18)$$

$$X_c = I_c \sum_{i=1}^n \alpha_i \lambda_i (1 - \rho_i) x_i \quad (19)$$

Within the zooplankton community, the fate of these nutritional excesses varies widely (e.g. Darchambeau et al. 2003; Jensen et al. 2006). This is however beyond the scope of this article. It was therefore assumed that food that is in surplus to that required by animals at any given temperature has no nutritional value and thus is voided via respiration decoupled from both maintenance and growth, the so-called "wastage respiration" (Anderson et al. 2005). As

a result, dividing equations 19 with I_c gives the fraction of total ingested C that is voided to satisfy the structural biochemical requirement of animals.

4.4 PARAMETER DETERMINATION AND MODEL APPLICATION

Unless stated, parameters employed here have the same values as those under chapter 3 of this thesis. As mentioned above, it is the aim of this study to investigate the effect of temperature on two co-occurring copepods: *Acartia tonsa* and *Temora longicornis*. Some parameter values are therefore specific to the individual species. *T. longicornis* has been classified as temperate copepod (Krause et al. 1995) but has a wide latitudinal range spanning from relatively warm Mediterranean (Halsban-Lenk et al. 2002) to colder Arctic waters (Chikin et al. 2003) where lipid demand for thermo-regulation by zooplankton is high (Kattner et al. 2007). Conversely, *A. tonsa* mostly inhabits temperate (Castro-Longoria 2003) to tropical waters where the lipid content of organisms is usually low (Lee et al. 2006). Based on these observations, one could question whether the physiological acclimation of *Acartia* to cold temperatures is different from that of *Temora*? To investigate this, here I conducted two scenario runs of the model: (1) temperature affects only energy requirement (Exp 1), and (2) temperature affects energy as well as structural lipid requirement of animals (Exp 2). The results are compared with egg production data of Holste et al. (Holste and Peck 2006; Holste et al. 2009). Consequently, β_i is calibrated to represent the biochemical composition of female copepod, while δ_i represents that of copepod eggs. Similarly, b is calibrated to represent the energy requirement for the maintenance of female copepod, while d represents the cost for producing an egg carbon.

The parameters for the minimum threshold of feeding behaviour (i.e. w_m , I_m and λ_m), and the intensity of the feeding response to food quality (Z_{ing} , Z_{ce} and η) could not be determined from empirical studies (see chapter 3 for the justification). Similarly, parameters for determining the optimum structural demand for total lipid (i.e., Z_{Lmn} , Z_{Lmx} , and h) were not determined from empirical data. These parameters were therefore determined by fitting the model to experimental data of Holste et al (Holste and Peck 2006; Holste et al. 2009). Specifically, the growth response of the copepod to changes in ambient temperature was simulated and compared with the experimental data of Holste et al. in order to determine the parameter values that could best describe the dynamics of egg production at all experimental temperatures. The best parameter values were taken as those that gave the least

sum of square errors between model predictions, and the observation (Haefner 2005). Other parameters for the model were determined as described below.

Biochemical composition of copepod adults and eggs

Protein (P), lipid (L) and carbohydrate (H) composition of female copepods and their eggs were estimated as described in chapter 2 of this thesis using a relationship between animal's dry weight and their total content of individual biochemical substance. Assumed dry weight for eggs and females of *Acartia tonsa* were 0.12 μg and 10.5 μg respectively (Kiørboe, 1989). Assumed dry weight for eggs and females of *Temora longicornis* were 0.1 μg and 33.45 μg respectively (Kreibich et al. 2008; Huntley and Lopez 1992).

In *Acartia* female, P, L and H were respectively determined to constitute 45.62, 10.38 and 3.1 % of dry weight. For *Temora* females, P, L and H constituted respectively 43.0, 13.2 and 4.0 % of dry weight. These give a total biochemical composition of 59.1 and 60.2 % in females of *Acartia* and *Temora* respectively. The remaining parts of female dry weight were taken to be substances not currently represented in the model, such as chitin. Eggs of *Acartia tonsa* were determined to be made up of 62.64, 35.12 and 1.78 % of P, L and H respectively. For *Temora* eggs, P, L, and H constituted 63.1, 34.7 and 1.8% of dry weight. These weights were converted into C, assuming C constitutes 53%, 75% and 40% respectively of P, L, and H in zooplankton (Ventura 2006). Following this assumption, P:L:H carbon mass ratios requirement for the structure of female copepods (i.e. $\beta_P : \beta_L : \beta_H$) were determined to be 0.73:0.24:0.04 for *Acartia* and 0.66:0.29:0.05 for *Temora*. Assuming protein has a C:N ratio (by mass) of 3.2 (Vollenweider, 1985), $\beta_P : \beta_L : \beta_H$ ratios translate into biomass-specific C:N ratio of 4.4 for *Acartia* and 4.8 for *Temora*, similar to what has been used in other studies (Touratier et al. 1999; Kuijper et al. 2004a; Mitra 2006). P:L:H ratios within eggs (i.e. $\delta_P : \delta_L : \delta_H$) were approximately 0.55:0.44:0.01 for both copepod species, which gives an equivalent C:N ratio of 5.8 for eggs, which is comparable with what has been used by others (Touratier et al. 1999; Kuijper et al. 2004a; Mitra 2006). These ratios were therefore taken as the default biochemical composition of animals, and were used unaltered when the effect of temperature on only energy expenditure by animals was investigated (i.e., Exp 1).

The model approach requires $\beta_P + \beta_L + \beta_H = 1$ and $\delta_P + \delta_L + \delta_H = 1$. The body weight of zooplankton however, could increase in the model (see text associated with equation 10). So whenever temperature affected the structural lipid requirement of the animals

(i.e., Exp 2), the relative contribution of each chemical substance to total carbon weight of the zooplankton during maintenance (β_i ; gC.gC⁻¹) and growth (δ_i ; gC.gC⁻¹) is calculated.

Activation energy for metabolism, ∂

Typically, oxygen consumption rate is used as a measure of metabolism in aerobic animals (e.g. Ikeda et al. 2001). Oxygen consumption V_o (volume individual⁻¹ time⁻¹) by female copepods was therefore used to determine the activation energy for metabolisms. Like all chemical processes, the rate of respiration depends on three major factors (Gillooly et al. 2001):

$$V_o \approx f(F_1, F_2, F_3) \quad (20)$$

where F_1 is substrate concentration, F_2 is flux of substrates and F_3 is the kinetic energy of the metabolic system. F_1 and F_2 are constrained by substrate supply and product removal rates, which are dependent upon the size of the organism. Because of allometric constraints on exchange surfaces and distribution networks (West et al. 1997; Enquist et al. 1998), the product of these two terms scales with body mass, ω . Thus

$$V_o \approx \omega^n \quad (21)$$

Temperature influences oxygen consumption through its effects on rates of metabolic reactions. This occurs via F_3 , whose effect on chemical reactions is governed by the Boltzmann factor $e^{-\partial/kT}$ (Gillooly et al. 2001). Thus

$$V_o \approx e^{-\partial/kT} \quad (21)$$

The combined effect of body weight and ambient temperature on oxygen consumption can therefore be approximated as

$$V_o \approx \omega^n e^{-\partial/kT} \quad (22)$$

From this, weight-specific oxygen consumption (V_m) by an animal could be represented using the relation below.

$$V_m \approx \frac{V_o}{\omega^n} \approx e^{-\partial/kT} \quad (23)$$

This consolidates almost all temperature effects on oxygen consumption into a single function with activation energy, ∂ , for oxygen consumption and by extension metabolism, being the only unknown quantity.

Following Gillooly et al. (2001), ∂ was determined from the slope ($= -\partial/k$) of an Arrhenius plot of V_m versus T . Published data on zooplankton females held at different temperatures were used (see Appendix 4). Only studies that reported respiration rate as volume individual-mass⁻¹ time⁻¹ or as volume time⁻¹ with reports of, or adequate information for estimating animals' dry weights were chosen.

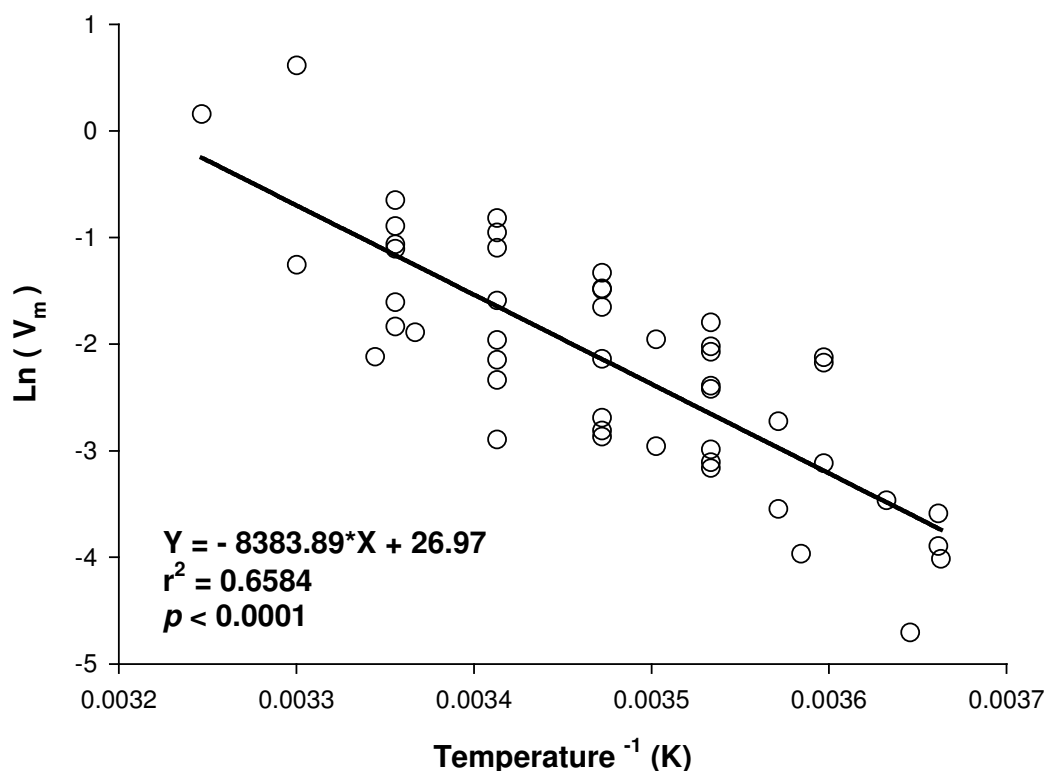


Figure 3. Effect of temperature on mass normalized oxygen consumption rate (V_m , $\mu\text{l O}_2 \cdot \mu\text{gDw}^{-1} \cdot \text{d}^{-1}$) of female copepods. See appendix 4 for source of data.

Several factors, including crowding, light, prey biochemical composition etc. affect oxygen consumption rate by animals (e.g. Omori and Ikeda 1984; Thor et al. 2002). However, these factors are rarely standardized in experimental studies to enable an inter-comparison of results. As a consequence, only a criterion that animals must not be food-quantity limited during oxygen uptake measurements was used for data selection. To satisfy this, data were taken only if organisms were cultured under food levels not below $1000 \mu\text{gC L}^{-1}$, a food concentration at which zooplankton growth is unlimited (Kiørboe et al. 1985). This was necessary to ensure that growth was not limited in the animals and that the reported oxygen

consumption measurements encompass respirations resulting from all biological activities (i.e. growth and non-growth processes). This criterion limited the number of published reports that could have otherwise been used. The Arrhenius plot of V_m versus T revealed a significant relationship with a slope of -8383.89 ± 890.33 K (mean \pm SE) (Figure 3). This gives average activation energy of $1.16\text{E-}19$ J for metabolism in female copepods (assuming Boltzmann constant, k , is $1.38\text{E-}23$ JK^{-1}), which is comparable with the average of 0.6 eV reported for aerobic metabolism in general (Gillooly et al. 2001: assuming 1 eV = $1.6\text{E-}19$ J).

Table 3. Estimation of egg formation cost at standard temperature for *Acartia tonsa*. P, H and L represent protein, carbohydrate and lipid respectively. Letters A to E show how calculations were done. E denotes energy per mol of ATP (assumed to be 305694.48 J; Campbell 1993). Values in parenthesis represent data for *Temora longicornis*. See text for source of data on egg biochemical composition and for further explanation.

Chem.	Percent composition	ng egg ⁻¹ A	°Monomer mol weight B	°mol ATP mol ⁻¹ monomer C	ATP egg ⁻¹ (mol *10 ⁻¹⁰) D = A*C/B	Cost egg ⁻¹ (J*10 ⁻⁵) D*E
P	62.64 (63.10)	75.17 (63.10)	112	4	26.84 (22.53)	8.21 (6.89)
H	2.24 (2.23)	2.69 (2.23)	162	2	0.33 (0.28)	0.10 (0.08)
L	35.12 (34.67)	42.14 (34.67)	535.75	7.25	5.70 (4.69)	1.74 (1.43)
Total	100.00 (100.00)	120 (100.00)	809.75	13.25	32.88 (27.50)	10.05 (8.41)

° Data extracted from Kiørboe et al. (1985). Lipid value is mean of all lipids-groups in Table 5 of the paper.

Energy cost for metabolism at standard temperature

The cost for a unit egg production at standard temperature, represented here by d_o , was determined based on the biochemical content required for eggs at different temperatures. Therefore, d_o varied with δ_i . Following previous studies (Kiørboe et al. 1985; Bämstedt et al. 1999), d_o was determined based on the amount of ATP required for egg formation. Table 3 contains a summary of the approach. All monomers required for biosynthesis were assumed to be derived from dietary sources. For the synthesis of protein and carbohydrates, I used the same monomer constituents as previously used by Kiørboe et al. (1985). Lipid, on the other hand, consists of a wide range of structurally different molecules with very different chemical

properties, but no repeating unit or monomers as in the chemical sense of the word. Therefore the conversion factor for lipid was based on the average of all value for the different lipid groups in Table 5 of Kiørboe et al. (1985). A mol of ATP was assumed to contain 7.3 kcal (\equiv 305694.8 J) of energy (Campbell 1993).

d_o was estimated to be $10.05 \cdot 10^{-5}$ J egg⁻¹ for *Acartia tonsa*, which is \sim 4.4 nmol ATP more than that determined for the same species by Kiørboe et al. (1985) based on the biochemical composition of other crustacean eggs. For *Temora longicornis*, d_o was estimated to be $8.41 \cdot 10^{-5}$ J egg⁻¹. Based on the assumed carbon content of biochemical substances, the per carbon energy requirement for egg production was determined to be 1377.96 and 1385.37 J. gC⁻¹ for *Acartia* and *Temora* respectively. These were taken to represent energy cost for the formation of eggs at standard temperature when lipid demand for the structure of the copepods was independent of ambient temperature (i.e., Exp 1). Whenever temperature affected egg lipid content (i.e., Exp 2), d_o was recalculated as described in Table 3 to reflect the changes in egg biochemical composition.

As mentioned above, the cost for maintenance at any temperature was calculated as one-third of that required for growth. The validity of this assumption has been demonstrated by several authors for a variety of zooplankton species (Kiørboe et al. 1985; Tsuda 1994; Thor 2000, 2003).

Prey biochemical composition

Rhodomonas sp. was the prey used in the experimental studies against which this model has been tested (see Holste and Peck 2006; Holste et al. 2009). The equivalent spherical diameter of this cryptophyte was assumed to be 6.5 μ m (Broglia et al. 2003), from which an algal cell volume of 143.7933 μ m³ was calculated. Carbon content (cell⁻¹) of the algae was estimated to be \sim 26.94 pg using equation 24, where S_f and C_f represent size (μ m³) and carbon (pg) per algal cell respectively.

$$\log C_f = a_1 \cdot \log S_f - \log a_2 \quad (24)$$

The relationship and constants “ $a_1 = 0.89$ ” and “ $a_2 = 0.49$ ” were determined as shown under appendix 5 of this thesis.

Total P, L and H contents (pg cell⁻¹) of the algae were also estimated using equation 24, but with C_f replaced by either P, L or H; S_f replaced by C_f ; the constant values are listed in Table 3.

Table 3. Constant values for the equation (24) describing the relationship between carbon and macromolecular biochemical content (pg cell^{-1}) of microalgae.

Biochemical substance	Constant values for equation 24	
	a_1	a_2
Protein	1.12	0.53
Lipid	1.65	1.02
Carbohydrate	1.10	1.14

These constants were determined based on published biochemical composition data for 68 phytoplankton species belonging to 8 different taxonomic groups. Details of the study can be found under appendix 2 of this thesis. Using this approach, P, L and H contents of the phytoplankton were respectively determined to be 11.80, 21.88 and 2.56 pg . Again with the assumed carbon fractions for individual compounds, P:L:H carbon mass ratios of 0.26:0.69:0.05 (i.e. $\alpha_P : \alpha_L : \alpha_H$) for *Rhodomonas* was determined.

Model implementation

The growth response of zooplankton to changes in ambient temperature is typically unimodal, with growth being highest only when temperature is optimum for growth (Holste and Peck, 2006 and references therein). To determine the possible behavioural/physiological basis for such a response, experimentally observed temperature-induced changes in egg production by *Acartia tonsa* (Holste and Peck 2006) and *Temora longicornis* (Holste et al. 2009) were simulated. Model-predicted feeding rates as well as the fate of ingested food were taken to reflect that of the animals under the experimental conditions. In both experimental studies employed for comparison, copepods were supplied with saturating amounts of the prey. A saturation food concentration of $1000 \mu\text{gC L}^{-1}$ (Kiørboe et al. 1985) was therefore assumed for the simulations.

Before initiating feeding, the model copepod first determines the quality (Q) of each prey, and growth limiting potential (L_i) of prey biochemical constituents based on its maximum capacity for food uptake (I_{mg} and λ_{mg}) as well as temperature-specific requirement for energy (b and d) and structural biochemical substances (β_i and δ_i). The equation (12) that describes food quality is not linear and can only be solved by

simultaneously satisfying the conditions for maintenance (i.e. equations 3 to 5) and growth (6 to 8). So during food quality determination, I_c was assigned the value of I_{mg} by setting $I_m = I_{mg}$ and $w_m = w_{mg}$ as described in chapter 3 of this thesis. Similarly, λ_i was assigned the value of λ_{mg} during food quality determination by setting $\lambda_m = \lambda_{mg}$. These were assumed to define the maximum sustainable rate of material flow through the copepods at any temperature. This assumption was necessary for simulating the potential impact of prey constituents on consumers. Since $I_{mg} > b / \sum_i^n \alpha_i \lambda_{mg} E_i$, energy as well as material requirements for maintenance (equations 2, 3 and 4) and growth (equations 5, 6 and 7) were calculated simultaneously using an iterative approach. This procedure predicts ρ_i , γ_i and x_i values that meet the requirements for maintenance and growth. U_i was afterwards determined. The same procedure as described in chapter 2 of this thesis was followed. The predicted U_i was then substituted into equations 11 and 12 respectively to determine L_i and Q . Temperature affect on lipid content, and hence on the biochemical composition of animals (β_i and δ_i) was determined prior to the determination of Q and L_i . As prey biochemical composition is assumed fixed, the calculated Q and L_i values indicated the potential impact of ambient temperature on growth as anticipated by the copepods. Food ingestion, assimilation, and respiration were then altered following the approach described in chapter 3 in order to maximise growth at any temperature.

4.5 RESULTS

Here, changes in ambient temperature may affect copepods' energy expenditure and/or lipid composition depending on the ambient temperature. These were investigated by running the model under two scenarios: (1) temperature affects only energy requirement (Exp 1), and (2) temperature affects energy as well as lipid requirement of animals (Exp 2). Values of all tuned parameters are listed in Table 4.

Acartia egg production and temperature

Figure 4 shows model results when temperature influences only energy demand of *Acartia tonsa* (Exp 1). Results show the cost for maintenance (b) and growth (d) increased exponentially with increasing temperature (Figure 4A), consistent with experimental observation (Gaudy et al. 2000; Ikeda et al. 2001).

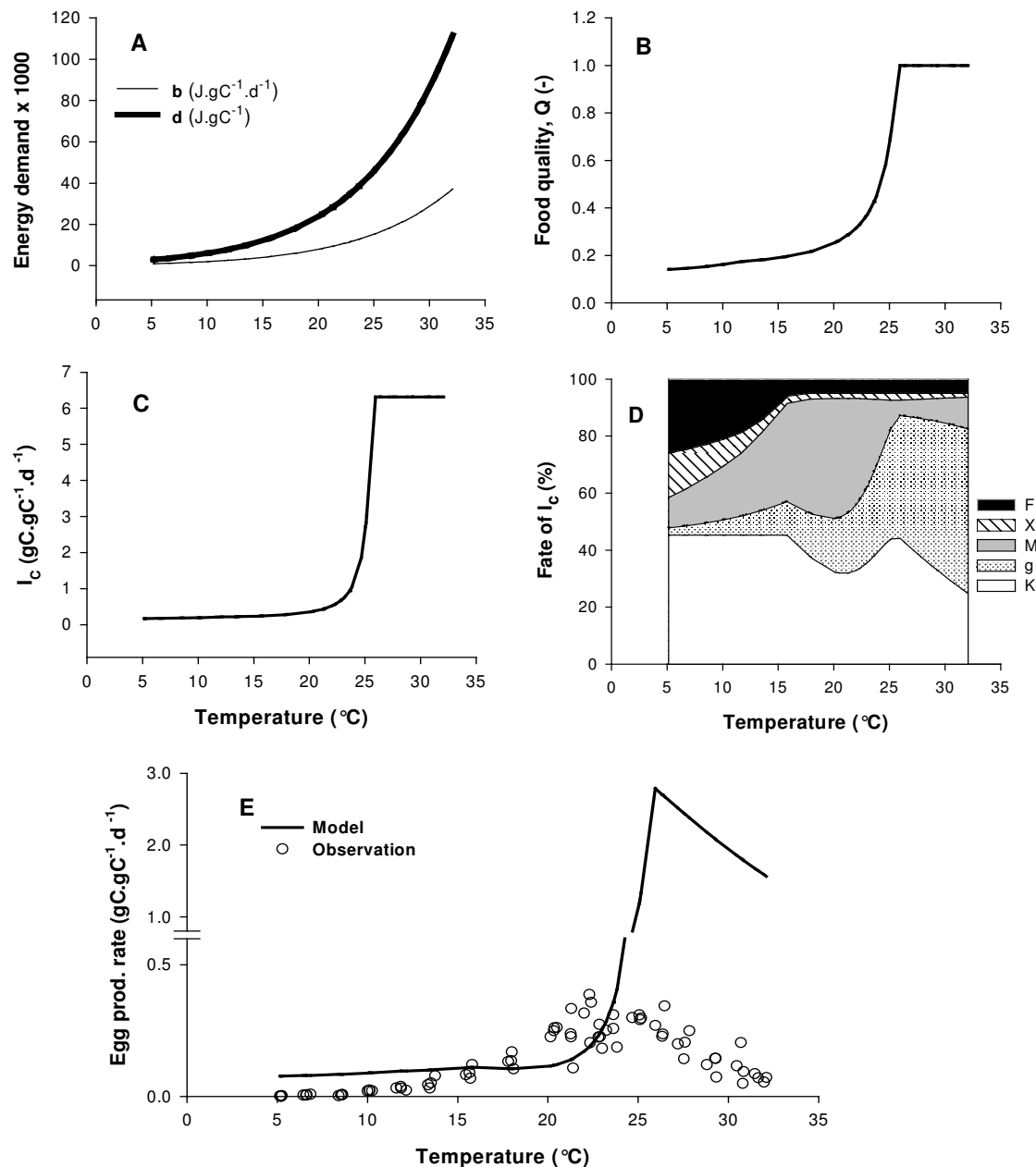


Figure 4. Model predictions when temperature influences only energy demand by *Acartia tonsa*. Results represent simulations when maximum threshold rate for food uptake (i.e., $I_{mg}\lambda_{mg}$), and activation energy for metabolism (∂) were input parameters. Predicted temperature effect on (A) energy demand for maintenance [b] and growth [d], (B) nutritional value of the prey, (C) carbon ingestion rate, I_c , (D) metabolic fate of the ingested carbon, and (E) carbon-specific growth rate. Open circles represent egg production data of Holste and Peck (2006). Fractions of subplot D are F = carbon released as faecal pellets; X = excess unutilized carbon; M = carbon respired for maintenance; g = carbon respired for energy to power growth; K = fraction of ingested carbon converted into eggs.

This led to an increase in the nutritional value (i.e. Q) of the algae, until the maximum nutritional value of one was achieved at $\sim 26^\circ\text{C}$ (Figure 4B). As a consequence, food ingestion rate (I_c) increased with temperature, again until the maximum is achieved (Figure 4C). This result is in agreement with observations demonstrating that under non-limiting food conditions, food ingestion rate increases with temperature (Deason 1980; Isla et al. 2008). Consistent with experimental observations (Deason 1980), carbon assimilation efficiency also increased with temperature. As a result, carbon egestion as faecal pellet decreased with increasing temperature (Figure 4D).

As expected, the metabolic fate of carbon assimilated by the copepod also varied with temperature (Figure 4D). Carbon catabolism for energy increased with ambient temperature, due to temperature effect on the cost of metabolism (both maintenance and growth). As a consequence, the fraction of ingested carbon that was voided in order to satisfy the structural biochemical requirement of the copepod decreased with increasing temperature. This led to a moderate fit between model and observation at warmer temperatures $> 23.6^\circ\text{C}$ (Figure 4E), with $\sim 51\%$ of the observed variations in egg production with temperature being explained by the simulation (regression of predicted versus observed egg production rates at temperature $> 23.6^\circ\text{C}$: gradient = 1.21 ± 0.17 , $r^2 = 0.5067$, $p < 0.0001$). Above this temperature however, the model significantly overestimated egg production by *Acartia*. Re-examination of the model revealed growth at higher temperatures to be significantly influenced by the parameters I_{mg} , λ_{mg} and ∂ . The modelling procedure was consequently altered by relying on the parameter estimation procedure described above (see section on model implementation) to determine I_{mg} , λ_{mg} and ∂ . The result was a significant improvement in model prediction over the entire temperature range (Figure 5E), with $\sim 62\%$ of the variation in the observed growth rate being explained by the model (gradient = 1.15 ± 0.10 , $r^2 = 0.6232$, $p < 0.0001$). This result was achieved with an I_{mg} of 1.0 day^{-1} , which is comparable with the value typically used in zooplankton models (e.g. Anderson et al. 2005). λ_{mg} was also determined to be 0.8 (dimensionless), comparable with the maximum food assimilation efficiency reported by Conover (1966). The estimated value of ∂ was $1.47\text{E-}19 \text{ J}$, which is close to the maximum activation energy of $1.92\text{E-}19 \text{ J}$, reported for aerobic metabolism (Gillooly et al. 2001).

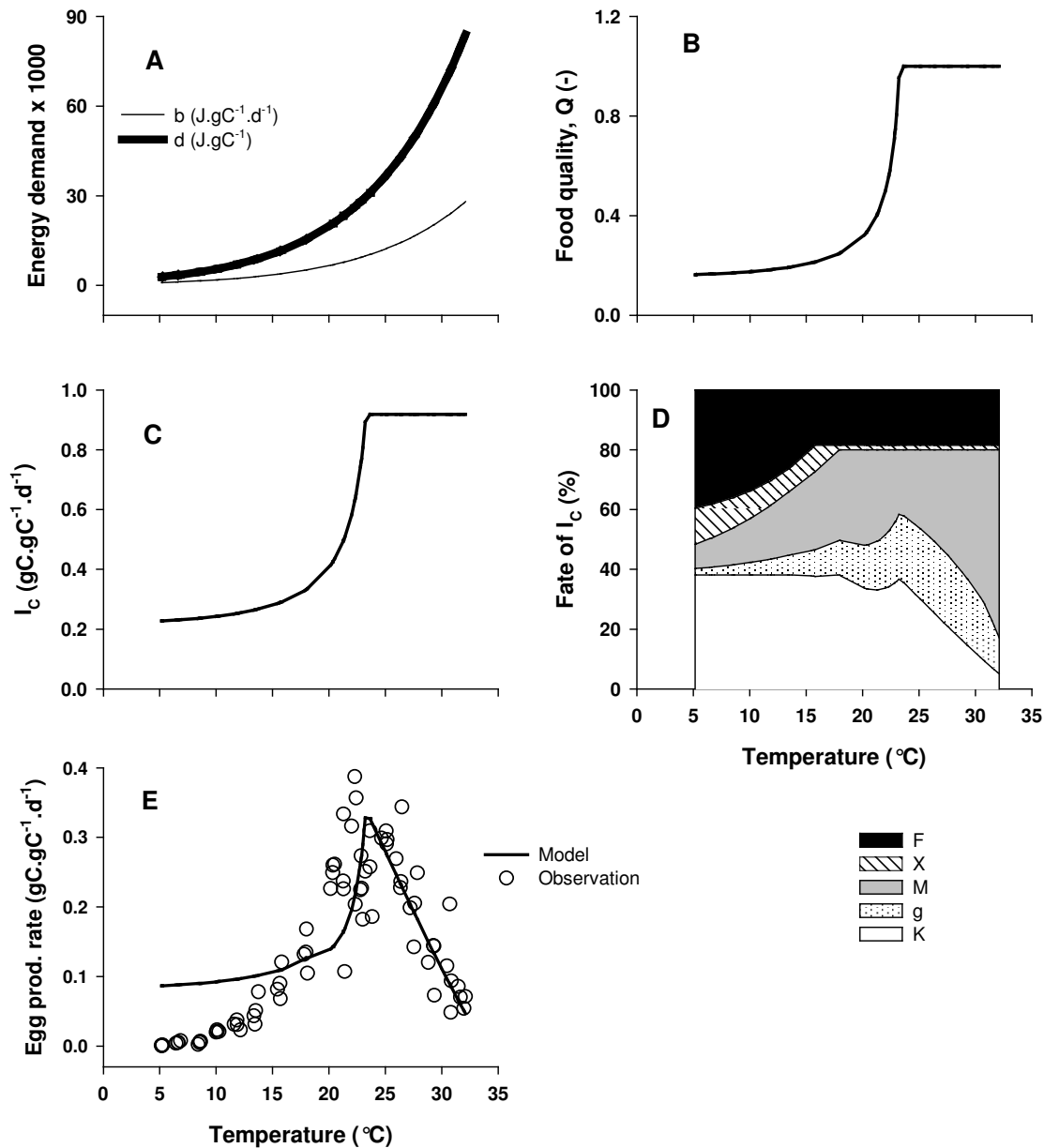


Figure 5. Model predictions when temperature influences only energy expenditure of *Acartia tonsa*. Results represents temperature effect on (A) energy demand for maintenance [b] and growth [d], (B) nutritional value of the prey, (C) carbon ingestion rate, I_c , (D) the metabolic fate of ingested carbon, and (E) carbon-specific growth rate. All other symbols and fractions of subplot D are the same as in Figure 4.

Similar to the results from the earlier simulation, food ingestion increased with temperature (Figure 5C). However, there was a very high increase in the fraction of ingested carbon catabolised for maintenance at higher temperatures (>25 °C) (Figure 5D). The availability of substrates after maintenance was thus reduced at temperatures >25 °C.

Consequently, the fraction of ingested carbon predicted to be converted into eggs (i.e. gross growth efficiency) decreased from ~40 % at 5°C to ~5 % at higher temperatures (> 30 °C), which is consistent with what has been observed by others (Davis and Massay 1977; Sullivan and McManus 1986). This gave rise to a growth response to temperature that was unimodal with a peak egg production rate at ~22.5 °C, which is within the optimum temperature range of 20 to 24.7 °C reported for the *Acartia tonsa* (Castro-Longoria 2003; Holste and Peck 2006).

Figure 6 shows the results for the scenario when temperature influences both energy demand and structural lipid content of *Acartia* (i.e., Exp 2). The lipid content of eggs produced by the copepod was constant at all test temperatures (Figure 5A). This finding is consistent with experimental data showing that the structural lipids (e.g. cholesterol) content of *Acartia* eggs does not change with the acclimation temperature of female copepods (Hasset and Crockett 2009). Total lipid demand for the structure of eggs was determined to be ~57.6 ng egg⁻¹. This is comparable to the maximum egg lipid content observed for the copepod (see appendix 1). Only structural lipid demand for the maintenance of female copepods decreased, from ~2.67 to 2.42 µg.female⁻¹, with increasing temperature (Figure 6A). These values are also comparable data from laboratory studies (see appendix 1).

To compensate for the elevated lipid demand for the structure of female copepods at cold temperatures, lipid catabolism for energy to power maintenance decreased by ~8%, compared to Exp 1 when structural lipid demand was indifferent to changes in ambient temperature (results not shown). This led to the catabolism of relatively more carbohydrates at temperatures < 15°C. As carbohydrate contains less energy than lipid (Table 1), the fraction of ingested carbon that is catabolised for maintenance at cold temperatures is significantly higher (Figure 6E), compared to results from Exp 1 (Figure 5D).

In addition, significant respiration of protein for energy to power egg production was predicted at temperatures < 15 °C (results not shown). As a result, the model fits the observation at cold temperatures markedly better, compared to results from EXP 1 (Figure 6F). Approximately, 78 % of the observed variations in egg production could be explained using EXP 2 (gradient = 0.75 ± 0.10 , $r^2 = 0.7812$, $p < 0.0001$). This is about 16 percentage points higher than the results from Exp 1. It is therefore concluded that lipid demand for the structure of female copepods at cold temperatures (< 15°C) may drive down the rate for egg production at those temperatures. Above 15°C, the fit between the model and observation is not markedly different from what was predicted when temperature affected only the energy expenditure of the copepod (Figure 6E).

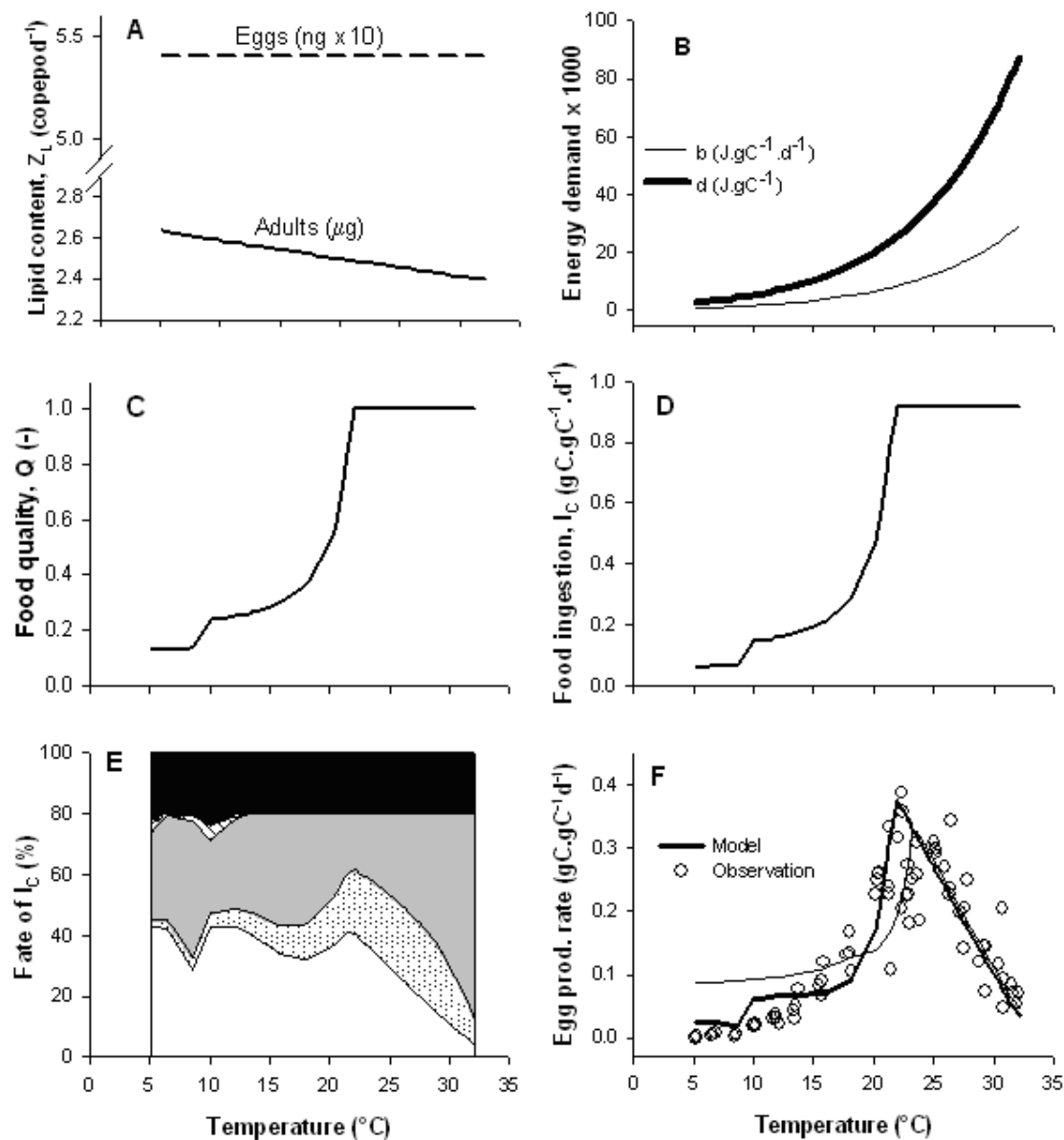


Figure 6. Model predictions when temperature influences *Acartia*'s energy expenditure and structural requirements for lipids. Results represents temperature effect on (A) lipid content of the copepods, (B) energy demand for maintenance [b] and growth [d], (C) nutritional value of the prey, (D) carbon ingestion rate, (E) metabolic fate of ingested carbon, and (F) carbon-specific growth rate. The model fits the observation at temperatures < 15°C markedly better, compared with results from experiment 1 (thin line) when only energy demand was influenced by temperature. All other symbols and fractions of subplot E are the same as in Figure 4.

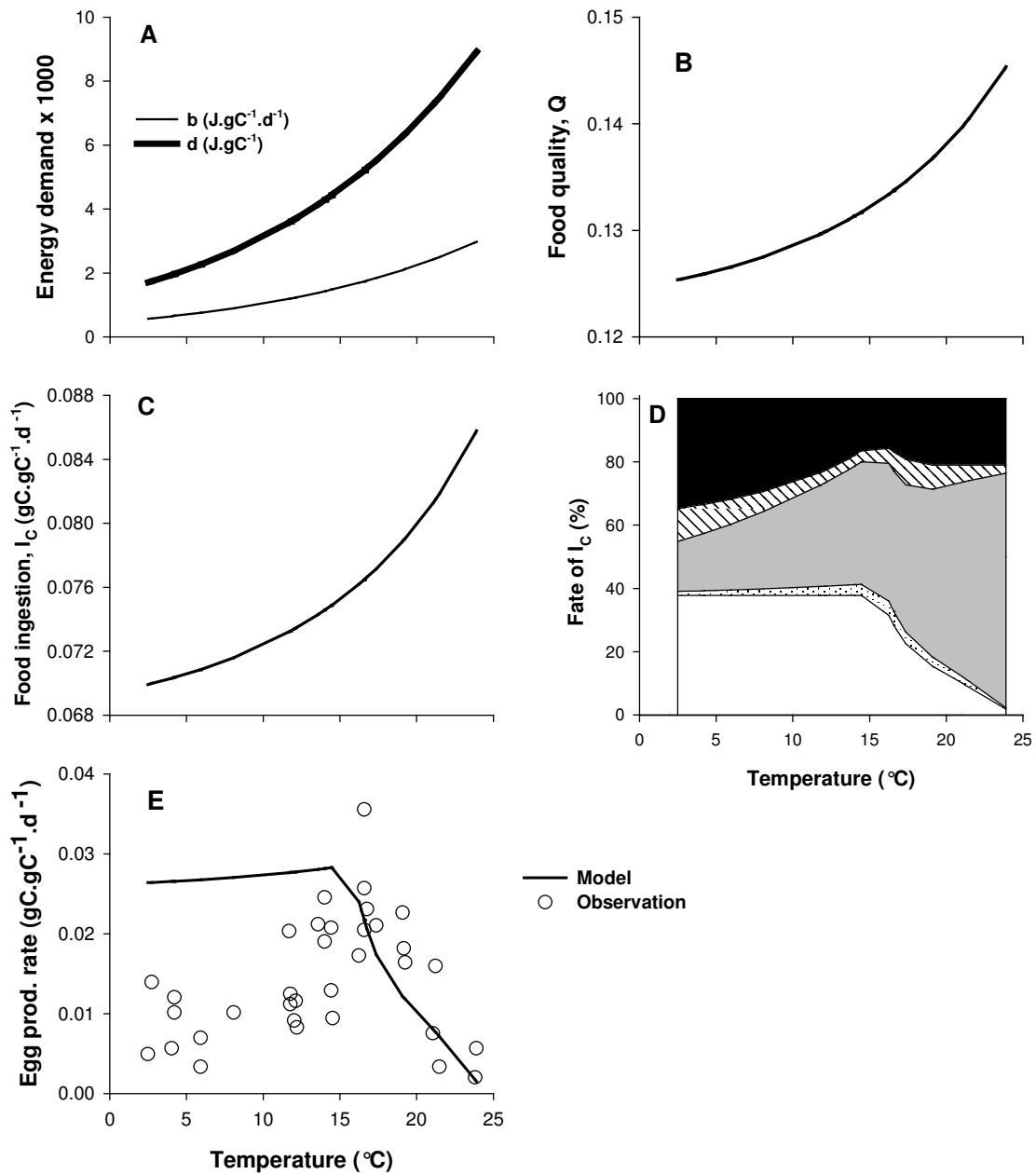


Figure 7. Model predictions when temperature influences only energy expenditure of *Temora longicornis*. Results represent temperature effect on (A) demand for energy for maintenance [b] and growth [d], (B) nutritional value of the prey, (C) carbon ingestion rate, (D) the metabolic fate of ingested carbon, and (E) carbon-specific growth rate. Open circles represent egg production data of Holste et al. (2009). Fractions of subplot D are the same as in Figure 4.

Table 4. Tuned values used for fitting model to observation. See Tables 1 and 2 for definitions. dl indicates dimensionless

Parameter	Unit	<i>Acartia tonsa</i>		<i>Temora longicornis</i>	
		Figure 5	Figure 6	Figure 7	Figure 8
∂	J	1.47E-19	1.51E-19	7.73E-20	9.12E-20
I_m	gC.gC ⁻¹ .d ⁻¹	0.01	0.01	0.003	0.003
I_{mg}	gC.gC ⁻¹ .d ⁻¹	1.0	1.0	1.0	1.0
Z_{ing}	dl	0.40	0.530	0.85	3.50
w_m	L.gC ⁻¹	50	50	50	50
Z_{eff}	dl	0.80	1.25	0.80	2.90
λ_m	dl	0.15	0.15	0.15	0.15
λ_{mg}	dl	0.80	0.80	0.80	0.80
η	dl	0.8	0.25	0.5	5.5
Fem	Z_{Lmn}		1.05E-6		6.02E-6
	Z_{Lmx}			9.61E-6	1.51E-5
	h	K ⁻¹		0.006	0.01
Eggs	Z_{Lmn}		5.76E-8		6.00E-8
	Z_{Lmx}			1.08E-7	9.00E-8
	h	K ⁻¹		0.91	0.91

***Temora* egg production and temperature**

Figure 7 shows model results when temperature influences only the energy demand of *Temora longicornis* (Exp 1). As expected, energy demand for maintenance (b) and growth (d) increased exponentially with increasing temperature, consistent with experimental observation (Gaudy et al. 2000; Ikeda et al. 2001). This lead to an increase in the nutritional value (i.e. Q) of the algae, thereby causing the rate for food ingestion (I_c) and assimilation efficiency to increase with temperature as demonstrated in previous studies (Deason 1980; Isla et al. 2008).

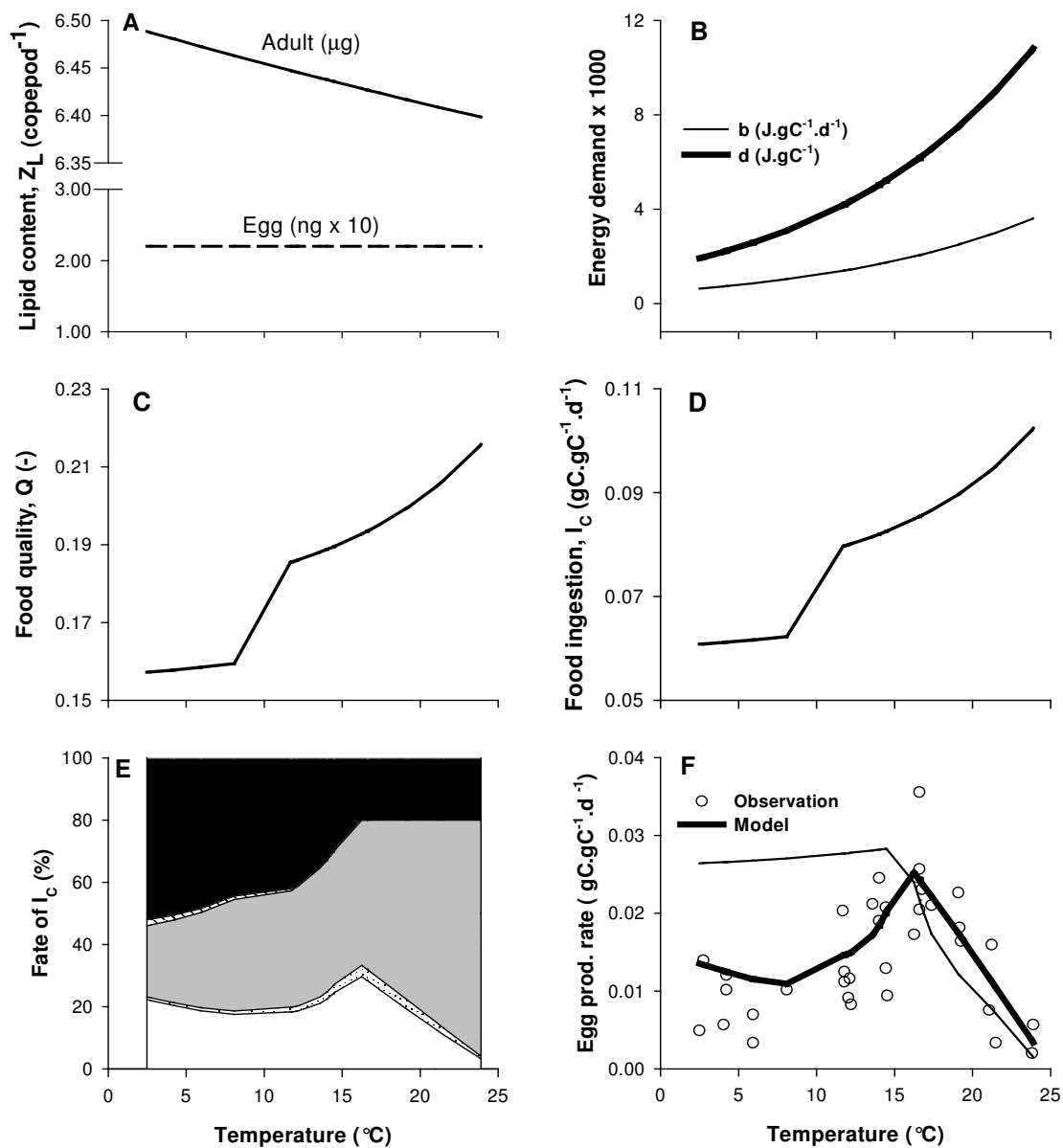


Figure 8. Model predictions when temperature influences *Temora*'s energy expenditure and structural requirements for total lipid. Results represents temperature effect on (A) the lipid content of the copepods, (B) energy demand for maintenance [b] and growth [d], (C) nutritional value of the prey, (D) carbon ingestion rate, (E) the metabolic fate of ingested carbon, and (F) carbon-specific growth rate. The model fits the observation at temperatures < 16.5°C markedly better, compared with results from experiment 1 (thin line) when only energy demand was influenced by temperature. All other symbols and fractions of subplots E are the same as in Figures 3

The metabolic fate of carbon assimilated by the copepod also varied with temperature (Figures 7E). My model assumes that energy cost for egg production is always higher than that of maintenance (Figure 7A). Despite this assumption, the results show that the cost for maintenance accounts for most of the carbon catabolised by *Temora*. However, the model overestimates egg production rate at temperatures < 16.5 °C (Figure 6E), thus suggesting other factors, in addition to energy cost for metabolism, may also influence *Temora* egg production at those temperatures. Above 16.5 °C however, there was a strong agreement between model-prediction and the observed decline in egg production with increasing temperature (gradient = 0.99 ± 0.21 , $r^2 = 0.6569$, $p = 0.0004$). This was due mainly to the high cost of maintenance at higher temperatures, which consumed a substantial portion of the ingested food and thus reducing the availability of substrate for egg production at temperatures < 16.5 °C (Figure 7D).

Figure 8 shows model results for the scenario when temperature influences both energy expenditure and the structural lipid content of *Temora* (i.e., Exp 2). The model could simulate the experimentally observed unimodal growth response of the copepod to changes in ambient temperature (Figure 8F; gradient = 1.09 ± 0.13 , $r^2 = 0.59$, $p < 0.0001$), with a peak egg production rate at ~ 16.3 °C, which is comparable with the experimentally observed temperature optimum of 16.6 °C (Holste et al. 2009).

Importantly, the model fits the observation at temperatures < 16.5 °C markedly better than it does when *Temora*'s lipid composition was indifferent to ambient temperature changes (Figures 8F). This was because significant use of lipid for cold temperature adaptation was predicted but only for *Temora* females, whose lipid content was predicted to increase with decreasing temperature was low (Figure 8A). This forced lipid sparing by females, causing energy poor proteins to be respired for maintenance at low temperatures (results not shown). Subsequently, the fraction of ingested C predicted to be respired for maintenance increased, from $\sim 16\%$, when lipid composition of females did not change with temperature (Figure 7D; temperatures < 5 °C), to $\sim 23\%$, when lipid content of females was temperature dependent (Figure 8E; at temperatures < 5 °C). This caused the model-predicted and observed egg production rates to be more comparable at temperatures < 16.5 (Figure 8F).

Comparison between Acartia and Temora

Figure 9 shows the comparison between the physiological responses of *Acartia* and *Temora* to changes in ambient temperature. Substrate demand for maintenance was predicted to be an important determinant of how both copepod species responded, in terms of egg

production, to changes in ambient temperature. In both species, growth was reduced where the fraction of ingested C catabolised for maintenance was high, and *vice versa*.

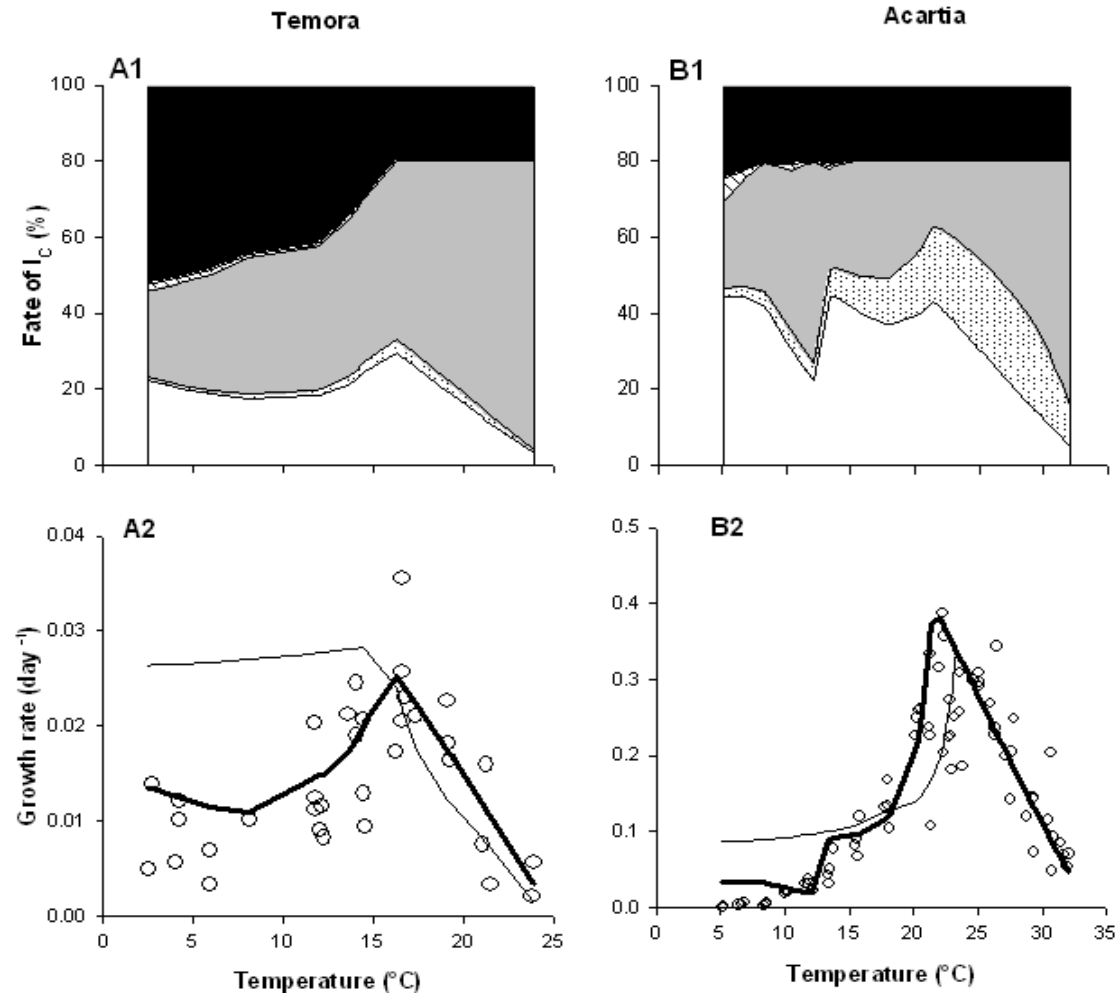


Figure 9. Comparing the responses of *Temora longicornis* (A1 & A2) and *Acartia tonsa* (B1 & B2) to temperature. Open circles represents experimentally observed growth rates. In both species, substrate demand for maintenance is a crucial determinant of egg production. Furthermore, the model fits the observations at lower temperatures markedly better when temperature influences energy expenditure and lipid content of the copepods (Exp 2: thick line), compared to results from the simulation when only energy demand was influenced by temperature (Exp 1: thin line). Model output tuned to species-specific response to ambient temperature. Tuned parameters same as in figures 7 and 8 for *Temora*, and figures 5 and 6 for *Acartia*. Subplots A1 and A2 represent results from Exp 2. All other symbols are the same as described under Figure 4.

Furthermore, temperature does not seem to influence the lipid content of eggs (i.e. Z_L) produced by both species. This is because all fits to the observed data were achieved with constant Z_L values for eggs, even when the simulation allowed temperature dependent regulation of egg lipid content (see Figures 6 and 8). Also, temperature induced changes in lipid content of females contributes to the reduction in egg production by both copepods at colder temperatures ($\leq \sim 16 \pm 1$ °C). The results also reveal important differences between *Acartia* and *Temora* in terms of how they respond to changes in ambient temperature.

The model predicts that the metabolic activation energy required by *Acartia* is higher (20 – 40%) than that of *Temora*. Similarly, the predicted minimum threshold for food ingestion for *Acartia* exceeded that of *Temora* (see Table 4) by ~4%, assuming an adult carbon weights of 4.2 μ g for *Acartia* (Kiørboe, 1989) and 13.38 μ g for *Temora* (Huntley and Lopez, 1992). These results suggest that *Temora*, despite being relatively bigger, may require less energy to kick-start its metabolism and for maintenance than *Acartia*. A metabolic advantage that may also explain *Temora*'s occurrence in polar waters (Klekowski and Weslavski 1990; Chikin et al. 2003; Lukashin et al. 2003) where primary production, and hence energy availability for primary consumers, is mostly low (Sargent and Falk-Petersen 1988). The results here may also explain why unlike *Acartia*, *Temora* can migrate below into deeper (i.e. below 50 – 70 m) waters (Schmidt 2006) where food conditions may be low.

Temperature, prey biochemical status and zooplankton production

The combined effects of temperature and prey's chemical composition on zooplankton production were investigated based on model predictions under variable temperature and food compositions. The investigation involved only *Acartia tonsa* as consumer. This was because it was the only species for which the model could more successfully simulate, with temperature-dependent regulation of animals' lipid content, the observed growth rates over the entire range of experimental temperatures (Figure 9). Variation in prey biochemical composition (α_i) was attributed to their C:N ratio (in terms of mass). α_P , α_L and α_H were estimated based on published relationships between algal C:N ratios and biochemical contents (see texts associated with Figure 3A of chapter 3). The relationships were developed based on data on diatoms (see Anderson 1994). Therefore, the parameters I_{mg} , I_m , z_{ceff} , z_{ing} , λ_{mg} and η had the same value as determined earlier for *Acartia* on diatom diet (see Tables 1 and 3 of chapter 3). Lipid content of *Acartia* females was assumed to be dependent of ambient

temperature based on the results associated with Figure 6 here. Therefore, all other parameters used for the simulation here were as listed in Table 4 for Figure 6.

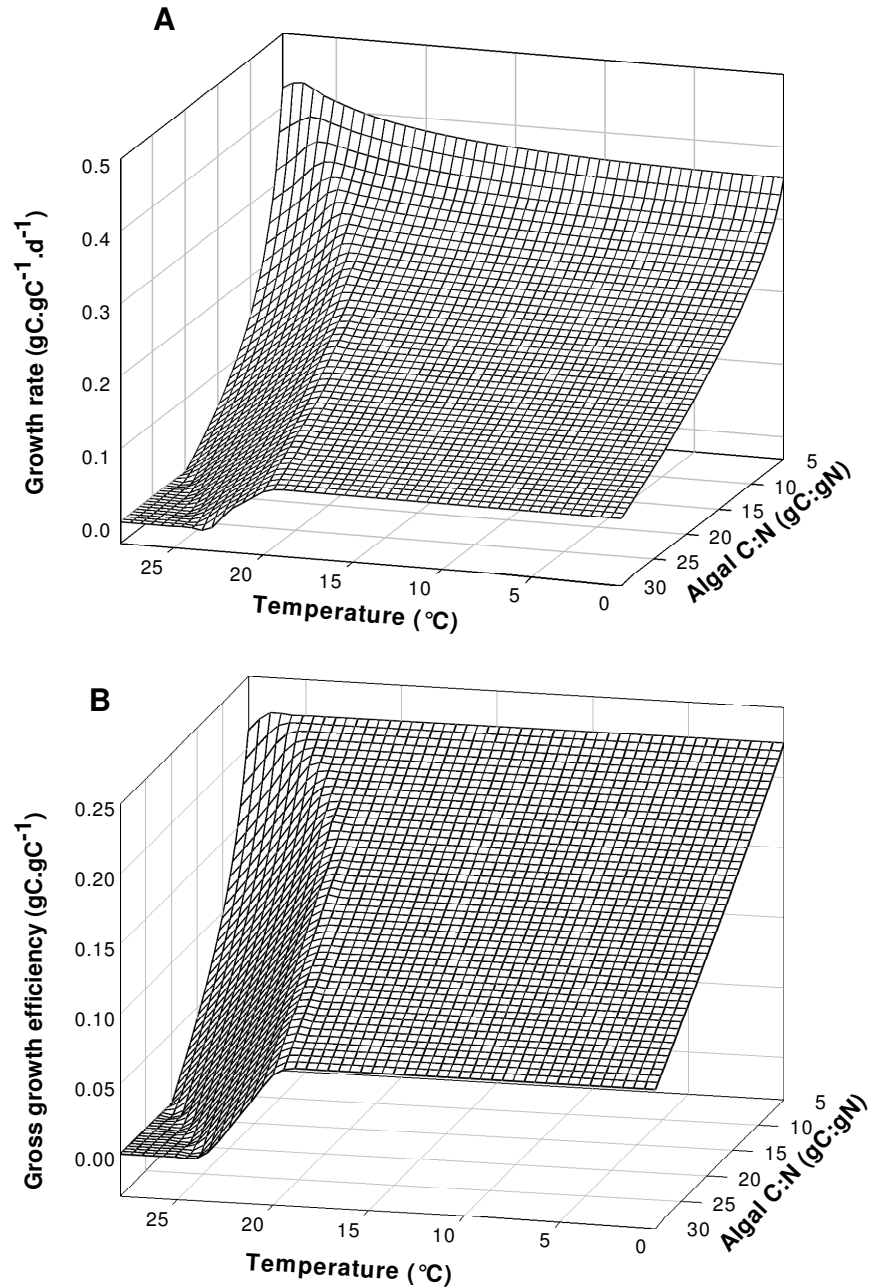


Figure 10. The interactive effect of temperature and prey biochemical composition on (A) the rate, and (B) gross efficiency for egg production by *Acartia tonsa*. Fitted parameters: $I_{mg} = 6.88$, $I_m = 0.24$, $\lambda_{mg} = 0.95$, $Z_{ing} = 0.22$, $Z_{eff} = 0.30$, and $\eta = 0.032$. All other parameters were same as listed in Table 4 for Figure 5. See text for further explanation.

Figure 10 shows how variations in ambient temperature and prey nutritional status may influence growth rate and growth efficiency in *Acartia tonsa*. Results are shown for the ambient temperature range of 0 to 28, and algal C:N of 5 to 30. Over the temperature range explored, the model predicts the rate for egg production to decrease with increasing algal C:N ratio (Figure 10). Similarly, gross efficiencies for egg production at the individual temperatures were predicted to decrease with increasing algal C:N. These findings are consistent with results from experiments and previous modelling studies (Checkley 1980; Kiørboe 1989; Touratier et al. 1999; Kuijper et al. 2004a; Mitra 2006). However, results here show that the relationship between prey nutritional status and predator's growth can be influenced by changes in ambient temperature. When food was N-rich (i.e. algae with C:N ratios ≤ 7), the rate for egg production was predicted to increase exponentially with increasing temperature. Here, copepod ingesting N-rich algae could maximize egg production because the C associated with low C:N algae could easily be assimilated for metabolisms (see Figure 6A of chapter 3). C assimilation efficiency however decreases with increasing algal C:N, as increasing C:N shifts algal macromolecular composition towards substances such as structural carbohydrates that are not easily assimilated by zooplankton. Accordingly, the results show that *Acartia* ingesting high C:N algae could maximize their growth only at low ambient temperatures (Figure 10), owing to the low energetic costs for metabolism at those temperatures. However, growth was predicted to decline when the cost for metabolism exceeded the acclimative capacity of the copepod. This decline in egg production rate began at different temperatures, depending on the biochemical composition of the food. Hence, the optimum temperature for egg production by *Acartia* decreased with increasing algal C:N (Figure 10). These results compare with experimental data demonstrating that the optimum temperature for egg production by marine copepods varies with the composition of their food (Holste et al. 2009 and references therein). Figure 10 thus shows that the effect of temperature on egg production by zooplankton may be influenced by the biochemical composition of the available prey, or vice versa. This, in addition to factors such as differences in salinity (Holste and Peck 2006; Holste et al. 2009) and female age (Parrish and Wilson 1978), may explain why experiments involving the same copepod species but different prey organisms often observed different optimum temperatures for egg production (Holste et al. 2009 and references therein).

4.6 DISCUSSION

Model assumptions and parameters

Before discussing the implications of the results with respect to the temperature tolerance of zooplankton and the effect of temperature on zooplankton production, it is worth commenting on the assumptions as well as parameters of the model. Many of the model parameters were set based directly on experimental data. The main unknown parameters were food intake threshold requirements (i.e. I_m , w_m and λ_m), feeding response parameters (z_{ing} , z_{eff} and η) and activation energy for metabolism (∂). These parameters were assigned values by fitting the model to experimental observation (Kiørboe 1989; Holste and Peck 2006; Holste et al. 2009). The result of the model is sensitive to variations in the values of model parameters. However, the basic patterns of C utilization exhibited by copepods in the model did not markedly change with the model parameters. It can therefore be said that the insight the model provides on the effect of temperature on feeding and physiological behaviour of the copepod probably reflects the natural behaviour of the copepod.

The functional nature of the relationship between temperature and lipid content of zooplankton is not known. Equation 9 was therefore used based on the fact that zooplankton species adapted to cold temperatures generally contain more lipid than their counterparts inhabiting relatively warmer waters (Farkas 1979; Nanton and Castell 1999; Lee et al. 2006). It is important to mention that lipid composition of zooplankton may also be influenced by several other factors such as food availability (Auel 2004), and prey biochemical composition (Ederington et al. 1995). Furthermore, it may be likely that copepods may rely only on structural lipids such as cholesterol (Isla et al. 2008; Hasset and Crokett 2009) and polyunsaturated phospholipids (Farkas 1979) for temperature acclimation. However, the amount and type of lipid required relative to temperature and feeding condition is not well understood. Further research on this issue is needed.

Temperature can also have a secondary effect on the reproduction of copepods through its influence on body size. The relationship between female weight and reproduction has been widely investigated (e.g. Mauchline 1998; Hirst and Bunker 2003). In most copepods, both clutch size and egg production rates have been found to increase with body mass (Corkett and McLaren 1978; Dzierzbicka-Glowacka and Zielinski 2004). Conversely, female body size is inversely related to temperature (Corkett and McLaren 1978; Lee et al. 2003). Consequently, a smaller female size at warmer temperatures (see for example Viitasalo et al. 1995) could potentially cause egg production to decrease. The experimental studies against which this model was tested involved a protocol that eliminated the effect of animals'

temperature history on egg production (See Holste and Peck 2006). Therefore, this study did not explicitly consider egg production to be influenced by temperature-induced changes in female size.

Model predictions

Zooplankton, like most living organisms, can adjust their behaviour to reflect changes in their environment, be it biotic or abiotic change (Levins 2005). Individual species however can experience adverse consequences if environmental change exceeds their acclimative capabilities. Whereas this is well recognized, acclimative behaviour and how it contribute to temperature tolerance of zooplankton is typically ignored or poorly represented in marine ecosystem models principally due to the lack of a realistic framework for its incorporation. To help address the problem, this article proposes a simple mechanistic framework for simulating temperature dependence of food ingestion, assimilation and metabolism by zooplankton. The model is based on the assumption that, prior to food ingestion, animals can “anticipate” (*sensu* Holland 1998) the nutritional value of their food at different temperatures and accordingly adjust their behaviour to optimise food intake and utilization. The form of the model makes it applicable to different zooplankton groups. Currently, it has been configured for egg production by two calanoid copepods: *Acartia tonsa* and *Temora longicornis*. In comparison with literature data, the model realistically predicts the rate of egg production, as well as C-specific gross growth efficiencies for egg production by the copepods under different food and temperatures conditions. Also, the optimum temperatures for egg production by the copepods are reasonably predicted.

In addition to reproducing observed effect of temperature on the rate of egg production, the added value of my model is that it shows the mechanisms that underlie temperature tolerance of animals. One striking outcome of my simulations is that the egg production response of copepods to changes in ambient temperature is driven mainly by temperature-induced changes in animals’ biochemical demand for maintenance. Here, egg production rate was reduced when the cost for maintenance cost, in terms of carbon, was high, and *vice versa* (see for example Figures 6 & 8). Others have also demonstrated the importance of adult maintenance in zooplankton production (e.g. Kuyper et al. 2004a; Anderson et al. 2005). Based on these observations and my results, it can be said that the minimum food concentration threshold (*sensu* Huntley and Boyd 1984) required by copepods for reproduction may have a temperature dependence, which may be related to the cost of maintenance. My model predicts maintenance cost, in terms of carbon, to be low at lower

temperatures and high at higher temperatures. This means that the minimum food concentration threshold for egg production may be reduced during colder temperatures, which may ensure adequate egg production in winter when food availability is usually low. This could give herbivorous copepods the potential to maintain a stock of premature stages pre-phytoplankton bloom, and thus being ready to utilize the algal spring bloom when it occurs (Fransz and Gonzalez 1991; Sommer et al. 2006). Clearly, the cost for maintenance is a major constraint on egg production by female copepods and thus needs to be explicitly considered in models for investigating the dynamics of zooplankton production.

Currently, energy demand for growth, rather than for maintenance is considered to be the major determinant of metabolic rates in copepods (Kiørboe et al. 1985; Thor et al. 2002). This is usually determined based on the oxygen consumption rate of animals, assuming a fixed amount of energy is gained per every unit of oxygen respired (see for example Ikeda et al. 2001; Isla and Perissinotto 2004). The justification for this approach is that protein synthesis, and hence new biomass formation, is the highest energy consuming process in animals (Grisolia and Kennedy 1966) and co-varies with oxygen uptake rates in a range of aquatic animals, including zooplanktonic crustaceans (Houlihan et al. 1990, 1997; Thor 2000; Whiteley et al. 2001). In animals however, the demand for oxygen is dictated by diverse respiratory substrates, with different catabolic requirements for oxygen. For example, whereas the complete oxidation of a molecule of palmitic acid consumes 46 atoms of oxygen, just 12 atoms of oxygen is required for catabolising a glucose molecule (White et al. 1964). In my model, copepods could exhibit plastic preference for respiratory substrate by respiring proteins, lipids or carbohydrates for energy (see model description). Furthermore, my model assumes that energy cost for egg production is always higher than that of maintenance (for example, see Figure 5A). Despite this assumption, the results show that respiratory consumption of carbon by zooplankton is mainly driven by the cost for maintenance (for example, see Figure 9 A1&B1). Based on this result, and the fixed respiratory quotient (i.e. CO₂ produced: O₂ consumed) for the catabolism of different substrates (Kiørboe et al. 1985; Mayzaud et al. 2005), one could argue that the reported increase in oxygen consumption rate with temperature (e.g. McAllen et al. 1999; Isla and Perissinotto 2004) may be more dependent on the cost for maintenance than that of new biomass formation (egg production). This may explain why the weight-specific respiration rates of male and female copepods are generally very similar (Gilbert and Williamson 1983 and references therein). It may also explain why female and male copepods of the same species can respond similarly, in terms of their weight-specific oxygen consumption, to changes in ambient temperature (Laybourn-

Parry and Tinson 1985; Isla and Perissinotto 2004), even though only females produce eggs. Hence the framework I have proposed has demonstrated the ability to address the effect of temperature on the physiological behaviour of zooplankton.

Here, temperature effect on the physiology of two calanoid copepods, i.e., *Acartia tonsa* and *Temora longicornis*, was investigated by apply the proposed framework. The results show that *Temora* may require relatively less energy to kick-start its metabolism and for maintenance than *Acartia* (Table 4). This suggests that *Temora* may be better adapted at exploiting conditions of low food availability than *Acartia*, despite its bigger size. Hence, during periods of low food availability, *Temora* may have a reproductive advantage over *Acartia* if both copepods depend on the same resource. Studies have also shown that adult *Temora* show diel vertical migration into deeper waters, for example below the halocline in the Baltic Sea (Schmidt 2006), where food availability and nutritional composition may be poor. Therefore, based on the results here, *Temora*'s ability to survive in deeper waters may be (partly) because the species require less energy for metabolism and for maintenance. The results also show that the minimum food concentration threshold for *Temora* production may be potentially lower than that of *Acartia*. The physiological importance of this may be insignificant in mid- and high- latitude waters since food availability is usually high, especially during high temperature seasons (Durbin et al. 1992). In low latitude waters on the other hand, egg production by zooplankton may be more sensitive to low food availability due to the high costs of maintenance at warmer temperatures. Hence, a low food concentration threshold may be advantageous for the production *Temora* within low latitude water (Chikin et al. 2003).

Another ecologically important prediction of my model is that prey biochemical composition significantly influences temperature-specific rate and gross efficiency for egg production by zooplankton (Figure 10). This is understandable, since no anabolic process can take place without nutrients, no matter what the ambient temperature is. Here, purely temperature-dependent growth was predicted only when food is nutritionally good (Figure 10A). This is because a nutritionally good prey, presented at an adequate concentration, presented little impediments to consumers' effort at meeting their temperature-specific requirements for metabolic substrates. However, once biochemical imbalance in the prey exceeds a specific limit (here determined to be algal C:N > 6), both the rate and efficiency of egg production were predicted to be dictated by both temperature and prey biochemical composition. Similar observations have been made by others (e.g. Cole et al. 2002; Masclaux et al. 2009). It can therefore be said that temperature effect on zooplankton growth may vary

with prey biochemical composition, with likelihood for a decrease in zooplankton production during high temperature and poor food conditions (Figure 10), because the high cost of metabolism at higher temperatures may exacerbate the nutritional inadequacy of prey organisms. This could have several implications for C cycling within marine ecosystems.

Consistent with experimental observations (e.g. Deason 1980; Isla et al. 2008), food ingestion was predicted to increase with temperature (Figures 4 – 8). The model also predicts the fraction of ingested C lost as faecal pellet to be high at low temperatures and low at high temperatures, consistent with observation (Sullivan and McManus 1986). These means that at higher ambient temperatures, most of the C fixed by algae during primary production may either be converted into: (1) new zooplankton biomass (egg production) that could be transferred to higher trophic levels via secondary consumption, or (2) metabolic by-product like CO₂, or organic compounds (Darchambeau et al. 2003; Darchambeau 2005; here via X_c , M_c , and g_c) that may be re-assimilated by autotrophs, and thus stimulating primary production. Moreover, faecal matter released at higher temperatures may potentially decompose before leaving the euphotic zone, due to the fact that microorganisms responsible for organic matter decomposition tend to be more abundant at higher temperatures (Laws et al. 2000). The net result of these could be fast C cycling (*sensu* Belovsky and Slade 2000) during warmer temperature seasons. Conversely at colder temperatures, the potential growth rates of heterotrophic microorganisms may be much slower and hence result in a low rate of organic matter decomposition. Consequently, the predicted low food consumption rates (i.e. low grazing rate and high faecal pellet production) suggest that C cycling by heterotrophs may be slow at colder temperatures. However, the impact of this on C exported into deeper waters, possibly as part of marine snow may be insignificant since phytoplankton production is also naturally low at colder temperatures.

This study has demonstrated that temperature and prey biochemical composition can interact to influence the dynamics of zooplankton production. These interactions have hitherto not been explicitly considered in zooplankton models. In aquatic systems, temperature and food are major determinants of animals' physiology, life history traits and habitat selection (Angilletta et al. 2004; Guisande 2006). As a result, further studies on the combined effects of temperature and food on zooplankton dynamics is necessary. The model presented here provides a base upon which further research can be developed.

Chapter 5

General Discussion

5.1 Modelling Approach

Copepods, like most animals, are heterotrophic and hence depend on complex organic molecules for nutrition. At the same time, dietary supply of chemical compounds vary substantially among different prey items, or within a single prey species due to changes in the oceanic environment (Morris et al. 1983; Dunstan et al. 1993). In order to compensate for the lack of sufficient nutrients in a specific prey type, copepods demonstrate varying feeding regulation mechanisms in order to satisfy their demands for energy and chemical substances. Such an acclimation via modification of feeding and incorporation by copepod would thereby determine the structure of food webs (Paine 1980; Levin 1998) and the dynamics of marine ecosystems. However, feeding regulation is a complex phenomenon dependent on a number of different environmental factors (e.g. temperature, predation), and therefore is difficult to capture within theoretical models for investigating marine ecosystems and their services. It is therefore encouraging that others have shown that food quality can serve as the basis for an intermediate complexity approach to modelling the complex dynamics of feeding among zooplankton (Mitra and Flynn 2005; Mitra 2006). To this end, chapter one of this thesis proposes an adaptive framework for food quality that could be useful. Here, I discuss how the implementation of the framework I have suggested contributes to our understanding of ecological processes.

Currently, optimum foraging theory (OFT) and ecological stoichiometry (ES) remains the only nutritionally explicit frameworks for modelling biochemical control of feeding behaviour in aquatic ecology (Raubenheimer et al. 2009). The shortfalls in the respective theories have been discussed under Chapters 1 and 2. A common weakness OFT and ES share is that they evaluate the nutritional quality (Q) of prey items by *a priori* identifying one food component (mostly energy for OFT-models; and N, P, or essential compounds for ES-models) as pre-eminent. This negates the importance of consumer physiology, and ignores biochemical constraints on the limiting role of chemical elements in animal production.

To address these issues, this thesis proposes a new adaptive model that bases Q on consumers' capacity for food uptake and metabolic physiology. Using this model, I investigated the impact of the macromolecular constituents of prey on carbon (C) and nitrogen (N) utilization for egg production by marine zooplankton. My model incorporates the stage-specific structural demand for biochemical substances by animals, has separate pathways for

the utilization of carbon (C) associated with proteins, lipids and carbohydrates, and considers food quality dependent regulation of food ingestion, assimilation and metabolism. In comparison with literature data (Checkley 1980; Kiørboe 1989; Holste and Peck 2006; Holste et al. 2009), the model realistically predicts the rate of egg production, as well as C- and N-specific gross growth efficiencies of egg production by copepods under different conditions of food and temperature (see chapters 3 and 4). However, contrary to popular opinion (White 1983; Elser and Hasset 1994; Touratier et al. 1999; Kuijper et al. 2004), results from my simulation suggest that C, and not N, limits the production of marine copepods due to lipid limitation and the refractory effect of complex polysaccharides (e.g. cellulose) on C assimilation (see text associated with Figure 6D & Table 4 of chapter 3). Hence, the C-limitation predicted here can be considered a form of relative resource limitation (*sensu* Schmitz, 2009) as the limitation was due not to inadequate C supply but rather mainly to how algal C is distributed between different compounds. Indeed, C-limitation of zooplankton production has also been predicted by other models using the principles of ecological stoichiometry and fixed food ingestion rate and utilization efficiency (e.g. Anderson and Pond 2000; Major et al. 2009). My approach however shows the mechanisms that underlie C-limitation of zooplankton production. It is therefore recommended as a more realistic approach to investigating biochemical control of animal production.

In my approach, assimilation efficiency of N was predicted to be almost constant (at ~0.9 to 0.93), while that of C decreased from 0.66 to 0.16 with increasing algal C:N (Figure 6 of chapter 3). These predictions are consistent with experimental data on zooplankton (Landry et al. 1984; Hasset and Landry 1988; DeMott et al. 1998). Based on these results, and the observation that N enrichment of phytoplankton enhances the rates of feeding by marine copepods (Checkley and Entzeroth 1985; Cowles et al. 1988), I conclude that herbivorous copepods may enhance egg production by feeding on N-rich algae, as the carbon in such algae can readily be assimilated. However, where C assimilation, and hence its supply for metabolism is limited by ingestible algal components, my analysis indicates that copepods may compensate by increasing N catabolism for energy (see Figure 6B of chapter 3). A number of studies have also demonstrated that zooplankton catabolise a substantial fraction of the N they ingest (Blazka 1966; Kuijper et al. 2004). It can therefore be said that copepods convert most of the algal N they ingest into (i) new copepod biomass (here via egg production) that could be transferred to higher trophic levels via secondary consumption or (ii) metabolic by-products such as ammonia that could be re-assimilated by phytoplankton. Conversely, the fraction of ingested carbon lost as faecal pellet was high, increasing with

algal C:N. By depleting faecal pellets of nitrogen, as the results here suggest (Figure 5 of chapter 3), copepods may increase the stoichiometric ratio between C and N within detritus. Previous studies have also shown that acclimative behaviour such as food selection by copepods (Huntley et al 1983; Roman 1984; Paffenhöfer and Van Sant 1985) alters prey community composition, and thereby increases C:N ratio of detritus (Schmidt 2008). This thesis does not treat prey selection, but food-quality mediated food selection has been shown by previous studies (e.g. Flynn and Davidson 1993; Jones and Flynn 2005). Hence, in addition to packaging algal material into rapidly sinking aggregates via grazing (Ebersbach and Trull 2008), copepods may also increase the C:N characteristics of the aggregated algal cells via food selection and differential assimilation of macromolecules. As a result, more algal C may be exported into deeper waters, possibly as part of marine snow if faecal pellets are not consumed or broken up by other organisms (Smetacek 1980).

It is important to mention that some of the assumptions underlying the framework presented here were informed by existing theories on animal nutrition. For example, the central premise of the thesis that food consumption and metabolism proceed in a manner that allows consumers to optimally utilise the food resources available to them, originated from OFT (Stephens and Krebs 1986). Also, ES informed the assumption that the catabolism of substrates proceeds according to consumers' own structural composition requirements (Sterner and Elser 2002; Grover 2003). In addition, the geometric framework (GF) for analysing animal nutrition in terrestrial ecology (Simpson and Raubenheimer 1995 and references therein) inspired how I separated prey and consumer biomass into their respective biochemical constituents. Like ES, GF treats food as multi-biochemical resource but unlike ES, GF is primarily an experimental framework. It determines the utility of prey organisms (i.e. food quality) mainly via graphic exploration of consumers' fitness in a multi-dimensional nutritional space. The application of GF would thus be limited in an environment where the number of relevant chemical substances is high (say >3), and where feeding is also a function of other environmental factors such as temperature and salinity. The food quality model presented in this thesis can consider several chemical substances as well as physical factors (see chapter 4 for example). It is therefore recommended as an alternative to the current approach to describing food quality in ecosystem models.

A dominant paradigm in feeding ecology is that animals feed to maximize their fitness (Yearsly et al. 2001; Raubenheimer et al. 2009). Here using egg production (growth) efficiency as a proxy for fitness, I assessed the viability of my macromolecular food quality model under different conditions of food and temperature. The model realistically captures

animals' ability to increase food ingestion and assimilation with increasing temperature (see, for example, Figure 7C of chapter 4), as demonstrated in experimental studies (Deason 1980; McAllen et al. 1999; Isla and Perissinotto 2004; Isla et al. 2008). The model however suggests that the rate for food ingestion may not always increase with temperature. Rather, it shows that the rate for food ingestion could be independent of temperature once the optimum temperature preference of the copepod is exceeded (Figure 5C of chapter 4). This observation is consistent with the fact that feeding is an optimization process that is constrained by consumers' own behaviour and physiological capabilities within different environments (Belovsky 1997). Hence, the model framework I have suggested has demonstrated the ability to address the effect of temperature on the feeding behaviour of zooplankton. Unlike previous models (e.g. Stegert et al. 2007), my model does not temperature directly to feeding rate. Rather, it determines food consumption rate based on animals' biochemical requirements (both energy and structural) for metabolic growth. It could therefore be used to explore temperature effect on the physiology of ectotherms.

Following this approach, the model predicts that lipid demand for the structure female copepods at cold temperatures, arguably to prevent lethal freezing (Kattner et al. 2007), increases protein catabolism for energy and thereby accelerates respiratory consumption of carbon assimilated by zooplankton (see Figures 6 & 8 of chapter 4). This physiological cold acclimation decreased the rate for egg at colder temperatures. In contrast, at warmer temperatures, the elevated cost of maintenance was predicted to decrease anabolism and other activities associated with production of new biomass for growth and reproduction. This result is consistent with empirical data (Hochachka and Somero 1984 and references therein) and emphasizes the cost of maintenance as a major constraint on zooplankton production. Another important outcome of my simulation is that the optimum temperature for egg production by *Acartia tonsa* increases with the nitrogen content of microalgae (Figure 10 of chapter 4). This is consistent with experimental observations (Cole et al. 2002) and suggests that the temperature acclimation of zooplankton production is contingent on the biochemical composition of their food.

Ultimately the need is for zooplankton models that reflect the broad pattern of acclimative behaviour that we observe, and yet are simple enough in their structure to find wide applicability. To that end, the physiological framework I have proposed requires no more parameters than previously documented models (e.g., Kuyper et al. 2004; Mitra 2006). My approach however extends the concept of food quality a step further, to include temperature conditions encountered by animals. This is critical since temperature influences

biochemical demand for metabolism (Farkas et al. 1984; Gaudy et al. 2000; Ikeda et al. 2001; Hassett and Crockett 2009; Sperfeld and Wacker 2009) and thereby determine food intake (Hutchings et al. 1999; Lafferty et al. 2006) and hence prey nutritional value. The framework I have proposed therefore advances our ability to realistically model feeding behaviour under different conditions of temperature and food.

The complexity of predator-prey interactions and other trophic processes such as food assimilation and utilization requires models intended for their investigation to “follow nature” (Hall 1988). This means grounding trophic ecology on the behaviour, physiology, and morphology of individual consumers (DeAngelis 1988; Raubenheimer et al. 2009) by (i) reducing community-level concepts, such as competitive exclusion and coexistence, to individual foraging strategies (Shoener 1986; Tilman 1987), and (ii) accounting for conditions encountered by individual consumers (fluctuating temperature, etc) and physiological effects of these conditions (DeAngelis 1988). These requirements cannot be realistically achieved without an appropriate approach for evaluating the quality of prey organisms prior to feeding initiation by model consumers. To address this issue, this thesis provides a mathematical framework with food quality being contingent upon molecular biochemical requirements, as well as the physiology and food uptake capabilities of consumers.

5.2 Future work

The framework presented here requires much work before it can be implemented within ecological models, *vis*:

- a. In addition to differences in biochemical characteristics, prey organisms may also differ in features such as size, cell structure, etc that influence predation. The influence of these factors on food consumption has not been treated in this thesis. This is because only systems involving single prey species were examined. However in a multi-prey environment, the food quality model (Q) presented here can for example be made part of a function that defines consumers' preference (p_i) for an individual prey (i) as $p_i = f(Q_i, S_i, s_i, n_i)$, where Q_i , S_i , s_i and n_i respectively represent the prey's quality, size, cell structure and any other feature(s) known to influence consumer's feeding behaviour. p_i could then be integrated into a function for feeding in a multi-prey environment (see Gentleman et al. 2003 for examples) in order to quantifying total intake, as well as how that intake might be derived from the various prey items.

- b. Structural biochemical requirement of consumers change with their stage of development (Brucet et al. 2005). Furthermore, male and female copepods may differ in their structural requirement for biochemical substances (see Morris and Hopkins 1983). As a consequence, the nutritional quality of a prey may vary with consumers' sex and stage of development. In the examples given here the model has been configured to represent only adults of female copepods and their eggs. However, the model can easily be re-parameterised to reflect energy and structural composition requirement of other developmental stages and sex of consumers. Setting $\delta_i = \beta_i$ (i.e. same chemical composition between old, β_i and new consumer biomass, δ_i) in the food quality model presented here would automatically simulate only how a non-reproducing animal might evaluate its prey organisms (see model description under chapter 2). Hence, the framework here can be implemented for non-reproducing copepods by simply following the approach described in the preceding chapters, if the parameter values for energy demand, structural constituents, as well as food consumption and physiological capabilities are known for such individuals. For non-adult consumers whose biochemical requirement might change over time (e.g. copepodids), a determination would have to be made about the temporal dynamics of β_i and energy consumption if one wishes to implement the model over the entire life cycle of a consumer.
- c. Most zooplankton communities are composed of many species. To describe the trophic behaviour of these communities using the individual-based food quality model presented here, one of three possible approaches could be used. (i) One could introduce separate equation and parameter sets for each species. This may be analytically manageable if the relevant species are few, else one risks having a massive and an analytically opaque system (as argued by DeAngelis 1988). (ii) One could argue that the community is dominated by just one species and choose to model only this species, or (iii) one could use a single equation set with parameters derived from some weighted average of the parameters appropriate to each species in the community (Broekhuizen et al. 1995).
- d. In nature, the number of chemical substances that may be relevant for the feeding behaviour of animals is high (i.e. > 30 different chemicals for most animals: Behmer and Joern, 2008). This thesis considers three major categories of biochemical compounds: lipids, carbohydrates and proteins. This ensures a simple model structure by decreasing the number of molecules that would otherwise be needed. This

categorization also agrees with previous findings showing that the availability of macromolecules determines the grazing behaviour (Cruz-Rivera and Hay 2000) as well as the fate of food ingested by zooplankton (Kuijper et al. 2004). However, the framework presented here can also be implemented for systems involving larger numbers of different biochemical substances. Here, one may require a different implementation procedure because the one described here (section 2.4 of chapter 2) is not tractable for systems involving more than 3 chemical substances. Here, the ecological simulator Gecko (Schmitz and Booth 1997) may offer the opportunity to explore the consequences of a rich variety of chemical substances on food quality and consumers' trophic behaviour. Gecko, like its progenitor Echo (Holland 1992, 1994), is essentially a game in which (i) organisms try to make the most of their environment, (ii) the capacity for interaction is coded at the individual organism level, (iii) resource production is specified, and (iv) available resources and general rules specify the magnitude of resource exchange between organisms (Schmitz and Booth 1997). In Gecko, organisms are consumers and their prey. The different resources could be taken as representing different chemical substances whose availability is defined by their relative composition in the prey (i.e. α_i). Consumers' capacity for food uptake (i.e. I_c and λ_i) could represent the magnitude of resource exchange, with the need to satisfy consumers' demand for energy (i.e. b and d via ρ_i and γ_i) and structural constituents (i.e. β_i and δ_i via ρ_i , γ_i and x_i) specifying the rules (i.e. equations 8, 10, and 11 for maintenance and 21, 22 and 23 for growth: see chapter 1) for resource exchange between prey and consumer.

5.3 Future issues

1. How much time does it take consumers to detect and react to changes in the biochemical composition of their food?
2. How do animals that undergo diapause as part of their life cycle (e.g. *Calanus hyperboreus*; Falk-Petersen et al. 2009) "interpret" their food environment prior to diapause?
3. How does individual feeding behaviour based on the food quality framework presented here extrapolates into that of an entire population or community?

Appendix 1

Biochemical Composition of Marine Zooplankton (Females and Eggs)

A1.1 ABSTRACT

Successful reproduction of zooplankton depends on several biochemical compounds, the availability of which could determine the dynamics of aquatic ecosystems. Hence, accounting for the biochemical requirements of zooplankton is a prerequisite for secondary production modelling. Lacking however are robust parameters, not restricted to specific species and/or habitat, for determining the biochemical characteristics of zooplankton. Presented in this study are parameters for determining protein (TP), lipid (TL), essential amino acid (EAAs) and essential fatty acids (EFAs) requirements of marine zooplankton females and their eggs. They were derived after reviewing published data on zooplankton species (42 female; 29 eggs) that inhabit different habitats and differ in morphology, trophic position and reproductive strategy. A strong log-log linear relationship between the size and biochemical composition of zooplankton was found. The data show that zooplankton can be classified into two main groups based on their content of TP and TL, these being those with $TP > TL$, and those with $TP < TL$. The critical dry weight that marks the transition between these two zooplankton groups was $\sim 3162 \mu\text{g}$ for adult, and $\sim 20 \mu\text{g}$ for egg. EFAs content of eggs was found to be independent of their TL composition. Conversely, lipid-poor females mostly contain more EFAs than lipid-rich ones, potentially due to difference in their habitat and food conditions. EAAs content of both eggs and adults was independent of the animals' protein composition.

A1.2 INTRODUCTION

Among other factors, the provision of biochemical substances is a major constraint on animal production (e.g. Anderson and Pond 2000; Anderson et al. 2004). Protein and lipids are the two most important biochemical substances animals require. Lipids serve as energy reserve, membrane components, antioxidants, etc (Kattner et al. 2007). Among others, proteins are major constituents of muscle tissues, metabolic enzymes and serve as carriers of genetic information (e.g. Ribonucleic acid, RNA). The availability of these substances can therefore control animals' behaviour, physiology, life history traits, as well as zoogeography (Guisande 2006). Furthermore, both protein and lipid have micromolecular sub-components that most animals cannot synthesize (i.e. essential compounds) but require for important life processes such as gene expression, cell differentiation (Xu et al. 1994; Anger 1998; Sessler

and Ntambi 1998) and thermal adaptation (Hassett and Crockett 2009; Sperfeld and Wacker 2009).

In aquatic systems, zooplankton constitute an important animal group. They are responsible for the transfer of a large proportion of the energy that moves between primary producers and tertiary (and higher) trophic levels; they are a common food source for adult fishes, and their eggs (and nauplii) are preyed upon by first-feeding larval fish (Cushing 1990), some actively prey on other animals, which may even affect the recruitment success of commercially important fish stocks (Bailey and Yen 1983; Yen 1987). Hence, the dynamics of aquatic systems depend therefore to a large extent on that of zooplankton. As a consequence, it is a prerequisite for aquatic ecosystem models to account for the biochemical composition/requirement of zooplankton (e.g. Anderson et al. 2005; Mayor et al. 2009; Flynn and Mitra 2009).

The biochemical composition of zooplankton has been extensively described in the literature, particularly that of lipids (Mauchline 1998; Ventura 2006 and references therein). Protein composition of crustacean zooplankton is known to be species-specific (Guisande et al. 2003). Similarly, lipid composition in marine zooplankton differs between species and can range between few percent to more than 60 % of individual dry mass (see review by Lee et al. 2006). Also, the biochemical composition of zooplankton may be dictated by several ambient conditions such as temperature (Farkas 1984; Hassett and Crockett 2009; Sperfeld and Wacker 2009), salinity (Anger 1998; Biagini et al. 2000), food availability (Guisande and Harris 1995; Appendix 3 of this thesis) and feeding history of the animals (Harvey 1937; Sykes 1990). In other words, different factors interact to determine the biochemical characteristics of zooplankton.

Hence, zooplankton production models could achieve greater robustness and applicability by employing biochemical parameters that are not restricted to specific species and/or habitat. Such parameters however do not currently exist. Also, how the difference in bulk biochemical contents of zooplankton is reflected in their essential compound composition has not yet been established. Since we are now aware that deficiencies in essential compounds could potentially overshadow the effect of bulk biochemical substances on animals (Jónasdóttir 1994; Guisande et al. 2000; Müller-Navarra et al. 2000), parameters for characterizing animals' composition of essential compounds are vital for modelling ecological processes that involve or are influenced by biochemical requirements of zooplankton, such as prey selection (Cowles et al. 1988; Jones and Flynn 2005) and C cycling (Darchambeau et al. 2003).

In this article, published biochemical composition data on marine zooplankton eggs and females from different habitats and laboratory conditions has been reviewed to: (1) establish parameters that could be used to determine total protein and lipid content of morphologically and nutritionally diverse zooplankton species, and (2) investigate the extent to which the essential amino and fatty acids requirements of zooplankton depends on their content of bulk protein and lipid.

A1.3 METHODS

Data base

Data on dry weight, protein, lipid, amino and fatty acids contents of adults and freshly produced eggs (i.e. eggs with no visible embryonic development) of zooplankton were extracted from published studies. A MATLAB[®] 7.0.4 software was used to digitize and extract data from figures. Biochemical constituent of zooplankton differs with ontogenic stage (e.g. Bruce et al. 2005). For the purpose of this thesis, the investigation here is restricted to the biochemical composition of only eggs and female adults. The species on which data were found are given in Tables 1 (for eggs) and 2 (for adults). Data on a total of 29 (eggs) and 42 (females) zooplankton species belonging to the most common taxonomic groups in marine habitats were found.

Several biotic and abiotic factors act together to determine the biochemical compositions of zooplankton. These factors exhibit great variability in space and time that cannot be ignored. However, they are rarely standardized in experimental studies to enable inter-comparison of results. Quantifying the major biochemical constituents of animals while accounting for the impact of the environmental conditions animals encounter therefore poses practical problems. Also, it is the aim of this study to establish robust parameters that could be applied under different environmental conditions. Hence, only a criterion that animals must not be food-quantity limited during sampling for biochemical analysis was used for data selection. Data from laboratory studies were taken only if organisms were cultured (in the case of adults) or produced (in the case of eggs) at food levels not below $1000 \mu\text{g C L}^{-1}$, a food concentration at which zooplankton are unlimited (Kiørboe et al. 1985). This was necessary because food availability affects zooplankton biochemical composition (e.g. Roman 1991; Guisande and Harris 1995; Appendix 3 of this thesis). All data from field studies were accepted under a presumption that animals in their natural habitats are less likely to be food limited.

Table 1. Published data used to describe the biochemical composition of crustacean eggs. Cop, Dec and Eup represent the taxonomic orders Copepoida, Decapoda and Euphausiacea respectively. Dw = dry weight; TL = total lipid; TP = total protein; FA = fatty acids; AA = amino acids

Order	Family	Species	Dw	TL	TP	FA	AA	Reference
Cop	Acartiidae	<i>Acartia omorii</i>	X	X		X		Shin et al. 2003
		<i>A. tonsa</i>	X		X	X		Appendix 3 of this thesis
		<i>A. tonsa</i>		X		X		Ederington et al., 1995
		<i>A. tonsa</i>	X		X			Drillet et al., 2008
		<i>A. tonsa</i>	X			X		Drillet et al., 2006
		<i>A. tonsa</i>				X	X	Drillet et al., 2006a
	Aetideidae	<i>Chiridius obtusifrons</i>	X	X				Auel, 2004
	Alpheoidea	<i>Neocalanus tonsus</i>	X	X				Ohman et al, 1989
	Calanidae	<i>Calanoides carinatus</i>	X	X				Borchers and Hutchings, 1986
		<i>Calanus finmarchicus</i>	X					Falk-Peterson et al., 2009
		<i>C. finmarchicus</i>	X	X				Ohman and Runge, 1994
		<i>C. helgolandicus</i>	X			X		Lacoste et al., 2001
		<i>C. helgolandicus</i>	X	X	X			Guisande and Harris, 1995
		<i>C. helgolandicus</i>				X	X	Laabir et al., 1999
		<i>C. simillimus</i>		X		X		Ward et al., 1996
		<i>C. simillimus</i>	X					Ward and Shreeve, 1998
		<i>Centropages tenuiremis</i>	X	X	X		X	Wang et al., 2005
		Euchaetidae	<i>Euchaeta japonica</i>	X	X			
	<i>Paraeuchaeta antarctica</i>		X	X	X			Alonzo et al., 2000
	<i>P. barbata</i>		X	X				Auel, 2004
	<i>P. glacialis</i>		X	X				Auel, 2004
	<i>P. norvegica</i>		X	X				Auel, 2004
	<i>P. polaris</i>		X	X				Auel, 2004
Euterpinidae	<i>Euterpina acutifrons</i>					X	Guisande et al., 1999; 2000	
Rhincalanidae	<i>Rhincalanus gigas</i>			X		X	Ward et al., 1996	
	<i>R. gigas</i>	X					Ward and Shreeve, 1998	
Dec	Hippolytidae	<i>Chorismus antarcticus</i>	X	X		X		Graeve and Wehrtmann, 2003
	Carpiliidae	<i>Xantho bidentatus</i>	X	X	X			Erri Babu, 1987
	Crangonidae	<i>Crangon crangon</i>	X	X	X			Pandian, 1967
		<i>Notocrangon antarcticus</i>	X	X		X		Graeve and Wehrtmann, 2003
	Majidae	<i>Hyas areaneus</i>	X	X	X			Petersen and Anger, 1997
	Nematocarcinidae	<i>Nematocarcinus lanceopes</i>	X	X		X		Graeve and Wehrtmann, 2003
	Nephropidae	<i>Homarus gammarus</i>	X	X	X	X	X	Rosa et al., 2005
		<i>H. gammarus</i>	X	X	X			Pandian, 1970
		<i>Nephrops Norvegicus</i>	X	X	X	X	X	Rosa et al., 2003
	Palaemonidae	<i>Macrobrachium rosenbergii</i>	X	X	X	X	X	Jun-jie et al., 2006
		<i>M. rosenbergii</i>	X	X	X			Clarke et al., 1990
	Paguridae	<i>Pagurus bernhardus</i>	X	X	X			Pandian and Schumann, 1967
Eup	Euphausiidae	<i>Euphausia crystallorophias</i>	X	X				Kattner and Hagen, 1998
		<i>E. superba</i>	X	X	X			Amsler and George, 1985
		<i>E. superba</i>	X	X				Clarke and Morris, 1983

Table 2. Published data used to describe the biochemical composition of adult crustaceans. Cop, Dec, Eup and Mys represent the taxonomic orders Copepoida, Decapoda, Euphausiacea and Mysida respectively. Dw = dry weight; TL = total lipid; TP = total protein; FA = fatty acids; AA = amino acids. F after species name represents females. Sex was not identified where it is not reported.

Order	Family	Species	Dw	TL	TP	FA	AA	Reference
Cop	Acartiidae	<i>Acartia clausi</i>	X	X	X	X	X	Rajkumar and Vasagam, 2006
		<i>A. clausi</i> F	X	X	X			Kapiris et al., 1997
		<i>A. omorii</i> F	X			X		Shin et al, 2003
		<i>A. tonsa</i> F	X		X	X		Chapter 7 of this thesis
		<i>A. tonsa</i>				X		Støttrup et al., 1999
		<i>A. tonsa</i>				X		Veloza et al., 2006
		<i>A. tonsa</i> F	X					Kjørboe et al., 1985
	Aetideidae	<i>Euchirella brevis</i> F	X	X				Lee and Hirota, 1973
		<i>E. galeata</i> F	X	X				Lee et al., 1971
		<i>E. pulchra</i> F	X	X				Lee et al., 1971
		<i>E. rostromagna</i> F	X	X		X		Hagen et al., 1995
	Alpheoidea	<i>Neocalanus plumchrus</i> F	X	X				Lee and Hirota, 1973
		<i>N. tonsus</i> F	X	X				Ohman et al., 1989
	Calanidae	<i>Calanus finmarchicus</i>	X	X				Evjemo et al., 2003
		<i>C. finmarchicus</i> F	X	X				Falk-Peterson et al, 2009
		<i>C. finmarchicus</i> F	X	X				Kattner and Krause, 1989
		<i>C. finmarchicus</i> F	X	X				Ohman and Runge, 1994
		<i>C. finmarchicus</i> F	X	X	X			Helland et al., 2003b
		<i>C. finmarchicus</i> F				X		Graeve et al., 2005
		<i>C. glacialis</i> F	X	X				Falk-Peterson et al., 2009
		<i>C. glacialis</i> F	X	X		X		Tande and Henderson, 1988
		<i>C. glacialis</i> F	X	X				Scott et al, 2000
		<i>C. glacialis</i> F	X	X				Lee and Hirota, 1973
		<i>C. glacialis</i>				X		Graeve et al., 1994
		<i>C. glacialis</i> F				X		Graeve et al., 2005
		<i>C. helgolandicus</i> F	X	X				Kattner and Krause, 1989
		<i>C. helgolandicus</i> F					X	Cowey and Corner, 1963
		<i>C. hyperboreus</i> F	X	X				Falk-Peterson et al., 2009
		<i>C. hyperboreus</i> F	X	X				Scott et al, 2000
<i>C. minor</i> F		X	X				Lee and Hirota, 1973	
<i>C. pacificus</i> F		X	X				Ohman, 1988	
<i>C. pacificus</i> F		X	X				Lee and Hirota, 1973	
<i>Calanus simillimus</i> CVI F		X	X				Ward et al., 1996	
Eucalanidae		<i>Eucalanus californicus</i> F	X	X				Ohman, 1988
	<i>E. bungii</i> F	X	X				Saito and Kotani, 2000	
Euterpinidae	<i>Euterpina acutifrons</i>				X	X	Guisande et al., 2000	
	<i>E. acutifrons</i>				X	X	Guisande et al., 1999	
	<i>E. acutifrons</i>	X					Zurlini et al., 1978	
Euchaetidae	<i>Euchaeta antarctica</i> F	X	X		X		Hagen et al., 1995	
	<i>Euchaeta japonica</i> F	X	X				Lee et al., 1974	
	<i>Paraeuchaeta antarctica</i> F	X	X	X			Alonzo et al., 2000	
	<i>P. antarctica</i> F	X	X				Auel and Hagen, 2005	
	<i>P. barbata</i> F	X	X				Auel and Hagen, 2005	
	<i>P. barbata</i> F	X	X				Lee, 1975	
	<i>P. cf. biloba</i> F	X	X				Auel and Hagen, 2005	
<i>P. glacialis</i> F	X	X				Auel and Hagen, 2005		

Table 2 continued

Order	Family	Species	Dw	TL	TP	FA	AA	Reference	
Cop	Euchaetidae	<i>Paraeuchaeta glacialis</i> F	X	X				Lee, 1975	
		<i>Paraeuchaeta norvegica</i> F	X	X			Auel and Hagen, 2005		
		<i>P. polaris</i> F	X	X			Auel and Hagen, 2005		
		<i>P. rubra</i> F	X	X			Lee et al., 1971		
	Metridinidae	<i>Metridia gerlachei</i> F	X	X				Graeve et al., 1994	
		<i>M. longa</i> F	X	X		X		Stevens et al., 2004	
		<i>M. longa</i> F	X	X				Lee, 1975	
		<i>M. okhotensis</i> F	X	X				Saito and Kotani, 2000	
		<i>M. pacifica</i> F	X	X				Ohman, 1988	
		<i>M. princeps</i> F	X	X				Lee et al., 1971	
		<i>Calanipeda aquae-dulcis</i>						X	Brucet et al., 2005
	Pseudodiaptomidae	<i>C. aquae-dulcis</i>	X						Brucet et al., 2006
		<i>Rhincalanus gigas</i> CIV F	X	X		X			Ward et al., 1996
	Rhincalanidae	<i>R. gigas</i> F	X	X					Lee and Hirota, 1973
		<i>R. nasutus</i> F	X	X					Ohman, 1988
		<i>R. nasutus</i> F	X	X					Lee and Hirota, 1973
		Temoridae	<i>Eurytemora velox</i>					X	
	<i>E. velox</i>		X						Brucet et al., 2006
	<i>Temora longicornis</i> F		X	X		X			Kreibich et al., 2008
Dec	Galatheididae	<i>Munida gregaria</i>			X		X	Burkholder et al., 1967	
Eup	Euphausiidae	<i>Euphausia crystallorophias</i>	X	X		X		Kattner and Hagen, 1998	
		<i>E. pacifica</i>					X	Suyama et al., 1965	
		<i>E. superba</i>	X		X		X	Srinivasagam et al., 1971	
		<i>E. superba</i>		X	X		X	Suyama et al., 1965	
		<i>E. superba</i>			X		X	Burkholder et al., 1967	
		<i>E. vallentini</i>					X	Suyama et al., 1965	
		<i>Meganyctiphanes norvegica</i>	X		X		X	Srinivasagam et al., 1971	

Dry weight and biochemical contents

Most studies reported dry weight for individual animals. Where dry weight was not reported, it was estimated either from: (i) animals' carbon weight assuming carbon constitute 40% of dry weight (Omori and Ikeda 1984; Bämstedt 1986), (ii) animals' length using published length – dry weight regression equations (for adults: Kimmerer and McKinnon 1987), or (ii) animals' size (length for adult; volume for eggs) using size – carbon regression equations (Uye 1982: for adults; Huntley and Lopez, 1992: for eggs) and then converted back to dry weight. Where these were not possible due to lack of data, dry weight data on the species from other published studies were used provided in addition to the food quantity criterion, it was measured under temperature conditions $\pm 1^\circ\text{C}$ (accuracy of most laboratory thermometers) similar to the study from which biochemical data were extracted. This was necessary because temperature influences the weight of most zooplankton species (Corkett and McLaren 1978; Huntley and Lopez 1992).

Reports on the individual fatty or amino acids compositions of zooplankton are often given in relative units, mostly either as a proportion of the animals' dry weight or total protein (amino acid) or lipid (fatty acid) content. Where values were expressed as proportions of animals' dry weights, mass of each compound was calculated by multiplying the reported proportions with the dry weight of the animal.

Lipid exists in two main forms: neutral and polar lipids. In animals, neutral lipids occur mainly as triacylglycerols, consisting of three molecules of fatty acid esterified to a molecule of glycerol, and wax esters made up of fatty acids and fatty alcohols. Polar lipids on the other hand, contain a constant non-fatty acid molecular fraction combined with a variable fatty acid part (Sargent and Falk-Peterson 1988). Most studies only reported the total fatty acid composition of lipids, without classification. This made the determination of the lipid source of the individual fatty acids impossible. Calculations involving essential fatty acids were therefore done without regard for their lipid source. Only studies giving fatty acids composition of organisms either in absolute units or as proportion of total lipid/fatty acids were used. Eicosapentaenoic acid (EPA) and docohexanoic acid (DHA) constitute the main essential fatty acids (EFAs) for zooplankton (see review by Müller-navarra 2008). They are thus the only EFAs considered for this study.

There are two main sources of amino acids in animals. These are protein-bounded amino acids and free amino acids (i.e. amino acids not bounded to proteins). Hence, only studies that reported total amino acid composition of animals were considered for this study. Where amino acid measurements were separated between free and protein-bounded types, the sum was estimated for each amino acid. Amino acids essential for zooplankton and considered for this study are histidine (HIS), arginine (ARG), threonine (THR), valine (VAL), lysine (LYS), isoleucine (ILE), leucine (LEU), phenylalanine (PHE) and methionine (METH) (Drillet et al., 2006; Jun-jie et al., 2006; Helland et al., 2003b).

Data analysis

When the biochemical composition of a species was extracted from different studies, the mean was estimated and used for that species. In both adults and eggs, there was a huge weight differences between animals (Tables 3 and 4). As a result, the distribution of the data was not normal. Therefore, prior to data analysis, all dry weight, total protein and lipid measurements were \log_{10} transformed to normalised the data (Sokal and Rohlf 1981).

Data analysis followed a procedure adapted from established methods for investigating size to biochemical content relationships in planktonic organisms (e.g.

Montagnes et al. 1994; Menden-Deuer and Lessard 2000). The relationship between organisms' dry weight and biochemical composition was examined to determine if it followed a linear or non-linear pattern by fitting the data to the power function below, using a non-linear curve fitting method (Sigmaplot 10.0):

$$Y = aX^c + b \quad (1)$$

where Y was either bulk content of protein (TP), or lipid (TL), X was animals' dry weight, and a , b and c were constants. The relationship between bulk substances and their total constituent of essential sub-units were also similarly examined, but with Y that represented either essential fatty acids (EFAs) or essential amino acids (EAAs), and X that was animals' content of bulk substances. The automatic initial-parameter estimator function in Sigmaplot was used. The program provides standard errors for estimated parameters. The error term associated with " c " was used to test the null hypothesis $c = 1$ (t-test, $\alpha = 0.05$, $df = n - 3$) against the alternate hypothesis that $c \neq 1$. If the null hypothesis could not be rejected, the relationship was considered linear. If $c \neq 1$, the relationship was considered non-linear; i.e. Y increases allometrically at a slower ($c < 1$) or greater ($c > 1$) rate than X . When a linear relationship could not be rejected, least squares regression was used to determine the slope, a , as well as the y-axis intercept, b , of the line.

The appropriateness of using Model I (fixed independent variable) instead of Model II (independent variable measured with errors) approach for the regression analysis here can be debated. The data in this study, like that of most oceanographic studies, violates an assumption that is explicitly part of the Model I theory, which is that at least the independent variable be under the control of the investigator (Laws and Archie 1981). Neither the dry weight nor biochemical composition of the zooplankton was a controlled variable in any of the studies referenced. However, the main goal of the analysis here is to establish a relationship and parameters that could be used to estimate animals' biochemical composition from dry weight. In such cases, analysis based on model I is considered appropriate (Menden-Deuer and Lessard 2000).

To compare the relative composition of individual essential compounds between zooplankton species, a 1-way ANOVA ($p < 0.05$) was conducted, with the different species being the factor. Due to size differences between species, the ANOVA test was based on the relative composition of essential compounds within organisms. For EAAs, these were calculated as the mass ratio between individual amino acids and total protein contents of organisms. For EFAs, they were calculated as the mass ratio between individual EFA and total fatty acid content of the animals.

Table 3. Dry weight (Dw), protein and lipid composition of female zooplankton

Family	Species	Dw (μg)		Total Lipid (% of dw)		Total protein (% of dw)	
		Mean \pm std	Range	Mean \pm std	Range	Mean \pm std	Range
Acartiidae	<i>Acartia clausi</i>	5.53 \pm 1.27	7 – 4.8	16.41 \pm 0.5		55.85 \pm 10.28	63.12 – 48.57
	<i>A. omorii</i>	5.23 \pm 0.35		9.05 \pm 0.00			
	<i>A. tonsa</i>	5.61 \pm 0.36	7.4 – 5.3	29.84 \pm 24.19	57.37 – 5.83		
	<i>Acartia sp.</i>					55.24	
Aetideidae	<i>Euchirella brevis</i>	352.94		17.00			
	<i>Euchirella galeata</i>	1500		4.0			
	<i>Euchirella pulchra</i>	416.66		12.00			
	<i>Euchirella rostromagna</i>	3265.0 \pm 91.9	3330 - 3200	26.45 \pm 3.37	28.83 – 24.06		
Alpheoidea	<i>Neocalanus tonsus</i>	500		30			
Calanidae	<i>Calanus finmarchicus</i>	213.39 \pm 75.73	342.62 – 95.0	23.83 \pm 12.37	46.1 – 5.49		
	<i>Calanus glacialis</i>	579.4 \pm 217.8	960.42 – 272.73	40.61 \pm 28.51	75.0 – 11.0		
	<i>Calanus helgolandicus</i>	162.50 \pm 29.27	236 - 133	11.68 \pm 5.19	17.96 – 7.71	39.40 \pm 0.15	39-67 – 39.33
	<i>Calanus hyperboreus</i>	2920 \pm 1500		62.2			
	<i>Calanus minor</i>	33.33		3			
	<i>Calanus pacificus</i>	232.9 \pm 37.17	259.3 – 206.7	17.85 \pm 12.94	27.0 – 8.7		
	<i>Calanus simillimus</i>	312.25 \pm 15.17	320 - 295	16.43 \pm 7.47	25.83 - 10		
Eucalanidae	<i>Eucalanus californicus</i>	363.1 \pm 23.5		11.5 \pm 2.1			
Euchaetidae	<i>Euchaeta Antarctica</i>	3720 \pm 1240	5133 - 2820	37.04 \pm 2.60	38.77 – 34.04		
	<i>E. japonica</i>	1153.84		52.00			
	<i>Paraeuchaeta antarctica</i>	4970 \pm 810	7550 - 4656	33.88 \pm 10.91	47.99 – 17.44	27.40 \pm 8.21	45.10 – 18.88
	<i>P. barbata</i>	6710.0 \pm 2828.7	9890 - 3680	44.04 \pm 3.55	46.90 – 39.30		
	<i>P. cf. biloba</i>	10370		58.50			
	<i>P. glacialis</i>	5530 \pm 2650	8410 - 4210	42.42 \pm 5.00	47.80 – 35.50		
	<i>P. norvegica</i>						
Euchaetidae	<i>Paraeuchaeta. norvegica</i>	4340 \pm 360	4770 - 3910	47.4 \pm 2.78	52.20 – 43.70		
	<i>Paraeuchaeta polaris</i>	3110 \pm 440		54.2 \pm 5.0			
Euphausiidae	<i>P. rubra</i>	2807.01		57			
	<i>Euphausia crystallorophias</i>	50800 \pm 14060	64400 – 31300	35.44 \pm 15.28	50.0 – 13.10		
	<i>E. pacifica</i>			1.48		16.65	
	<i>E. superba</i>	54770 \pm 410	60530 – 49010	24.65		42.73 \pm 15.61	56.21 – 17.75
	<i>Meganyctiphanes norvegica</i>	93040 \pm 6760	97820 – 88260			46.90 \pm 4.38	50.0 – 43.80

Table 3 continued

Family	Species	Dry weight, dw (μg)		Total Lipid (% of dw)		Total protein (% of dw)	
		Mean \pm std	Range	Mean \pm std	Range	Mean \pm std	Range
Euterpinidae	<i>Euterpina acutifrons</i>	3.89 \pm 2.23	5.47 – 2.31			47.56 \pm 1.45	46.53 – 48.58
Galatheidae	<i>Munida gregaria</i>			38.08		13.19	
Metridinidae	<i>M. longa</i>	433.5 \pm 62.6	520 - 370	29.96 \pm 16.77	57.00 – 10.81		
	<i>M. okhotensis</i>	490		74.83			
	<i>M. pacifica</i>	101.0 \pm 11.0		8.2 \pm 1.1			
Pseudodiaptomidae	<i>Calanipeda aquae-dulcis</i>	120.92					
Rhincalanidae	<i>Rhincalanus gigas</i>	1557 \pm 1279	3004 – 579.7	35.97 \pm 29.94	69.00 – 10.59		
	<i>R. nasutus</i>	266.3 \pm 27.5	285.7 – 246.8	37.90 \pm 5.80	42.00 – 33.80		
Temoridae	<i>Eurytemora sp.</i>					57.26	
	<i>Eurytemora velox</i>	219.89					
	<i>Temora longicornis</i>	33.45 \pm 9.97	40.50 – 26.40	4.85 \pm 2.47	6.60 – 3.10		

Separate tests were conducted for each essential compound. Prior to the ANOVA test, the relative composition data were arcsine transformed and verified for normality via Kolmogorov–Smirnov test. Whenever significant differences were found, the Tukey Test was applied to find the responsible species (Sokal and Rohlf 1981).

A1.4 RESULT

Protein and lipid contents

Total protein and lipid composition of the animals used in this study are shown in Tables 3 and 4. Bulk biochemical compositions of zooplankton varied between as well as within zooplankton species. In adults, lipids and proteins constituted on average between 3.0 to 74.8%, and 13 to 58.5% of individual dry weight respectively. In eggs, the average composition was between 3.9 to 93.1%, and 33 to 66.0% of dry weight respectively for lipids and proteins.

Table 4. Dry weight (Dw), protein and lipid content of zooplankton eggs

Family	Species	Dw (μg)		Total Lipid (% of dw)		Total protein (% of dw)	
		Mean \pm std	Range	Mean \pm std	Range	Mean \pm std	Range
Acartiidae	<i>Acartia clausi</i>						
	<i>A. omorii</i>	0.06		32.30			
	<i>A. tonsa</i>	0.12 \pm 0.02	0.12 – 0.08	46.70 \pm 20.36	61.10 – 32.30	53.46 \pm 11.42	61.54 – 45.38
Aetideidae	<i>Chiridius obtusifrons</i>	10		71.9			
Alpheoidea	<i>Neocalanus tonsus</i>	0.21		66			
Carpiliidae	<i>Xantho bidentatus</i>	71		30.50		54.3	
Calanidae	<i>Calanoides carinatus</i>	0.8		75			
	<i>Calanus finmarchicus</i>	0.56 \pm 0.08	0.63 – 0.47	9.24 \pm 8.15	15.0 – 3.47		
	<i>Calanus helgolandicus</i>	0.81		35.50		58.5	
	<i>Calanus simillimus</i>	2.07		3.92			
	<i>Centropages tenuiremis</i>	0.12		31.22		54.85	
Crangonidae	<i>Crangon crangon</i>	17.0		32.6		58.7	
	<i>Notocrangon antarcticus</i>	687 \pm 27	714 - 660	14.3			
Euchaetidae	<i>Euchaeta japonica</i>	62.5		64			
	<i>Paraeuchaeta antarctica</i>	12.56 \pm 3.51	21.14 – 9.51	52.29 \pm 10.55	62.50 – 44.94	46.29 \pm 13.16	64.09 – 22.71
	<i>P. barbata</i>	130		59.7 \pm 0.6			
	<i>P. glacialis</i>	60		63.80 \pm 3.10			
	<i>P. norvegica</i>	30		59.00 \pm 0.90			
	<i>P. polaris</i>	110		93.1			
Euphausiidae	<i>Euphausia crystallophias</i>	23		52.17			
	<i>E. superba</i>	28.95 \pm 1.82	30.00 – 26.86	33.15 \pm 2.61	35.00 – 31.30	57.4	
Euterpinae	<i>Euterpina acutifrons</i>	0.04				63.67	
Hippolytidae	<i>Chorismus antarcticus</i>	830 \pm 73	903 - 757	18.8			
Majidae	<i>Hyas areaneus</i>	69		30.60		33.90	
Nematocarinidae	<i>Nematocarinus lanceopes</i>	277		18.1			
Nephropidae	<i>Homarus gammarus</i>	1250 \pm 520	799.2 - 1700	35.00 \pm 12.44	43.80 – 26.20	52.63 \pm 7.40	57.86 – 47.40
	<i>Nephrops Norvegicus</i>	399.74		15.17 \pm 1.15		50.25	
Palaemonidae	<i>Macrobrachium rosenbergii</i>	45.85 \pm 5.95	51.0 – 40.7	30.810 \pm 3.41	33.22 – 28.40	53.59 \pm 10.91	61.30 – 45.87
Paguridae	<i>Pagurus bernhardus</i>	27		29.50		66.0	

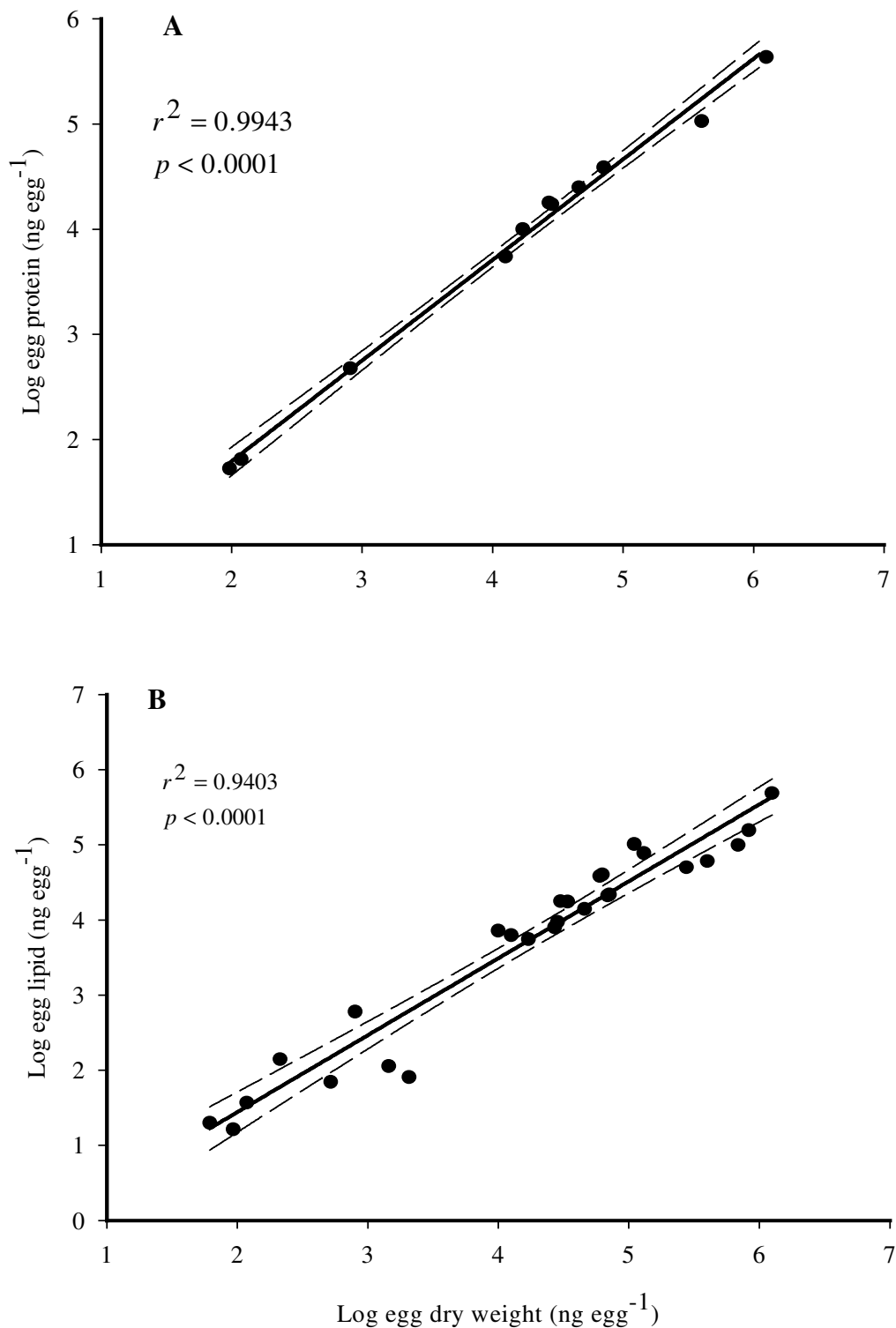


Figure 1. The relationship between dry weight and total protein (A) and total lipid (B) composition of zooplanktonic crustacean eggs. Each point represents a single species. Solid lines through data represent the least-square regression and broken lines are 95% confidence intervals. Regression statistics are given in Table 5. Axes scales are equal

These huge differences in biochemical compositions between organisms could generally be explained by the differences in the animals' dry weights. Figure 1 shows a plot of eggs' dry weight versus their total composition of lipid and protein. Figure 2 is a similar plot for adults. I should note that only organisms for which data on both dry weight and total biochemical contents were reported have been used for these plots. The parameters for the linear fits to the data are given in Table 5. The results show that for crustacean zooplankton as a group, the log-log relationship between dry weight and each bulk compound is linear (i.e. $c = 1$, t-test, $p < 0.05$). For both life stages, the slopes of the regression lines, which indicate the extent to which bulk biochemical composition of animals depended on the animals' dry weight, were all positive and highly significant ($p < 0.0001$), having coefficients of variations $\leq 5.1\%$ with adjusted r^2 values being ≥ 0.90 (Table 5). These statistics indicate a good predictive relationship between dry weight and the bulk biochemical composition of zooplankton. Generally stated, the heavier the animal, the bigger its composition of proteins and lipids. The biochemical composition of smaller individuals did not seem to vary more or less than that of bigger ones (Figure 1 and 2). Therefore, the scatter in the data around the regression lines cannot be attributed to the weight of individual animals.

Differences between lipid and protein composition of zooplankton were determined by comparing the parameters for the lines that describe their respective relationship with animals' dry weights (t-test, $df = N_1 + N_2 - 6$, $p < 0.05$, where N_1 and N_2 represent the number of data points used for determining protein and lipid regression lines respectively). Figure 3 shows the results. In eggs, the elevation (y-axis intercept, b) of the Dw:TP regression line is higher than that of Dw:TL ($p < 0.05$). However, the slope of Dw:TL line is slightly higher, albeit insignificant ($p > 0.05$), than that of egg Dw:TP line. The plot shows that eggs with dry weight $\leq 20\mu\text{g}$ may contain more proteins than lipids. Conversely, eggs with dry weights $\geq 20\mu\text{g}$, such as those produced by species that mostly inhabit colder waters like the deep-sea copepods *Chiridius obtusifrons*, and *Paraeuchaeta Antarctica*, may contain more lipids than proteins (Table 4). Among adults, the slope of the Dw:TL regression line was significantly higher than that of Dw:TP. However, the elevation of the Dw:TP regression line was higher ($p > 0.05$). As a result, the Dw:TL and Dw:TP regression lines crossover at adult dry weight of $\sim 3162.29 \mu\text{g}$ (Figure 3B). Adults with dry weight $\geq \sim 3162.29 \mu\text{g}$ may therefore contain more lipids than proteins. The reverse may be true for those with dry weight $\leq \sim 3162.29 \mu\text{g}$.

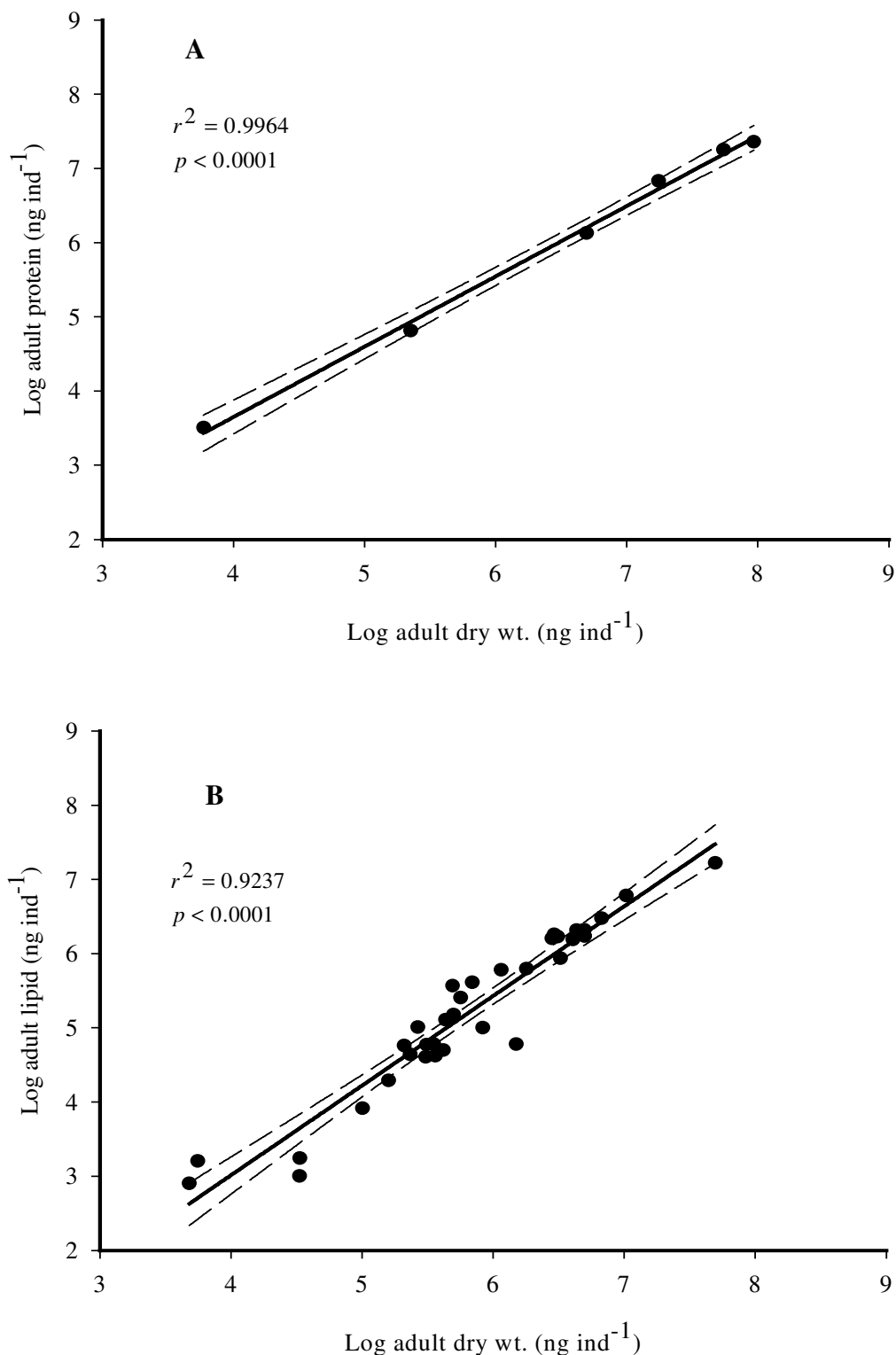


Figure 2. The relationship between dry weight and total protein (A) and total lipid (B) composition of zooplanktonic crustacean adults. Each point represents a single species. Solid lines through data represent the least-square regression and broken lines are 95% confidence intervals. Regression statistics are given in Table 5. Axes scales are equal.

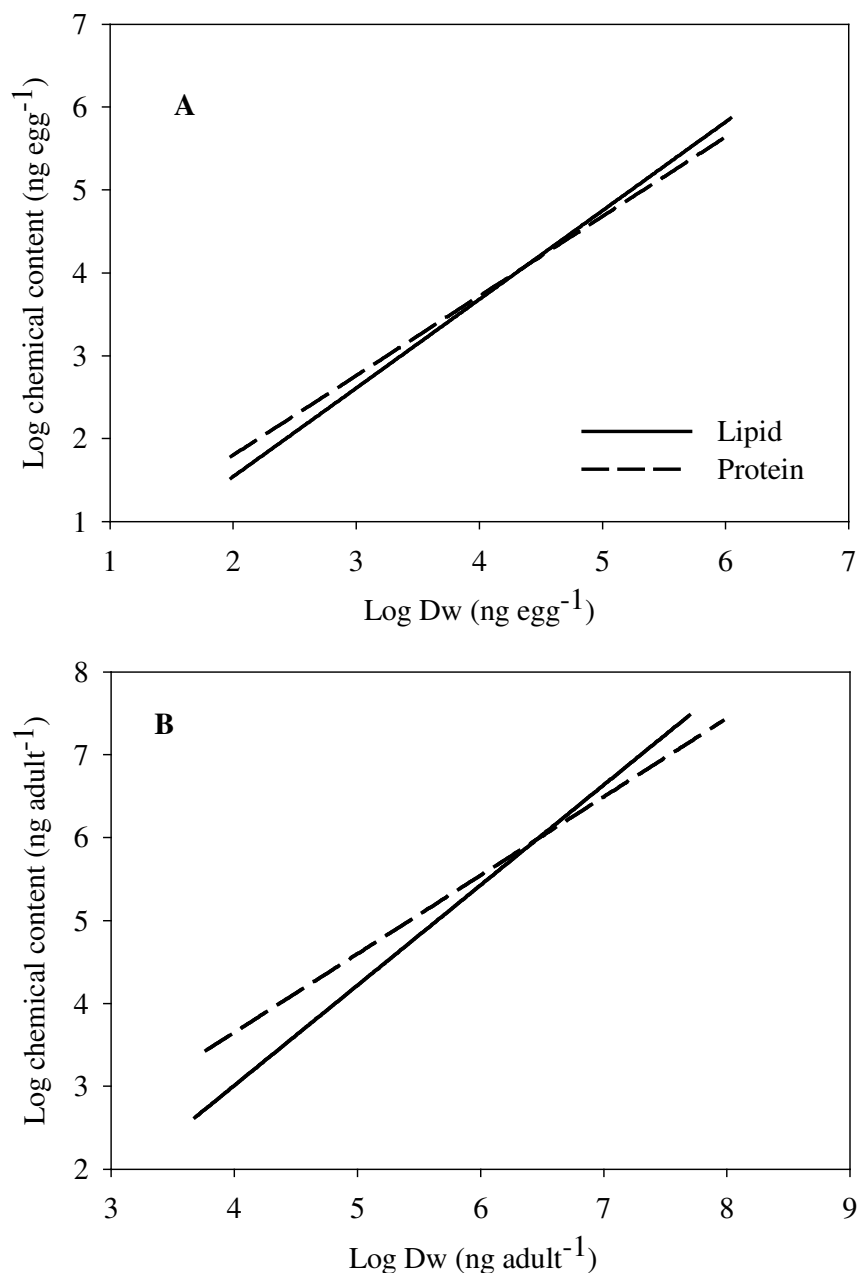


Figure 3. Comparison of total protein and lipid constituents of zooplankton eggs (A) and adults (B). Axes scales are not equal.

Essential amino and fatty acids contents

Tables 6 and 8 contain the essential amino and fatty acids composition of the zooplankton species. Generally, there were variations in total EAAs and EFAs composition of both eggs and adults. However, only the variations in EFAs content of the adults could be explained by the animals' composition of total lipid (Figures 4 and 6). Total essential fatty acid content of adults decreased linearly with their content of total lipid (Figure 5A).

Table 5. Parameters for the formula $\log Y = a \cdot \log X + \log b$ that describes the relationship between zooplankton dry weight (Dw, ng individual⁻¹) and their total composition (ng individual⁻¹) of lipid (TL), protein (TP), and essential fatty acids (EFA). a and b are constants. CV represents coefficient of variation in %.

Life stage	Variable		Parameter	Statistics			
	X	Y		Estimate	CV	P-value	Adjusted r ²
Egg	Dw	TL	a	1.07	5.04	<0.0001	0.9077
			b	-0.60	37.36	0.0362	
	Dw	TP	a	0.96	2.64	<0.0001	0.9921
			b	-0.12	102.50	0.3521	
Adult	Dw	TL	a	1.21	5.08	<0.0001	0.9213
			b	-1.83	19.77	<0.0001	
	Dw	TP	a	0.95	3.02	<0.0001	0.9954
			b	-0.14	13.00	0.5058	
	*L	*EFA	a	-1.05	19.46	0.0009	0.7385
			b	50.55	9.12	<0.0001	

* The relationship and parameters were estimated on normal (i.e. not log) axes. L = total lipid (% of individual dry weight); EFA (% of individual total fatty acid content).

On the other hand, total EAA content of adults were independent of their content of total protein (Figure 5B). In eggs, total EFA was independent of the total lipid contents (Figure 4A). Similarly, total EAA composition of eggs was not significantly influenced by their bulk protein contents (Figure 4B).

Tables 6 to 9 show the comparison of individual essential compounds between zooplankton species. Among adults, there were significant differences between species in both EPA and DHA. However, the post hoc test showed these differences to be limited to very few species (Tables 6 and 7). Also EPA and DHA fractions of the total fatty acids were generally not different within most species. This, together with the results shown in Figure 5A, suggests that lipid-rich zooplankton females may generally contain less essential fatty acids than lipid-poor ones. There were significant differences in individual amino acids between species of female copepods (Tables 6 and 7). However, the differences were not related to the bulk protein composition of the animals (Figure 5B).

Significant differences in all essential compounds except Leucine were found between eggs produced by different copepod species. However, the post hoc test showed these differences to be limited to eggs produced by few species (Tables 8 and 9). Noting that total protein and lipid content of eggs increased with their dry weight, the results here suggest that among planktonic crustacean, egg production requirement for essential compounds may be dependent on egg size/weight.

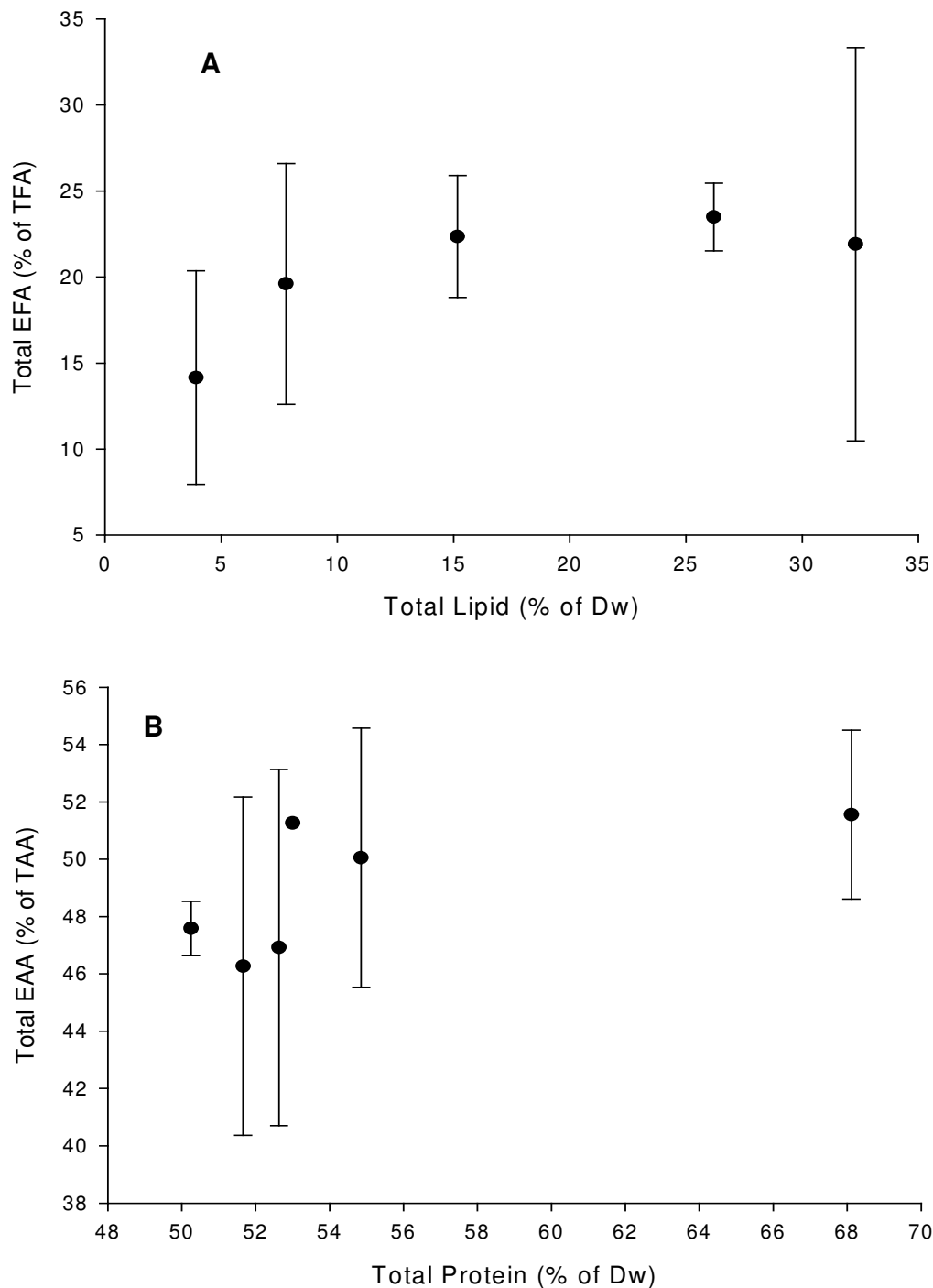


Figure 4. The relationship between bulk biochemical substances and their essential compound constituents of zooplankton eggs. **(A)** Total lipid versus essential total fatty acids (EFA), **(B)** Total protein versus total essential amino acids (EAA). Each point represents a species. Dw represents animals' dry weight. Axes scales are equal. Regression lines not fitted because they were not significant at $p < 0.05$. For A: $y = [0.24 \pm 0.11] * x + [16.25 \pm 2.31]$, $r^2 = 0.4514$, $p = 0.1301$. For B: $y = [0.23 \pm 0.13] * x + [48.94 \pm 7.33]$, $r^2 = 0.4317$, $p = 0.1563$.

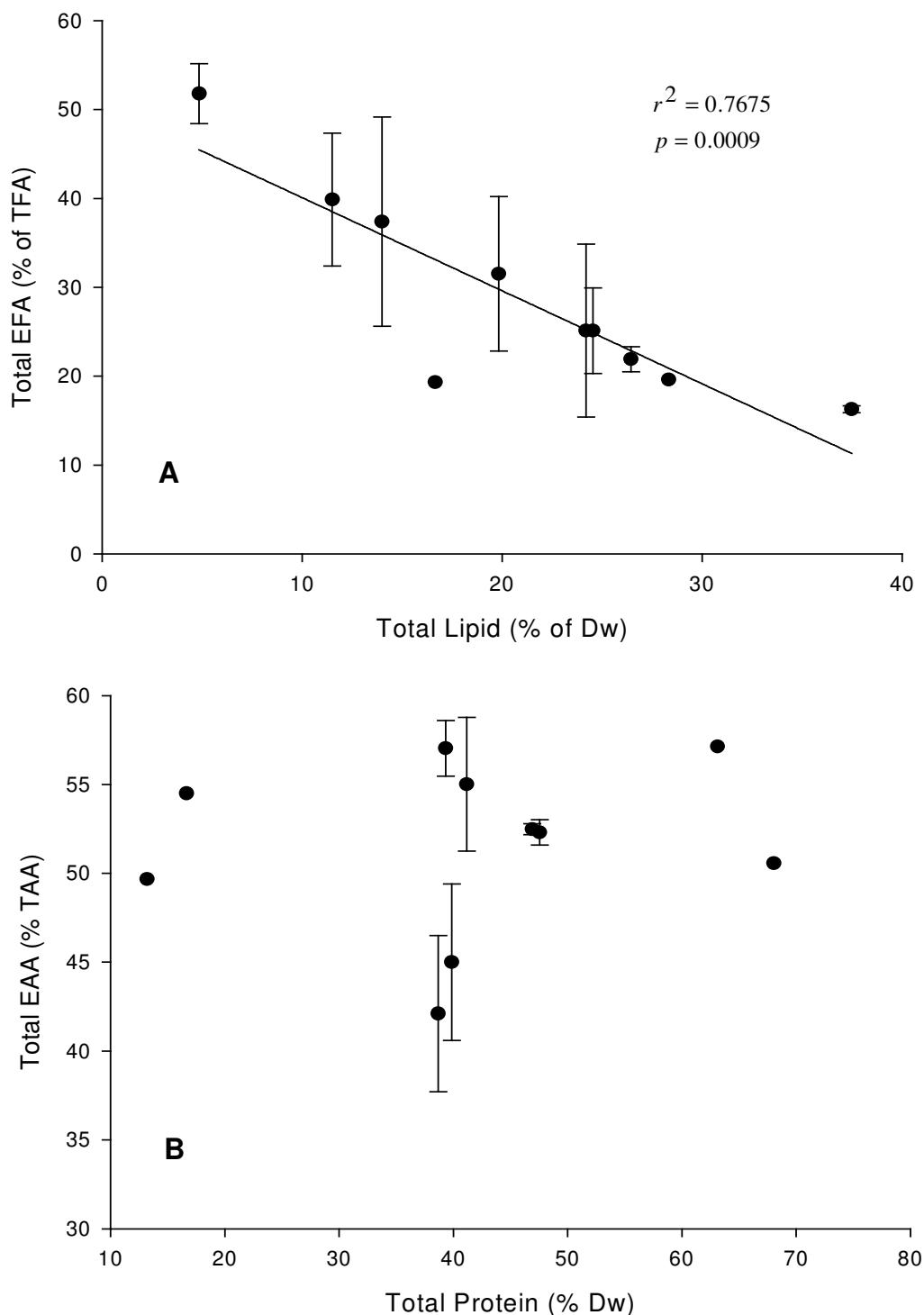


Figure 5. The relationship between bulk biochemical substances and their essential compound constituents of zooplankton adults. (A) Protein versus total essential amino acids (EAA). (B) Lipid versus total essential fatty acids (EFA). Each point represents a single species. Regression statistics for fitted line are given in Table 7. No fit was made for subplot B because its regression is not significant at $p < 0.05$ ($y = [0.04 \pm 0.10] * x + [49.89 \pm 4.47]$, $r^2 = 0.0200$, $p = 0.6964$).

Within the range of the available data, some eggs contained more proteins than lipids (Figure 3A), which suggest their requirements for essential amino acids may also exceed that of essential fatty acids. Conversely, eggs that contained more lipids than proteins may require more EFAs than EAAs.

A1.5 DISCUSSION

Bulk protein and lipid contents

Proteins and lipids composition of zooplankton have been extensively treated in the literature. The general knowledge has been that total protein and lipid content of zooplankton are proportionally related to the weights of the animals (e.g. Helland et al. 2003a,b; Guisande 2006). The results here support such conclusions (Figures 1 and 2). Bigger sized animals have obviously more cells as they may rely on greater mass of muscle for movement than smaller ones. While muscle tissues are made up of mainly proteins, lipidal compounds constitute the main components of cell membranes, in addition to serving as energy reserves (Falk-Petersen 1988). These could explain why both lipid and protein contents of zooplankton were directly proportional to their weight, being high in heavier adults and low in less heavy ones (Table 3).

Reproductive success is a major criterion for determining the adaptation of zooplankton to their habitat conditions. Based on difference in reproductive strategy (i.e. time and source of lipid used for reproduction), Kattner et al. (2007) classified zooplankton into 2 main groups, these being animals that depend on food intake for successful reproduction, and those that do not depend on food intake for successful reproduction. These two groupings were largely preserved when both protein and lipid contents were used to discriminate between the animals (Figure 3B; Table 3), with species compositions similar to those previously given by Kattner et al. (2007).

Group 1 comprised of zooplankton with dry weight $\geq 3162.29 \mu\text{g}$ and containing more lipid than protein. It consisted mostly of species belonging to the taxonomic families euchaetidae, euphausiids and some *Calanus* species like *C. hyperboreus* (Table 3). These animals tend to inhabit or show diel vertical migration into meso- and bathy-pelagic environments (Ohman and Townsend 1998) where food availability is often low. In addition, the available data show that members of this group produce relatively heavier eggs that mostly contain more lipid than protein (Tables 1 and 4).

Table 6. Essential compounds of zooplankton adults. Different superscript letters within columns represent significant differences ($p < 0.05$)

Species	Essential amino acid (% of Total amino acids)								
	HIS	ARG	THR	VAL	LYS	ILE	LEU	PHE	METH
<i>A. clausi</i>	3.50 ^a	8.57 ^a	2.50 ^k	9.57 ^a	11.04 ^a	2.17 ^a	9.92 ^a	1.05 ^a	1.98 ^a
<i>C. aquae-dulcis</i>	2.5 ± 0.3 ^{b c d e}	7.4 ± 1.3 ^a	5.2 ± 0.4 ^a	5.4 ± 0.4 ^b	8.5 ± 1.0 ^b	4.9 ± 0.3 ^b	7.0 ± 0.5 ^{b c d}	4.1 ± 0.4 ^b	0.0 ^b
<i>C. helgolandicus</i>	3.8 ± 0.3 ^a	16.5 ± 0.6 ^b	3.7 ± 0.0 ^b	6.9 ± 1.9 ^{b c}	10.7 ± 1.7 ^a	3.8 ± 0.3 ^c	6.2 ± 0.3 ^{b c}	2.5 ± 0.5 ^{c f}	1.5 ± 0.4 ^c
<i>E. acutifrons</i>	2.6 ± 0.1 ^{b c d e}	12.7 ± 4.3 ^b	4.9 ± 0.1 ^{a c}	5.5 ± 0.1 ^{b c}	7.5 ± 0.1 ^{b c}	4.9 ± 0.1 ^b	7.2 ± 0.2 ^{b c d}	3.5 ± 0.2 ^{b d}	0.0 ^b
<i>E. pacifica</i>	2.2 ^{b c d e}	5.95 ^a	4.83 ^{a c d}	5.19 ^{b c}	7.84 ^{b d}	5.16 ^b	7.83 ^{b d}	6.5 ^{c e}	3.25 ^d
<i>E. superba</i>	2.8 ± 0.5 ^{b c d}	7.2 ± 0.7 ^a	4.5 ± 0.4 ^{c d e}	5.5 ± 0.6 ^{b c}	9.3 ± 0.6 ^{a b}	4.8 ± 0.3 ^b	7.5 ± 0.9 ^{b c d}	2.8 ± 0.5 ^{d f g}	2.4 ± 0.2 ^a
<i>E. vallentini</i>	2.1 ^{b c e}	5.92 ^a	4.8 ^{a e f}	5.35 ^{b c}	8.55 ^b	5.28 ^b	8.35 ^{b d}	6.64 ^e	3.18 ^d
<i>E. velox</i>	2.6 ± 0.1 ^{b c d e}	5.5 ± 0.6 ^a	5.9 ± 0.4 ^g	5.8 ± 0.5 ^{b c}	7.4 ± 0.5 ^b	4.6 ± 0.6 ^b	6.1 ± 1.3 ^{b c}	4.2 ± 0.3 ^{b g}	0.0 ^b
<i>M. gregaria</i>	2.43 ^{b c d e}	7.02 ^a	4.78 ^{a e h}	5.70 ^{b c}	6.15 ^{c d}	3.63 ^c	6.71 ^{b c d}	4.35 ^b	2.21 ^a
<i>M. norvegica</i>	3.0 ± 0.3 ^{a b d}	7.4 ± 0.2 ^a	4.5 ± 0.0 ^{c d f h}	4.9 ± 0.2 ^b	9.7 ± 0.0 ^{a b}	4.6 ± 0.1 ^b	7.2 ± 0.0 ^{b c d}	4.6 ± 0.0 ^b	2.3 ± 0.2 ^a
Species	Essential Fatty acids (% of total fatty acids)								
	EPA	DHA	EPA:DHA						
<i>A. clausi</i>	9.95 ^a	9.37 ^{a b}	1.06						
<i>A. omorii</i>	13.2 ± 7.9 ^{a b}	22.0 ± 5.6 ^{a b}	0.60						
<i>A. tonsa</i>	6.1 ± 5.7 ^a	15.5 ± 11.9 ^{a b}	0.39						
<i>C. finmarchicus</i>	16.8 ± 9.8 ^{a b}	20.6 ± 12.9 ^{a b}	0.82						
<i>C. glacialis</i>	12.2 ± 5.1 ^{a b}	12.9 ± 9.7 ^{a b}	0.95						
<i>C. helgolandicus</i>	16.6 ± 4.3 ^{a b}	23.3 ± 7.5 ^{a b}	0.71						
<i>C. hyperboreus</i>	11.4 ± 0.4 ^{a b}	7.0 ± 0.3 ^{a b}	1.62						
<i>C. simillimus</i>	5.16 ^a	9.0 ^{a b}	0.57						
<i>E. antarctica</i>	6.2 ± 1.8 ^a	9.4 ± 0.4 ^{a b}	0.66						
<i>E. crystallorophias</i>	15.2 ± 1.9 ^{a b}	16.3 ± 8.7 ^{a b}	0.93						
<i>E. rostromagna</i>	12.6 ± 0.2 ^{a b}	9.3 ± 0.3 ^{a b}	1.35						
<i>M. longa</i>	14.3 ± 3.1 ^{a b}	10.8 ± 4.8 ^{a b}	1.32						
<i>P. elongatus</i>	13.8 ± 8.5 ^{a b}	15.2 ± 3.6 ^{a b}	0.91						
<i>R. gigas</i>	16.5 ^{a b}	3.1 ^{a c}	5.32						
<i>T. holothuriue</i>	14.2 ± 7.8 ^{a b}	24.7 ± 7.7 ^{a b}	0.58						
<i>T. longicornis</i>	25.9 ± 5.7 ^b	25.8 ± 3.4 ^{a b}	1.00						

Table 7. Comparison of essential biochemical compounds within crustacean zooplankton adults. A summary and description of the units of each compound are given in Table 6

Variable	df	MS	F	<i>p</i>
EPA				
Species	15	76.482	2.9298	0.00522
Error	32	26.105		
Total	47			
DHA				
Species	15	143.823	3.4213	0.001698
Error	32	42.0375		
Total	47			
HIS				
Species	9	0.81608	16.3216	5.72E-8
Error	21	0.05		
Total	30			
ARG				
Species	9	33.1777	16.9039	4.13E-8
Error	21	1.9627		
Total	30			
THR				
Species	9	2.3329	48.4178	1.21E-12
Error	21	0.048182		
Total	30			
VAL				
Species	9	5.4965	13.6175	2.98E-7
Error	21	0.40364		
Total	30			
LYS				
Species	9	6.4552	15.4363	9.56E-8
Error	21	0.41818		
Total	30			
ILE				
Species	9	2.4173	40.9081	6.86E-12
Error	21	0.059091		
Total	30			
LEU				
Species	9	3.4566	13.0215	4.45E-07
Error	21	0.26545		
Total	30			
PHE				
Species	9	7.9552	109.3841	2.22E-16
Error	21	0.072727		
Total	30			
METH				
Species	9	4.3555	191.6412	0.0000
Error	21	0.022727		
Total	30			

Consequently, their strategy for successful reproduction involves substantial maternal investment, in terms of biochemical substances, into egg biomass (Alonso et al. 2000; Auel 2004; Lee et al. 2006). Hence, animals here mostly rely on the reserves they accumulate during favourable feeding conditions for reproduction (Lee et al. 2006), a strategy also known to be associated with marine and freshwater zooplankton species that reproduce under poor feeding conditions (e.g. during winter) (Vanderploeg et al. 1992; Ventura and Catalan 2005; Lee et al. 2006).

Production of heavy eggs by species within this group can be interpreted as an adaptation to living in and/or reproducing under food-limited conditions. This is because heavy lipid-rich eggs would have enough internal energy reserves to enable lecithotrophic ontogenetic development, thus making freshly hatched animals relatively independent of their ambient food conditions (Auel 2004). For instance, among eggs from the euchaetid *Euchaeta japonica*, feeding does not begin until 20 to 30 days after hatching (Lee et al. 1974). Therefore the higher lipid than protein content of species within this group could be interpreted as part of their adaptation to poor feeding conditions.

Conversely, group 2 is made up of individuals that mostly contained more proteins than lipids (Figure 3B), with dry weights $\leq 3162.29 \mu\text{g}$ (Table 3). Most of the species within this group, such as *Acartia* and most *Calanus spp*, inhabit epi-pelagic environments, where food availability is mostly high. They are therefore known to be strongly dependent on ambient food conditions for successful reproduction, and females do not or invest very little of their biomass reserves into egg production (Kattner et al. 2006; Lee et al. 2006). The available data show that species in this group mostly produce eggs that contain more proteins than lipids (Table 4, Figure 3A). Eggs produced in this zone probably do not require a lot of energy reserves, as they could benefit from the abundant food supply within the upper water layer. High lipid content, as potential reserve for reproduction, in members of this group may therefore not be necessary. Moreover, eggs may derive some advantages from containing more proteins than lipids. Some crustacean eggs swell by osmotically absorbing ambient water in order to aid the rupture of egg membrane during hatching, a process that has been attributed to a high internal osmolality within embryos caused by amino acids (Rosa et al. 2005). Therefore, habitat conditions as well as physiological needs of the animals can both be determining factors for the biochemical composition of eggs. It has to be mentioned that some species in this group are not strictly epi-pelagic. For example *C. glacialis* and *C. finmarchicus* are capable of vertical migration below the surface layer, have substantial lipid contents and can reproduce without feeding (see Hirche and Kattner 1993).

However, it has been shown that even among these species, egg production dependence on external food supply is sometimes very strong (see Diel and Tande 1992; Hirche and Kattner 1993; Nielsen and Hansen 1995).

Table 8. Essential compound composition of zooplankton eggs. Different superscript letters within columns represent significant differences ($p < 0.05$)

Species	Essential amino acid (% of Total amino acids)									
	HIS	ARG	THR	VAL	LYS	ILE	LEU	PHE	METH	
<i>A. tonsa</i>	3.0 ^{ab}	5.0 ^{ab}	5.0 ^{ab}	7.0 ^{ac}	5.0 ^a	4.0 ^a	8.0 ^a	5.0 ^a	2.0 ^a	
<i>C. helgolandicus</i>	4.8 ± 1.9 ^{ab}	11.5 ± 2.7 ^{ab}	6.9 ± 2.5 ^{ab}	5.7 ± 1.3 ^{ac}	7.2 ± 4.1 ^a	2.2 ± 1.0 ^b	4.1 ± 2.0 ^a	2.7 ± 0.9 ^{ab}	1.3 ± 0.9 ^a	
<i>C. tenuiremis</i>	3.1 ± 0.6 ^{ab}	5.4 ± 0.4 ^a	5.3 ± 0.7 ^{ab}	8.0 ± 2.0 ^{ac}	7.7 ± 0.2 ^a	3.9 ± 0.0 ^{ac}	6.9 ± 2.5 ^a	4.3 ± 1.1 ^a	1.5 ± 0.0 ^a	
<i>E. acutifrons</i>	2.8 ± 0.6 ^{ab}	9.0 ± 3.1 ^{ab}	4.7 ± 0.6 ^{abc}	5.1 ± 0.2 ^{ac}	8.6 ± 0.8 ^a	4.9 ± 0.3 ^a	6.6 ± 0.3 ^a	4.1 ± 0.6 ^a	0.0 ± 0.0 ^b	
<i>H. gammarus</i>	1.71 ± 0.1 ^a	4.3 ± 0.2 ^a	3.4 ± 0.2 ^{ac}	3.9 ± 0.2 ^a	4.2 ± 0.1 ^a	2.8 ± 0.2 ^{bc}	4.5 ± 0.2 ^a	2.8 ± 0.1 ^{ab}	2.0 ± 0.1 ^a	
<i>N. norvegicus</i>	3.6 ± 0.2 ^{ab}	3.3 ± 0.2 ^a	3.0 ± 0.1 ^{ac}	0.1 ± 0.1 ^b	1.3 ± 0.1 ^b	2.5 ± 0.1 ^b	4.1 ± 0.1 ^a	2.2 ± 0.1 ^b	1.3 ± 0.1 ^a	
	Essential Fatty acids (% of total fatty acids)									
	EPA		DHA		EPA:DHA					
<i>A. tonsa</i>	4.4 ± 4.1 ^{ab}		3.8 ± 1.3 ^a		0.87					
<i>A. omorii</i>	16.1 ± 9.6 ^{ab}		20.2 ± 5.8 ^{bc}		0.79					
<i>C. helgolandicus</i>	0.8 ± 1.3 ^a		10.5 ± 4.9 ^{abc}		0.08					
<i>C. simillimus</i>	0.0 ± 0.0 ^a		3.6 ± 6.2 ^a							
<i>C. antarcticus</i>	22.8 ± 4.4 ^b		7.4 ± 4.2 ^a		3.08					
<i>E. crystallorophias</i>	11.8 ^{ab}		7.1 ^a		1.66					
<i>H. gammarus</i>	13.0 ± 0.4 ^{ab}		10.5 ± 0.3 ^{ab}		1.24					
<i>N. lanceopes</i>	14.0 ± 1.9 ^{ab}		5.8 ± 2.9 ^a		2.41					
<i>N. norvegicus</i>	7.6 ± 0.7 ^{ab}		14.8 ± 0.5 ^{bc}		0.51					
<i>N. antarcticus</i>	12.2 ± 7.8 ^{ab}		4.2 ± 4.3 ^a		2.91					
<i>R. gigas</i>	3.0 ± 5.2 ^a		1.0 ± 1.8 ^a		3.00					

Egg sizes are known to increase linearly with female body mass, though the extent of the increase differs significantly between egg-carrying and broadcast-pawning zooplankton (Mauchline 1988; Kiørboe and Sabatini 1994; Ohman and Townsend 1998). Contrary, for some of the species in this study, egg size, measured as dry mass, cannot be explained by the weight of the females using a positive linear relationship (data not shown). For example, even though all species of the copepods genus *Paraeuchaeta* carry their eggs in egg sac (Auel 2004), eggs produced by the relatively small *P. polaris* adults were heavier than those from their bigger counterparts such as *P. glacialis* and *P. norvegica* (Table 3). Similarly, among *Calanus* species, eggs produced by *C. helgolandicus* were heavier than those from *C. finmarchicus* even though adults of the latter species may be heavier than those of the former.

Essential amino and fatty acids contents

Animals' composition of compounds with primarily structural roles is more constant (Ventura and Catalan 2005). These include proteins and by extension essential amino acids and the membrane phospholipids EPA and DHA. The relationship shown here between these micro-compounds and their respective bulk compound content (expressed in percent of dry mass and hence independent of the mass of the species) reveal important characteristics of crustacean zooplankton (Figures 4 and 5). It demonstrates animals' requirement for and/or accumulation (only by adults) of essential compounds relative to their total composition of bulk compounds.

There was no significant relationship between total lipid and essential fatty acids contents of eggs (Figure 4A). Moreover, the contribution of both EPA and DHA to the total fatty acids content of organisms was not significantly different between most species (Tables 7 and 9). These suggest that on average, there may be similarities between zooplankton species in terms of their essential fatty acid requirements for egg production. Among adults on the other hand, the results here suggest that lipid-poor species may contain more EPA and DHA, while the opposite may be true for lipid-rich species (Figure 5A). This is consistent with existing knowledge regarding lipid accumulation by zooplankton (Sargent and Henderson 1986; Olsen 1999; Evjemo et al. 2003).

The ability for lipid accumulation is most highly developed in zooplankton species that inhabit polar regions of the world, where primary production, and hence seasonal energy input to the low trophic levels, is of high intensity but short duration (Sargent and Falk-Petersen 1988; Lee et al. 2006). Zooplankton in these environments generally contain more neutral lipid (i.e. wax esters) than polar lipids (e.g. Lee and Hirota 1973).

Table 9. Comparison of essential biochemical compounds within crustacean zooplankton eggs. A summary and description of the units of each compound are given in Table 8

Variable	df	MS	F	<i>p</i>
EPA				
Species	10	153.2752	8.2788	8.87E-6
Error	24	18.5142		
Total	34			
DHA				
Species	10	98.5191	8.103	1.07E-5
Error	24	12.1583		
Total	34			
HIS				
Species	5	3.0737	3.5088	0.043184
Error	12	0.876		
Total	17			
ARG				
Species	5	28.9459	8.444	0.002328
Error	12	3.428		
Total	17			
THR				
Species	5	5.8849	4.1129	0.02732
Error	12	1.4308		
Total	17			
VAL				
Species	5	21.3675	18.484	9.17E-5
Error	12	1.156		
Total	17			
LYS				
Species	5	21.852	6.2399	0.007046
Error	12	3.502		
Total	17			
ILE				
Species	5	3.1099	13.6398	0.000339
Error	12	0.228		
Total	17			
LEU				
Species	5	6.0795	2.9256	0.069867
Error	12	2.078		
Total	17			
PHE				
Species	5	2.6509	5.5227	0.010711
Error	12	0.48		
Total	17			
METH				
Species	5	1.4269	8.5956	0.002175
Error	12	0.166		
Total	17			

In this study, most of the lipid-rich adults with low relative EPA and DHA contents are polar species (Tables 3 and 6). Experiments have also shown that EPA and DHA fraction of fatty acids in copepods increases when animals are starved (e.g. Evjemo et al. 2003). It has therefore been suggested that zooplankton that experience long periods of food shortage generally accumulate neutral lipids and as a result tend to have reduced levels of membrane phospholipids (Sargent and Falk-Petersen 1988 and references therein). Hence, the variability in the EFAs composition of female (Figure 5A) may be attributed to animals' adaptation to their ambient food conditions. The extrapolation of the regression line in Figure 5A to zero percent of total lipid (i.e. crossing the y-axis) shows the maximum level of EFA zooplankton adults may require in the absence of lipid accumulation. This was estimated to be 50.55 ± 4.61 % of the total fatty acids (Table 5), which is comparable with what has been estimated for starving copepods (e.g. Evjemo et al. 2003), and hence may represent the minimum requirement threshold for essential fatty acid by female zooplankton. Further research is needed on this.

Total essential amino acid content of zooplankton was independent of species' protein contents (Figures 4B and 5B), and only Leucine content of eggs was significantly different between species (Tables 7 and 9). Essential amino acids constituted approximately half of all the amino acids in animals (48.94 ± 7.33 % in eggs and 49.89 ± 4.47 % in adults). This suggests that the total requirement for essential and non-essential amino acids in both stages of zooplankton may be the same. However, the composition of individual amino acids was significantly different between adults of most species (Table 6). Animals in this study differ in what they feed on or were raised with different prey species. While some are herbivorous (e.g. *Calanus* spp.), others are omnivorous (e.g. *Acartia* spp., *Paraeuchaeta* spp.). Significant diet effect on the amino acids contents of copepod species has been demonstrated by experimental studies (e.g. Guisande et al. 2002). Therefore the differences in individual essential amino acids among adult species in this study could be attributed to difference in diet. Unlike adults however, the composition of individual EAA within eggs were similar in most species (Tables 8 and 9). Similarly, the essential fatty acids contents of eggs were independent of the total lipid requirement for egg production (Figure 4A). Also, the composition of EPA and DHA within eggs was not significantly different between most species. It can therefore be said that egg production requirement for both essential amino acids and essential fatty acids may be similar among zooplankton species.

Conclusion

It has been shown that the biochemical (total protein, lipid, EAAs and EFAs) composition of zooplankton (both eggs and adult females) can be determined from the animals' dry weight (in the case of total protein and lipid) and bulk compound content (in the case of essential amino and fatty acids) by using a simple log-log linear relationship. The relationships were derived from data on zooplankton that inhabit different habitats and also differ in morphology, trophic position, as well as reproductive strategy. Hence, they are not restricted to any specific species or environment. The parameters determined here (Table 5) could therefore be used (by modelers) to estimate the compound requirement of female zooplankton and their eggs.

One of the most important findings of this study is that, DHA + EPA composition of eggs may not reflect that of adults (Compare figures 4A and 5A). This enjoins modellers to account for stage-specific biochemical requirements of zooplankton.

Appendix 2

Biochemical Composition of Phytoplankton

A2.1 ABSTRACT

Changes in ambient conditions like temperature, nutrient richness, light etc. affect the chemical composition of phytoplankton. Since herbivores, particularly zooplankton, rely on optimum supply of chemical substances for successful reproduction, changes in the biochemical status of algae could impact the dynamics of aquatic systems. However, modelling investigations of the link between autotrophic production and the availability of substrates for secondary production have so far been hampered by the lack of robust parameters for characterizing the biochemical composition of algae. Existing parameters have been derived either from studies conducted under steady state conditions, and so do not capture natural variability in habitat conditions, or based on algal-size, which may not directly reflect cellular biochemical quotas. In this study, published data on the biochemical composition of algae have been synthesized to determine new parameters for estimating protein (TP), lipid (TL), essential amino acid (EAA), and essential fatty acids (EFA) composition of phytoplankton cells. Data on 68 phytoplankton species belonging to 7 taxonomic classes that were cultured in diverse degrees of nutrient richness under different conditions of temperature (16-30°C), photoperiod (constant illumination - 10 hours of light), and light intensity (20-100 $\mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) were used in order to ensure that the new parameters could be applied under diverse habitat conditions. The parameters are based on algal carbon-quotas for the simple reason that carbon constitutes a significant fraction of all organic compounds. The data show that phytoplankton cells can be classified into two main groups based on their content of TP and TL. Independent of species, some cells have TP>TL, while in others TP<TL. The critical cellular C-quota that marks the transition between these two phytoplankton groups is ~4 pg. This suggests elasticity in how algae split the carbon they assimilate between protein and lipid synthesis. No significant difference in EAA composition was found between algal taxonomic groups. Conversely, EFAs composition of algae varies significantly between algal groups. The ecological implications of the results *vis-à-vis* aspects of herbivore ecology have been discussed.

A2.2 INTRODUCTION

Algal biochemical composition is a major controlling factor in the production of aquatic animals (Boersma et al. 2008; Müller-Navarra 2008). The biochemical characteristics of phytoplankton can be as important as food quantity in controlling herbivore growth (see

Müller-Navarra and Lampert 1996; Österblom et al. 2008;), or can even account solely for the carbon transfer efficiencies in aquatic systems (Müller-Navarra et al. 2000). Particularly important is the fact that most aquatic primary consumers cannot synthesize or are less efficient at synthesizing certain amino and fatty acids (i.e. essential compounds), and so rely on their provision in autotrophs (Müller-Navarra 2008 and references therein). Furthermore, phytoplankton are characterized by secondary metabolites, such as unsaturated aldehydes in diatoms, which may interfere with food consumption and processing by aquatic herbivores (Pohnert 2004). The biochemical characteristics of phytoplankton are therefore at the heart of aquatic ecosystem models (e.g. Mitra 2006; Flynn and Mitra 2009; this thesis).

Algal biochemical composition changes with ambient conditions. Most studies characterize phytoplankton based on their composition of elements, often C:N, C:P and N:P ratios and the data show that these ratios are not constant but vary even under nutrient-replete conditions. Molar ratio values for N:P range from 5 to 19, C:N range from 3 to 17 and C:P range from 27 to 135 (Geider and Roche 2002). These can be attributed in part to inter- and intra-specific chemical differences between algae, as well as to differences in growth conditions (Sakshaug et al. 1983; Terry et al. 1983; Geider and Roche 2002). In phytoplankton, N occurs mostly in proteins, nucleic acids, chlorophylls and N-containing osmolytes (e.g. glycine betaine). P occurs mostly in nucleic acids (RNA, DNA), phospholipids, and in carriers of energy and information (e.g. coenzymes, ATP). The availability of these compounds in phytoplankton can be influenced by environmental factors such as nutrient concentration, day length, irradiance, and temperature (e.g. Thompson et al. 1992; Lynn et al. 2000; Teoh et al. 2004). Thus, variability in algal elemental composition may arise from changes in algal compound composition as influenced by ambient conditions (Geider and Roche 2002). Hence, aquatic ecosystem models requiring parameters for algal biochemical composition may achieve greater robustness and applicability by employing parameters that are not restricted to specific growth and/or habitat conditions. Such parameters however do not currently exist.

Existing parameters for characterizing the biochemical composition of phytoplankton have been derived from studies conducted under steady state conditions or on limited temporal scale (e.g. Montagnes et al. 1994). Such parameters may be very useful for determining algal biochemical composition within limited space and/or time where habitat conditions may not vary significantly. However, they may not be applicable for determining the biochemical composition of phytoplankton over wider spatio-temporal scale, because ambient conditions may vary. For such cases, algal biochemical parameters derived from algae cultured under variable and wider range of ambient conditions might be more suited.

Also, several of the existing parameters have been derived for relationships that predict cellular biochemical compositions based on algal size (Montagnes et al. 1994 and references therein). Cell size varies with ambient condition, algal physiological state as well as cell cycle (Flynn 2008). For example, algal cells tend to be larger in size when cultured under P-limited conditions (Lehman 1976a; Gotham and Rhee 1981; John and Flynn 2002), while N-limited phytoplankton cells are typically smaller (Davidson et al. 1992; Wood and Flynn 1995). Algal biochemical composition however does not always reflect algal size (Flynn 2008). Hence, the robustness of size-based biochemical parameters may be limited. Consequently, it has been advised that parameters for determining algal biochemical composition should be based on C-quota (Flynn 2008). This does not suggest that algal C-quota does not vary with growth or habitat conditions. Contrary, there are reports of changes in C-quota with abiotic factors (e.g. Thompson et al. 1992; Falkowski et al. 1985). Carbon however constitutes a significant portion of organic compounds, and can constitute as much as 84% of some compounds (Laws 1991; Fernandez et al. 1996; Geider and Roche 2002). Hence, parameter(s) for determining algal biochemical composition may better capture the biochemical variations in algal cells if they are based on C-quota, rather than on cell size.

Here, published biochemical composition data on microalgae from both marine and freshwater habitats, and cultured with different levels of nutrients as well as under different conditions of light, and temperature have been reviewed to: (1) establish a parameters that could be employed to estimate the biochemical composition of algae based on their total carbon content, and independent of culture/habitat conditions, and (2) investigate quality differences between algal taxonomic groups. Here, the criterion for algal quality was their essential amino and fatty acids composition.

A2.3 METHODS

Data source

This study considers only the major compounds of protein, lipid, carbohydrate, amino acids and fatty acids. Low molecular weight compounds like RNA, DNA were not considered due to lack of adequate data. Data on total carbon, carbohydrate (TH), protein (TP), lipid (TL), as well as individual amino and fatty acids constituents of phytoplankton harvested at different growth phases were extracted from published studies. A MATLAB[®] 7.0.4 software was used to digitize and extract data from figures. Data on a total of 68 phytoplankton species belonging to 8 different taxonomic groups from both marine and freshwater habitats, and cultured under diverse conditions of temperature (16 to 30°C), photoperiod (constant to 10 hours of

illumination), light intensity (20 to 100 $\mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and growth media with diverse degrees of nutrient richness were acquired (Table 1). It is the aim of this study to establish robust parameters that could be applied under different environmental conditions. Hence, acquired data were analysed independent of the conditions under which algae were cultured.

Zooplankton constitute major herbivores in aquatic systems (Tiselius 1988; Durbin et al. 1995). Amino acids essential for these animals are histidine (HIS), arginine (ARG), threonine (THR), valine (VAL), lysine (LYS), isoleucine (ILE), leucine (LEU), phenylalanine (PHE) and methionine (METH) (Drillet et al., 2006; Jun-jie et al., 2006; Helland et al., 2003). Eicosapentaenoic (EPA) and docohexanoic acids (DHA) constitute the main essential fatty acids (EFAs) for zooplankton (see review by Müller-navarra 2008). The yardstick for algal quality was therefore based on the relative composition of these micromolecules in their respective bulk compounds (i.e. protein for amino acids, and lipids for fatty acids).

Data analysis

Due to the huge carbon quota differences between algal cells (range: 1.2 to >100 pg cell^{-1}), the distribution of the biochemical data was not normal. Therefore, prior to the analysis, all data were normalised by \log_{10} transformation (Sokal and Rohlf, 1981). Statistical tests followed established procedure for investigating algal biochemical composition (Montagnes et al. 1994; Menden-Deuer and Lessard 2000). Biochemical constituents of algae were plotted against that of total carbon and examined for possible relationship(s), by fitting the data to a power function (equation 1) using a non-linear curve fitting method (Sigmaplot 10.0):

$$Y = aX^c + b \quad (1)$$

where Y was bulk biochemical substance (TP, TL or TH), X was carbon quota, a , b and c were constants. The relationship between bulk substances and their total constituent of essential sub-units were also similarly examined, but with Y that represented either EFAs or EAAs, and X that was algal quota of bulk substances. The automatic initial-parameter estimator function in Sigmaplot was used. The program provides standard errors for estimated parameters. The error term associated with “ c ” was used to test the null hypothesis $c = 1$ (t-test, $\alpha = 0.05$, $\text{df} = n-3$) against the alternate hypothesis that $c \neq 1$. If the null hypothesis could not be rejected, the relationship was considered linear. Where $c \neq 1$, the relationship was considered non-linear; i.e. Y increases allometrically at a slower ($c < 1$) or greater ($c > 1$) rate than X . When a linear relationship could not be rejected, least squares regression was used to determine the slope, a , as well as the y-axis intercept, b , of the line.

Table 1. Published data used to describe the biochemical composition of phytoplankton. GM = growth medium; T = temperature in °C; LI = light intensity in $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; L:D = light:dark cycle in hours; GP represents the growth phase at which cells were harvested where L = logarithmic phase, S = stationary phase, e = early, m = mid, l = late; Reported biochemical data are C = carbon, TP = total protein, TL = total lipid, TH = total carbohydrate, FA = fatty acids and AA = amino acids. Blank spaces or – indicate data on culture condition and chemical substance were not reported

Reference	Phytoplankton		Habitat	Culture condition				Culture technique	GP	Biochemical data reported					
	Class	Species		GM	T	LI	L:D			C	TP	TL	TH	FA	AA
Knuckey et al., 2002	Bacillariophyceae	<i>Attheya septentrionalis</i>	M	f/2	17.5	45	12:12	Semi-continuous	L	X	X	X	X		
Knuckey et al., 2002		<i>A. septentrionalis</i>	M	f/2	17.5	45	14:10		L	X	X	X	X		
Brown, 1991		<i>Chaetoceros calcitrans</i>	M	f/2	20	70-80	12:12		IL	X	X	X		X	
Enright et al., 1986		<i>C. calcitrans</i>	M	f/2	22	300	12:12			X				X	
Brown, 1991		<i>C. gracilis</i>	M	f/2	20	70-80	12:12		IL	X	X	X		X	
Enright et al., 1986		<i>C. gracilis</i>	M	f/2	22	300	12:12			X				X	
Renaud et al., 2002		<i>Chaetoceros sp.</i>	M	f/2	25	80	12:12		IL	X	X	X			
Renaud et al., 2002		<i>Chaetoceros sp.</i>	M	f/2	27	80	12:12		IL	X	X	X			
Renaud et al., 2002		<i>Chaetoceros sp.</i>	M	f/2	30	80	12:12		IL	X	X	X			
Brown and Jeffrey, 1995		<i>Cylindrotheca fusiformis</i>	M	f/2	20	70-80	12:12		L	X	X	X		X	
Montagnes et al., 1994		<i>Detonula pumilla</i>	M	EASW	16	20-60	14:10		mL	X	X				
Knuckey et al., 2002		<i>Entomoneis cf punctulata</i>	M	f/2	17.5	45	12:12		L	X	X	X	X		
Knuckey et al., 2002		<i>E. cf punctulata</i>	M	f/2	17.5	45	12:12		S	X	X	X	X		
Knuckey et al., 2002		<i>Extubocellulus spinifera</i>	M	f/2	17.5	45	12:12		L	X	X	X	X		
Knuckey et al., 2002		<i>E. spinifera</i>	M	f/2	17.5	45	12:12		S	X	X	X	X		
Brown and Jeffrey, 1995		<i>Lauderia annulata</i>	M	f/2	20	70-80	12:12		IL	X	X	X		X	
Renaud et al., 1994		<i>Melosira sp.</i>	M	f/2	25	80	12:12		IL	X	X	X	X		
Brown and Jeffrey, 1995		<i>Nitzschia closterium</i>	M	f/2	20	70-80	12:12		IL	X	X	X		X	
Brown, 1991		<i>N. closterium</i>	M	f/2	20	70-80	12:12		IL						
Renaud et al., 1994		<i>N. closterium</i>	M	f/2	25	80	12:12		IL	X	X	X	X		
Renaud et al., 1994		<i>Nitzschia (frustulum)</i>	M	f/2	25	80	12:12		IL	X	X	X	X		
Brown and Jeffrey, 1995		<i>Navicula jeffreyi</i>	M	G	20	70-80	12:12		IL	X	X	X		X	
Renaud et al., 1994		<i>Navicula sp.</i>	M	f/2	25	80	12:12		IL	X	X	X	X		
Brown, 1991	<i>Phaeodactylum tricorutum</i>	M	f/2	20	70-80	12:12	IL	X	X	X		X			
Brown and Jeffrey, 1995	<i>Skeletonema costatum</i>	M	f/2	20	70-80	12:12	IL	X	X	X		X			

Table 1 continued

Reference	Phytoplankton		Habitat	Culture condition						Biochemical data reported					
	Class	Species		GM	T	LI	L:D	Culture technique	GP	C	TP	TL	TH	FA	AA
Brown, 1991	Bacillariophyceae	<i>Skeletonema costatum</i>	M	f/2	20	70-80	12:12		IL	X	X	X		X	
Enright et al., 1986		<i>S. costatum</i>	M	f/2	22	300	12:12			X				X	
Enright et al., 1986		<i>S. menzeli</i>	M	f/2	22	300	12:12			X				X	
Brown and Jeffrey, 1995		<i>Skeletonema sp.</i>	M	f/2	25	70-80	12:12		IL	X	X	X		X	
Lynn et al., 2000		<i>Stephanodiscus minutulus</i>	FW	Normal COMBO	16	80	14:11		S	X	X	X	X		
Lynn et al., 2000		<i>S. minutulus</i>	FW	Low Si COMBO	16	80	14:12		S	X	X	X	X		
Lynn et al., 2000		<i>S. minutulus</i>	FW	Low N COMBO	16	80	14:13		S	X	X	X	X		
Lynn et al., 2000		<i>S. minutulus</i>	FW	Low P COMBO	16	80	14:14		S	X	X	X	X		
Knuckey et al., 2002		<i>Thalassiosira oceanica</i>	M	f/2	17.5	45	12:12		L		X	X	X	X	
Knuckey et al., 2002		<i>T. oceanica</i>	M	f/2	17.5	45	12:12		S		X	X	X	X	
Brown, 1991		<i>T. pseudonana</i>	M	G/2	20	70-80	12:12		LL		X	X	X		X
Montagnes et al., 1994		<i>T. pseudonana</i>	M	EASW	16	20-60	14:10	Semi-continuous	ML	X	X				
Jonasdottir, 1994		<i>T. weissflogii</i>	M	f/2	16		10:10	Batch	EE	X	X		X	X	
Jonasdottir, 1994		<i>T. weissflogii</i>	M	f/2	16		10:10	Batch	ME	X	X		X	X	
Jonasdottir, 1994		<i>T. weissflogii</i>	M	f/2	16		10:10	Batch	LE	X	X		X	X	
Jonasdottir, 1994		<i>T. weissflogii</i>	M	f/2	16		10:10	Batch	S	X	X		X	X	
Jonasdottir, 1994		<i>T. weissflogii</i>	M	f/2	16		10:10	Batch	EE	X	X		X	X	
Jonasdottir, 1994	<i>T. weissflogii</i>	M	f/2	16		10:10	Batch	S	X	X		X	X		
Montagnes et al., 1994	<i>T. weissflogii</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					
Renaud et al., 1994	Chlorophyceae	<i>Ankistrodesmus sp.</i>	FW	WC/2	25	80	12:12		LL		X	X	X	X	
Montagnes et al., 1994		<i>Chlamydomonas sp.</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X				
Renaud et al., 1994		<i>Chlamydomonas sp.</i>	FW	WC/2	25	80	12:12		LL		X	X	X	X	
Brown and Jeffrey, 1992		<i>Chlorella protothecoides</i>	FW	MBLNB2	20	70-80	12:12		L		X	X	X		X
Dunstan et al., 1992		<i>C. protothecoides</i>	FW	MBLNB2	0	70-80						X		X	
Brown and Jeffrey, 1992		<i>Chlorella sp.</i>	M	f/2	20	70-80	12:12		L		X	X	X		X
Brown and Jeffrey, 1992		<i>Chlorella sp.</i>	M	f/2	21	70-81	12:12		L		X	X	X		X
Brown and Jeffrey, 1992		<i>Chlorella sp.</i>	M	f/2	27	70-80	12:12		L		X	X	X		X
Dunstan et al., 1992		<i>Chlorella sp.</i>	M	f/2	0	70-80						X		X	
Dunstan et al., 1992		<i>Chlorella sp.</i>	M	f/2	0	70-81						X		X	
Renaud et al., 1994		<i>Chlorella sp.</i>	M	f/2	25	80	12:12		LL		X	X	X	X	
Renaud et al., 1994		<i>Chlorella sp.</i>	M	f/2	25	80	12:12		LL		X	X	X	X	

Table 1 continued

Reference	Phytoplankton		Habitat	Culture condition						Biochemical data reported						
	Class	Species		GM	T	LI	L:D	Culture technique	GP	C	TP	TL	TH	FA	AA	
Renaud et al., 1994	Chlorophyceae	<i>Dunaliella salina</i>	M	J/1	25	80	12:12		LL		X	X	X	X		
Brown, 1991		<i>D. tertiolecta</i>	M	f/2	20	70-80	12:12		LL		X	X	X		X	
Enright et al., 1986		<i>D. tertiolecta</i>	M	f/2	22	300	12:12				X				X	
Montagnes et al., 1994		<i>Dunaliella tertiolecta</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					
Renaud et al., 1994		<i>Scenedesmus dimorphus</i>	FW	WC/2	25	80	12:12		LL		X	X	X	X		
Renaud et al., 1994		<i>S. quadricauda</i>	FW	WC/2	25	80	12:12		LL		X	X	X	X		
Renaud et al., 1994		<i>Selenastrum sp.</i>	FW	WC/2	25	80	12:12		LL		X	X	X	X		
Brown and Jeffrey, 1992		<i>Stichococcus sp.</i>	M	f/2	20	70-80	12:12		L		X	X	X		X	
Dunstan et al., 1992		<i>Stichococcus sp.</i>	M	f/2	0	70-80						X		X		
Brown, 1991		Cryptophyceae	<i>Chroomonas salina</i>	M	f/2	20	70-80	12:12		LL		X	X	X		X
Montagnes et al., 1994			<i>C. salina</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X				
Montagnes et al., 1994			<i>C. salina</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X				
Montagnes et al., 1994			<i>Chroomonas sp.</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X				
Montagnes et al., 1994			<i>Cryptomonas profunda (1)</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X				
Montagnes et al., 1994	<i>C. profunda (2)</i>		M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					
Renaud et al., 2002	<i>Cryptomonas sp.</i>		M	f/2	25	80	12:12		LL		X	X	X			
Renaud et al., 2002	<i>Cryptomonas sp.</i>		M	f/2	27	80	12:12		LL		X	X	X			
Montagnes et al., 1994	<i>Cryptomonas sp.</i>		M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					
Montagnes et al., 1994	<i>Pyrenomonas salina</i>		M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					
Jonasdottir, 1994	<i>Rhodomonas lens</i>		M	f/2	16		10:10	Batch	EE	X	X		X	X		
Jonasdottir, 1994	<i>R. lens</i>		M	f/2	16		10:10	Batch	LE	X	X		X	X		
Jonasdottir, 1994	<i>R. lens</i>		M	f/2	16		10:10	Batch	S	X	X		X	X		
Jonasdottir, 1994	<i>R. lens</i>		M	f/2	16		10:10	Batch	EE	X	X		X	X		
Jonasdottir, 1994	<i>R. lens</i>	M	f/2	16		10:10	Batch	S	X	X		X	X			
Montagnes et al., 1994	<i>R. lens</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X						
Enright et al., 1986	<i>Rhodomonas sp.</i>	M	f/2	22	300	12:12				X				X		
Jonasdottir, 1994	Dinophyceae	<i>Prorocentrum minimum</i>	M	f/2	16		10:10	Batch	EE	X	X		X	X		
Jonasdottir, 1994		<i>P. minimum</i>	M	f/2	16		10:10	Batch	LE	X	X		X	X		
Montagnes et al., 1994		<i>Gymnodinium sanguineum</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					
Montagnes et al., 1994		<i>G. simplex</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					

Table 1 continued

Reference	Phytoplankton		Habitat	Culture condition						Biochemical data reported						
	Class	Species		GM	T	LI	L:D	Culture technique	GP	C	TP	TL	TH	FA	AA	
Mansour et al., 2003	Dinophyceae	<i>Gymnodinium sp.</i>						Batch	L			X		X		
Mansour et al., 2003		<i>Gymnodinium sp.</i>						Batch	L			X		X		
Mansour et al., 2003		<i>Gymnodinium sp.</i>						Batch	linear			X		X		
Mansour et al., 2003		<i>Gymnodinium sp.</i>						Batch	S			X		X		
Montagnes et al., 1994		<i>G. vitiligo</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					
Montagnes et al., 1994		<i>Gyrodinium aurealum</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					
Montagnes et al., 1994		<i>G. uncatenum</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					
Brown and Jeffrey, 1993	Eustigmatophyceae	<i>Nannochloropsis oculata</i>	M	f/2	22	100	12:12	Batch	L		X	X	X		X	
Brown and Jeffrey, 1993		<i>N. oculata</i>	M	f/2	22	100	12:12	Semi-continuous	L		X	X	X		X	
Brown and Jeffrey, 1993		<i>N. oculata</i>	M	f/2	22	100	12:12	Batch	S		X	X	X		X	
Brown, 1991		<i>N. oculata</i>	M	f/2	20	70-80	12:12		LL		X	X	X		X	
Dunstan et al., 1993		<i>N. oculata</i>	M	f/2	22	100	12:12	Batch	L			X			X	
Dunstan et al., 1993		<i>N. oculata</i>	M	f/2	22	100	12:12	Semi-continuous	L			X			X	
Dunstan et al., 1993		<i>N. oculata</i>	M	f/2	22	100	12:12	Semi-continuous	L			X			X	
Dunstan et al., 1993		<i>N. oculata</i>	M	f/2	22	100	12:12	Batch	S			X			X	
Dunstan et al., 1993		<i>N. oculata</i>	M	f/2	22	100	12:12	Batch	S			X			X	
Brown et al., 1998			<i>Nannochloropsis-like sp.</i>	M	f/2	20	70-80	12:12		LL		X	X	X		
Ponis et al., 2006	Pavlovophyceae	<i>Diacronema vlkianum</i>	M		19-20	35-50	CI		E		X	X	X		X	
Ponis et al., 2006		<i>D. vlkianum</i>	M		19-20	35-50	CI		S		X	X	X		X	
Dunstan et al., 1992	Prasinophyceae	<i>Cocoid prasinophyte</i>										X			X	
Montagnes et al., 1994		<i>Mantoniella squamata</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					
Montagnes et al., 1994		<i>Micromonas pusilla</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X					
Dunstan et al., 1992		<i>M. pusilla</i>	M	G/2	0	30							X		X	
Dunstan et al., 1992		<i>M. pusilla</i>	M	G/2	0	30							X		X	
Brown and Jeffrey, 1992		<i>M. pusilla</i>	M	G/2	20	30	12:12		L		X	X	X			X
Brown and Jeffrey, 1992		<i>M. pusilla</i>	M	G/2	27	30	12:12		L		X	X	X			X
Brown and Jeffrey, 1992		<i>Pyramimonas cordata</i>	M	G/2	20	70-80	12:12		L		X	X	X			X
Brown and Jeffrey, 1992		<i>Pycnococcus provasolii</i>	M	f/2	16	70-80	12:12		L		X	X	X			X
Dunstan et al., 1992		<i>P. provasolii</i>	M	f/2	0	70-80							X		X	
Brown and Jeffrey, 1992		<i>Tetraselmis chui</i>	M	f/2	20	70-80	12:12		L		X	X	X			X
Brown, 1991		<i>T. chui</i>	M	f/2	20	70-80	12:12		LL		X	X	X			X
Dunstan et al., 1992		<i>T. chui</i>	M	f/2	0	70-80							X		X	

Table 1 continued

Reference	Phytoplankton		Habitat	Culture condition					Biochemical data reported					
	Class	Species		GM	T	LI	L:D	Culture technique	GP	C	TP	TL	TH	FA
Brown, 1991	Prasinophyceae	<i>Tetraselmis suecica</i>	M	f/2	20	70-80	12:12		LL	X	X	X		X
Montagnes et al., 1994	Prymnesiophyceae	<i>Chrysochromulina herdlensis</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X			
Montagnes et al., 1994		<i>Coccolithus pelagicus</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X			
Fernandez et al., 1996b		<i>Emiliania huxleyi</i>	M	K-media	17	75		0.24 DR		X	X	X	X	
Fernandez et al., 1996b		<i>E. huxleyi</i>	M	K-media	17	75		0.35 DR		X	X	X	X	
Fernandez et al., 1996b		<i>E. huxleyi</i>	M	K-media	17	75		0.53 DR		X	X	X	X	
Fernandez et al., 1996b		<i>E. huxleyi</i>	M	K-media	17	75		0.75 DR		X	X	X	X	
Montagnes, 1994		<i>E. huxleyi</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X			
Brown, 1991		<i>Isochrysis galbana</i>	M	f/2	20	70-80	12:12		LL	X	X	X		X
Brown, 1991		<i>I. aff. galbana</i>	M	f/2	20	70-80	12:12		LL	X	X	X		X
Enright et al., 1986		<i>I. aff. galbbna</i>	M	f/2	22	300	12:12			X				X
Fidalgo et al., 1998		<i>I. galbana</i>	M	Nitrate	18	115			L	X	X	X	X	X
Fidalgo et al., 1998		<i>I. galbana</i>	M	Nitrate	18	115			ES	X	X	X	X	X
Fidalgo et al., 1998		<i>I. galbana</i>	M	Nitrate	18	115			LS	X	X	X	X	X
Fidalgo et al., 1998		<i>I. galbana</i>	M	Nitrite	18	115			L	X	X	X	X	X
Fidalgo et al., 1998		<i>I. galbana</i>	M	Nitrite	18	115			ES	X	X	X	X	X
Fidalgo et al., 1998		<i>I. galbana</i>	M	Nitrite	18	115			LS	X	X	X	X	X
Fidalgo et al., 1998		<i>I. galbana</i>	M	Urea	18	115			L	X	X	X	X	X
Fidalgo et al., 1998		<i>I. galbana</i>	M	Urea	18	115			ES	X	X	X	X	X
Fidalgo et al., 1998		<i>I. galbana</i>	M	Urea	18	115			LS	X	X	X	X	X
Montagnes et al., 1994		<i>I. galbana</i>	M	EASW	16	20-60	14:10	semi-continuous	L	X	X			
Brown and Jeffrey, 1993		<i>Isochrysis sp</i>	M	f/2	22	100	12:12	Batch	L	X	X	X		X
Brown and Jeffrey, 1993		<i>Isochrysis sp</i>	M	f/2	22	100	12:12	semi-continuous	L	X	X	X		X
Brown and Jeffrey, 1993		<i>Isochrysis sp</i>	M	f/2	22	100	12:12	Batch	S	X	X	X		X
Dunstan et al, 1993		<i>Isochrysis sp.</i>	M	f/2	22	100	12:12	Batch	L		X		X	
Dunstan et al, 1993		<i>Isochrysis sp.</i>	M	f/2	22	100	12:12	Semicontinuous	L		X		X	
Dunstan et al, 1993		<i>Isochrysis sp.</i>	M	f/2	22	100	12:12	Semicontinuous	L		X		X	
Dunstan et al, 1993		<i>Isochrysis sp.</i>	M	f/2	22	100	12:12	Batch	S		X		X	
Brown and Jeffrey, 1993		<i>Pavlova lutheri</i>	M	f/2	22	100	12:12		L	X	X	X		X

Table 1 continued

Reference	Phytoplankton		Habitat	Culture condition						Biochemical data reported					
	Class	Species		GM	T	LI	L:D	Culture technique	GP	C	TP	TL	TH	FA	AA
Brown and Jeffrey, 1993	Prymnesiophyceae	<i>Pavlova lutheri</i>	M	f/2	22	100	12:12	semi-continuous	L	X	X	X		X	
Brown and Jeffrey, 1993		<i>P. lutheri</i>	M	f/2	22	100	12:12	Batch	S	X	X	X		X	
Brown and Jeffrey, 1993		<i>P. lutheri</i>	M	f/2	22	100	12:12	semi-continuous	S	X	X	X		X	
Brown, 1991		<i>P. lutheri</i>	M	f/2	20	70-80	12:12		LL	X	X	X		X	
Dunstan et al., 1993		<i>P. lutheri</i>	M	f/2	22	100	12:12	Semi-continuous	L		X		X		
Dunstan et al., 1993		<i>P. lutheri</i>	M	f/2	22	100	12:12	Semi-continuous	L		X		X		
Dunstan et al., 1993		<i>P. lutheri</i>	M	f/2	22	100	12:12	Batch	S		X		X		
Dunstan et al., 1993		<i>P. lutheri</i>	M	f/2	22	100	12:12	Batch	S		X		X		
Montagnes et al., 1994		<i>P. lutheri</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X				
Brown et al., 1998		<i>P. pinguis</i>	M	f/2	20	70-80	12:12		LL	X	X	X			
Brown, 1991		<i>P. salina</i>	M	f/2	25	70-80	12:12		LL	X	X	X		X	
Montagnes et al., 1994		<i>Phaeocystis pouchetii</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X				
Montagnes et al., 1994		<i>Prymnesium parvum</i>	M	EASW	16	20-60	14:10	Semi-continuous	L	X	X				
Ponis et al., 2006		<i>Pseudoisochrysis paradoxa</i>	M		19-20	35-50	CI		E	X	X	X	X		
Ponis et al., 2006		<i>P. paradoxa</i>	M		19-20	35-50	CI		S	X	X	X	X		

Nutritional quality of algal taxonomic groups were compared based on two proxies: i) protein quality, which equalled essential amino acid composition (relative to total protein) of algae, and ii) lipid quality, which was inferred from EFA components of algal total fatty acids (TFA) by first determining the TFA fraction of algal lipid, and then the EFA fraction of TFA. The approach here was necessary because most studies only reported the fatty acids composition of the algae (Table 1). All comparisons were done using a 1-way ANOVA ($p < 0.05$), with the different taxonomic groups being the factor. Prior to the ANOVA test, all data expressed in relative units were arcsine transformed and verified for normality via a Kolmogorov–Smirnov test. Whenever significant differences were found, the Tukey Test (for ANOVA) was applied to find the responsible taxonomic group (Sokal and Rohlf 1981). Separate tests were conducted for each type of compound.

A2.4 RESULTS

A criterion for algal physiological health is the Redfield C:N ratio (Montagnes et al. 1994). On atom:atom basis, the C:N ratio of the algae in this study ranged from 5 to 10, which is comparable to the Redfield ratio of 6.625 for a typical phytoplankton (Redfield 1934). Hence, it can be concluded that the data for this study were derived from algae in good physiological state. Inconsistencies in the data may arise from differences in experimental methodology, measurement specificity and accuracy of the studies referenced for this review. Nonetheless, by pulling together the different observations on individual phytoplankton groups, these inconsistencies have been partially alleviated.

Bulk biochemical composition

Figure 1 shows the relationship between algal carbon quota and cellular composition of total protein, lipid and carbohydrate. All three compounds share a log-log linear relationship with algal carbon content (i.e. “ c ” was not significantly different from 1 at $p < 0.05$). The parameters for the linear fits to the data are given in Table 2. The coefficient of variation within individual parameter was $\leq 26\%$. The slopes of the regression lines, which indicate the extent to which algal compound composition was dependent on algal carbon quota, were all highly significant ($p < 0.0001$) with adjusted r^2 values being ≥ 0.63 . These statistics suggest a good predictive relationship between total carbon and the bulk biochemical composition of algal cells.

Differences in protein, lipid and carbohydrate composition of the phytoplankton cells were determined by comparing the parameters for the lines that describe their respective relationship with carbon-quota (t-test, $df = N_1 + N_2 - 6$, $p < 0.05$, where N_1 and N_2 respectively represent total number of data points used for determining the regression lines 1 and 2 for the compared substances). Figure 2 shows the results. It shows no significant difference in slope between the regression lines for protein and carbohydrate ($p > 0.05$). However, the elevation (y-axis intercept, b) of the protein-carbon regression line is higher than that of the carbohydrate-carbon line ($p < 0.05$). Hence, microalgae may generally contain more protein than carbohydrate. The y-axis intercept value for the lipid-carbon line is less than that of protein-carbon line ($p < 0.05$), but not significantly different from that of the carbohydrate-carbon line. Moreover, the slope of the lipid-carbon regression line is only different from that of carbohydrate-carbon line ($p < 0.05$). As a result, lipid-carbon and protein-carbon regression lines crosses-over at algal carbon quota of $\sim 4 \text{ pg cell}^{-1}$ (Figure 2). Consequently, cells with

carbon contents ≥ 4 pg may contain more lipid than protein. The reverse may be true for algal cells with carbon content ≤ 4 pg.

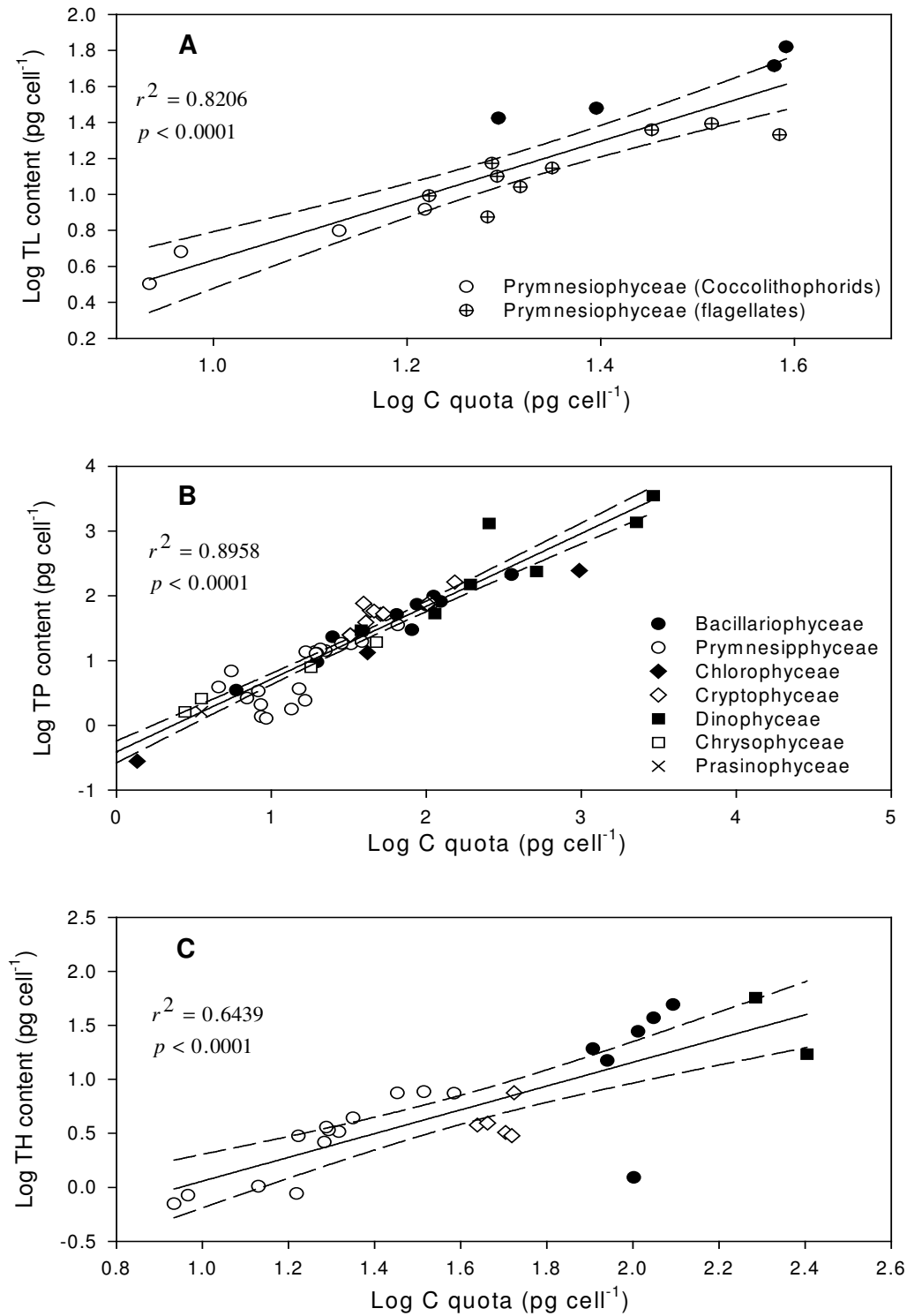


Figure 1. Log-log relationships between algal carbon quota and cellular composition of (A) total lipid, TL, (B) protein, TP, and (C) carbohydrate, TH. See Table 2 for parameters for the regression lines. Axes scales are not the same.

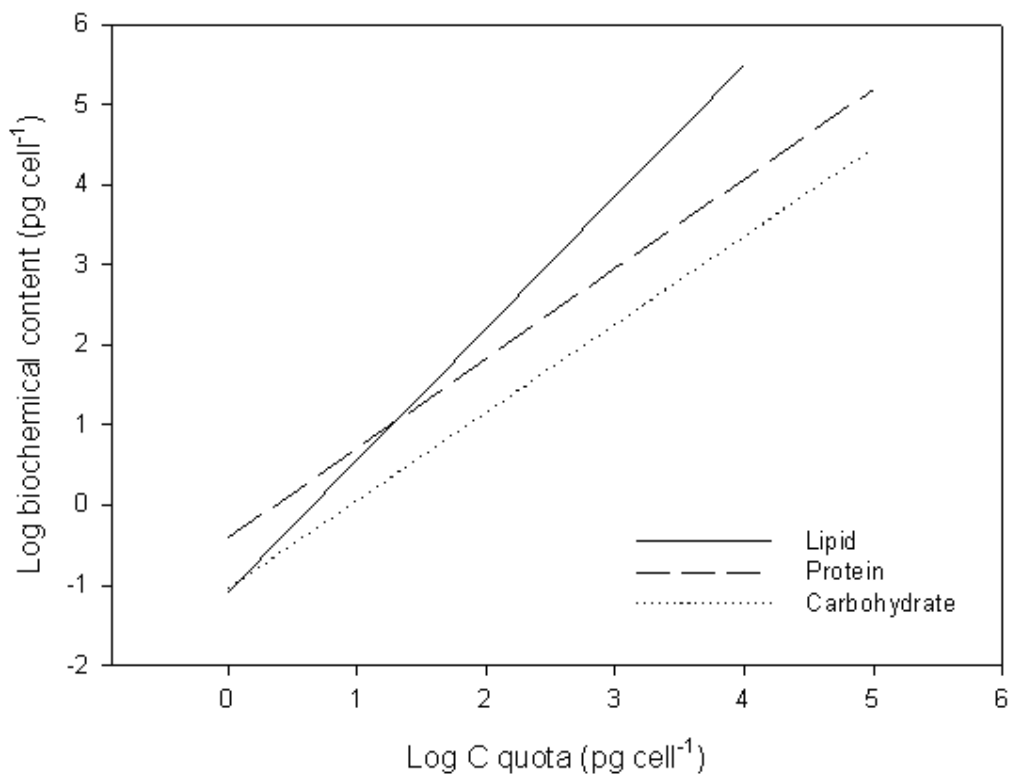


Figure 2. Comparison of the bulk biochemical constituents of phytoplankton

Table 2. Parameter estimates for a relationship (i.e. $\log Y = a \cdot \log X + \log b$) between the cellular constituents of phytoplankton grown at diverse conditions. C = total carbon, TP = Total protein, TL = Total lipid, TH = Total carbohydrates, TFA = Total fatty acids, TEFA = Total essential fatty acids, TEAA = Total essential amino acids, CV = coefficient of variation (%)

Variable		Parameter	Statistics			
Independent (x)	Dependent (y)		Estimate mean	CV	p-value	Adjusted r ²
C	TL	a	1.65	12.07	<0.0001	0.8087
		b	-1.09	24.32	0.0009	
TL	TFA	a	1.03	5.02	<0.0001	0.8686
		b	-0.51	11.74	<0.0001	
TFA	TEFA	a	1.20	5.69	<0.0001	0.7880
		b	-1.01	7.55	<0.0001	
C	TP	a	1.12	4.44	<0.0001	0.8940
		b	-0.41	20.56	<0.0001	
TP	TEAA	a	1.02	0.47	<0.0001	0.9980
		b	-0.34	1.45	<0.0001	
C	TH	a	1.10	15.18	<0.0001	0.6291
		b	-1.04	26.43	0.0009	

Essential amino (EAA) and fatty acids (EFA) contents

The ratio between total essential and non-essential amino acids constituents of proteins was not significantly different from 1 for most species (data not shown). Hence, there was a strong log-log linear relationship between total protein and total EAA of the cells (Figure 3C). This suggests general similarities between algal cells, in terms of the ratio between their respective constituents of total EAA and protein. It was therefore concluded that the quality of phytoplankton protein could not be attributed to their total composition of essential amino acids. Comparison of the individual amino acids from different phytoplankton groups was therefore necessary. Figure 4 shows the composition of EAAs among 5 algal taxonomic groups. Tables 3 and 4 contain the results for the comparison of individual amino acids between phytoplankton groups. Generally, the composition of individual EAAs in different phytoplankton species followed similar trend. Graded in increasing order, these were HIS = METH < ILE ≤ THR < PHE = VAL < LYS < ARG < LEU. Between taxonomic groups, there were significant differences in only 4 EAAs. These were HIS, VAL, ILE and METH (Table 3). With the exception of ILE, most the differences here occur between only few (3 or less) taxonomic groups.

The term lipid generally encompasses several compounds that include fatty acids and others (e.g. waxes, cholesterol). Most of the papers referenced for this study only reported fatty acid composition of the algae. Hence, in order to determine algal lipid quality, I needed first to know the proportion of algal lipid made up of fatty acid. Figure 3A shows a significant log-log linear relationship between algal lipid and total fatty acids content. Consequently, I inferred algal lipid quality from the relationship between EFA and total fatty acids content of algae.

Figure 3B shows a significant log-log linear relationship between total fatty acid and EFA contents of algae. The parameters for the fitted line to the data had coefficient of variations ≤ 6% with an adjusted r^2 value of 0.79 (Table 2). Given the linear relation between algal carbon and lipid contents (Figure 1A), one could estimate total EFA content of algae stepwisely, first by calculating total lipid from carbon quota, then total fatty acids from lipid and finally total EFA from total fatty acids. The relationships between these groups of compound were all positively linear, which suggest general similarities between algal cells, in terms of the ratio between their respective constituents of total EFA and lipid. Hence, the quality of algal lipid could not be attributed to their total composition of essential fatty acids. It was therefore necessary to compare the composition of individual EFAs between phytoplankton groups. Figure 5 shows EPA and DHA composition of 6 phytoplankton groups.

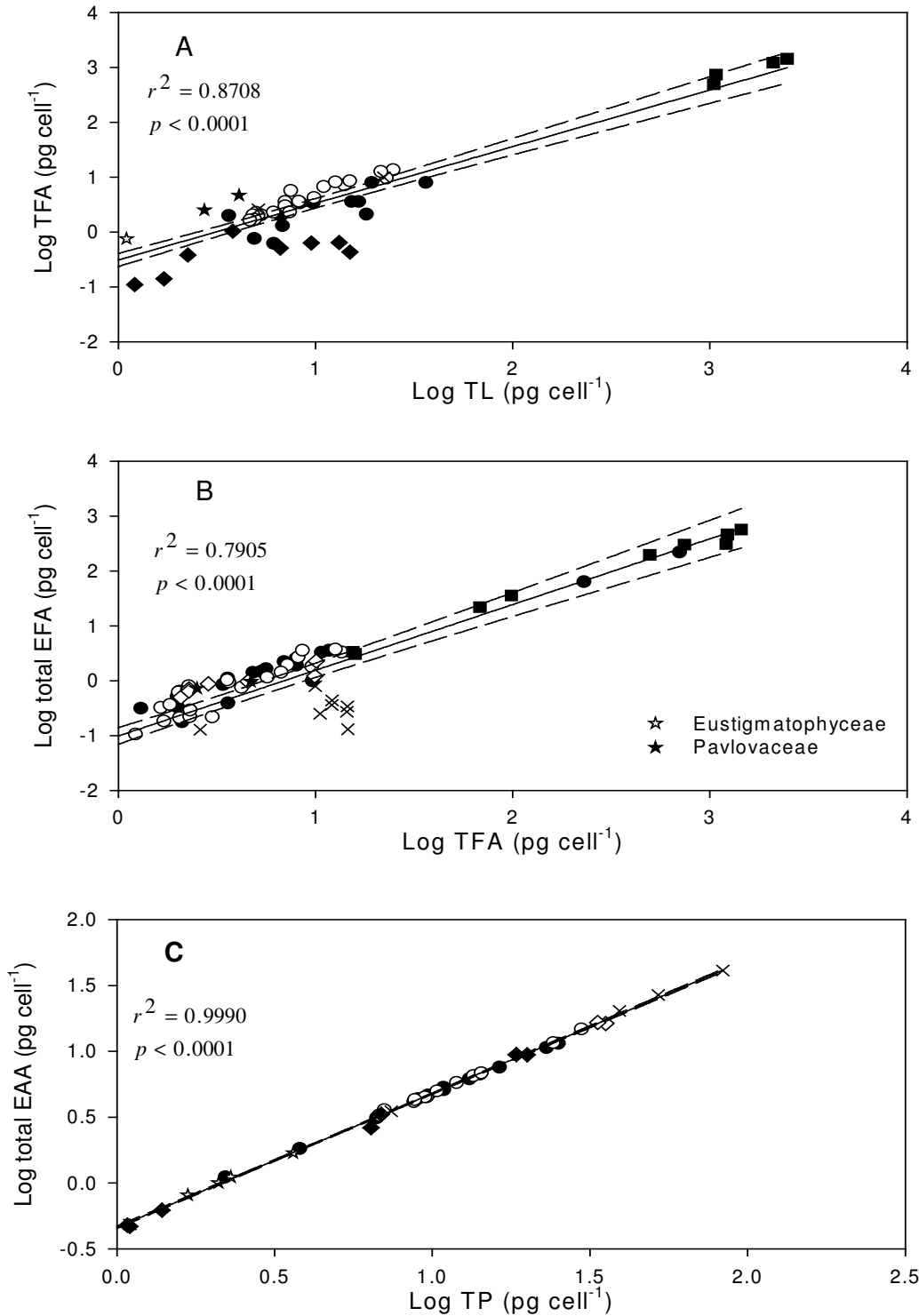


Figure 3. Log-log relationship between algal constituents of (A) total lipid, TL and total fatty acid, TFA; (B) TFA and total essential fatty acids, EFA; and (C) between total protein, TP and total essential amino acids, EAA. See Table 2 for regression line parameters. Axes scales are not the same. Apart from those for eustigmatophyceae and pavlovaceae, the rest of the data symbols are as described in figure 1.

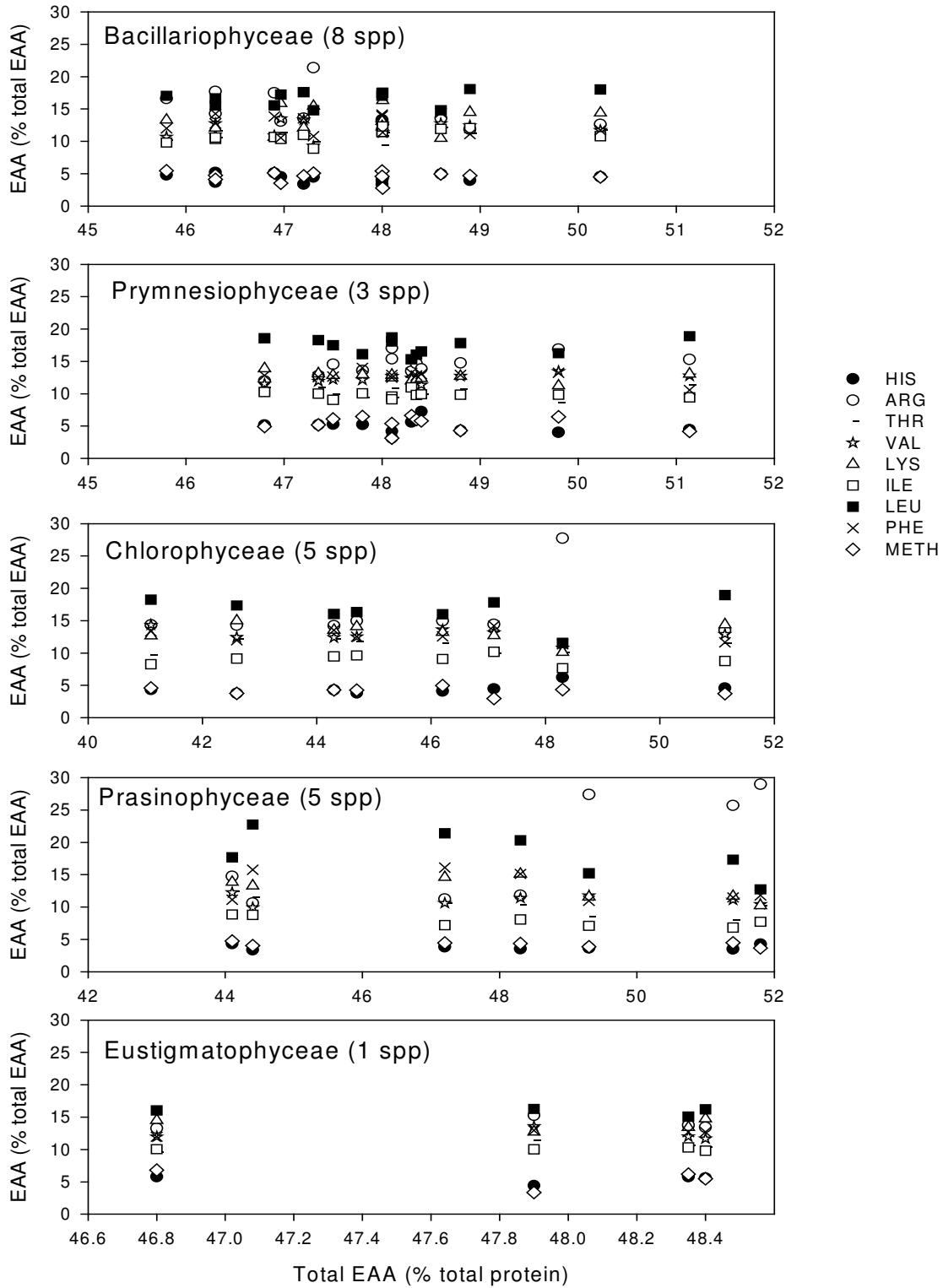


Figure 4. Individual essential amino acid (EAA) composition of algae grouped into taxonomic classes. The amino acids are histidine (HIS), arginine (ARG), valine (VAL), Lysine (LYS), isoleucine (ILE), leucine (LEU), phenylalanine (PHE) and methionine (METH). SPP represents number of species

Apart from the prasinophytes, DHA content of algae generally did not vary much within taxonomic groups. Conversely, EPA content of algae did vary within algal taxonomic groups for which data on more than one species were found. There are significant differences in EPA and DHA composition of algae between taxonomic groups (Table 3). Table 4 contains the results of the post-hoc comparison of EFA between algal groups. Based on those results, phytoplankton groups may be graded according to their content of EFA as dinophyceae > prymnesiophyceae > cryptophyceae > prasinophyte > bacillariophyceae = chlorophyceae = eustigmatophyceae for DHA; and eustigmatophyceae \geq prymnesiophyceae \geq bacillariophyceae \geq cryptophyceae \geq dinophyceae > prasinophyceae \geq chlorophyceae for EPA.

A2.5 DISCUSSION

Changes in the oceanic environment affect both the physical and chemical composition of algae. This has been shown by both laboratory and field studies (e.g. Kattner et al. 1983; Morris et al. 1983; Mayzaud et al. 1989; Lynn et al. 2000; Teoh et al. 2004). Microalgae are responsible for almost half of the primary production in our biosphere (Field et al. 1998) and serve as major source of nutrition for aquatic herbivores. As the nutritional/functional outcome of herbivory depends in part on algal biochemical composition (see Darchambeau et al. 2003; Pohnert 2004; Jones and Flynn 2005), knowledge on how phytoplankton vary in terms of their biochemical composition is vital for assessing the importance of prey selection, as well as quantifying nutritional intake by herbivores.

Compound composition of algae varies considerable depending mainly on their growth conditions. For example, algae accumulate proteins when grown in N-sufficient media because protein-bounded N is used to sustain growth when external nitrogen source is depleted (Dortch, 1982). Genetic differences may also cause the biochemical composition of algae to differ. However, the results here (Figures 1 and 2) show that the (inter-, intra-specific) differences in algal biochemical composition may be explained by changes in algal C-quota. Carbon constitutes a mass-specific proportion of all organic compounds (Laws 1991; Fernandez et al. 1996; Geider and Roche 2002), and algae fix carbon only via photosynthetic production of organic compounds. Hence, the result that algal composition of TL, TP and TH varied directly with C-quota should not be surprising.

Interestingly however, the distribution of algal carbon between protein, lipid and carbohydrate was not similar for all algal cells (Figure 2). The results show algae may generally contained more protein than carbohydrate, with TP:TH of ~ 4 (mass-ratio) that does not change with algal C-quota.

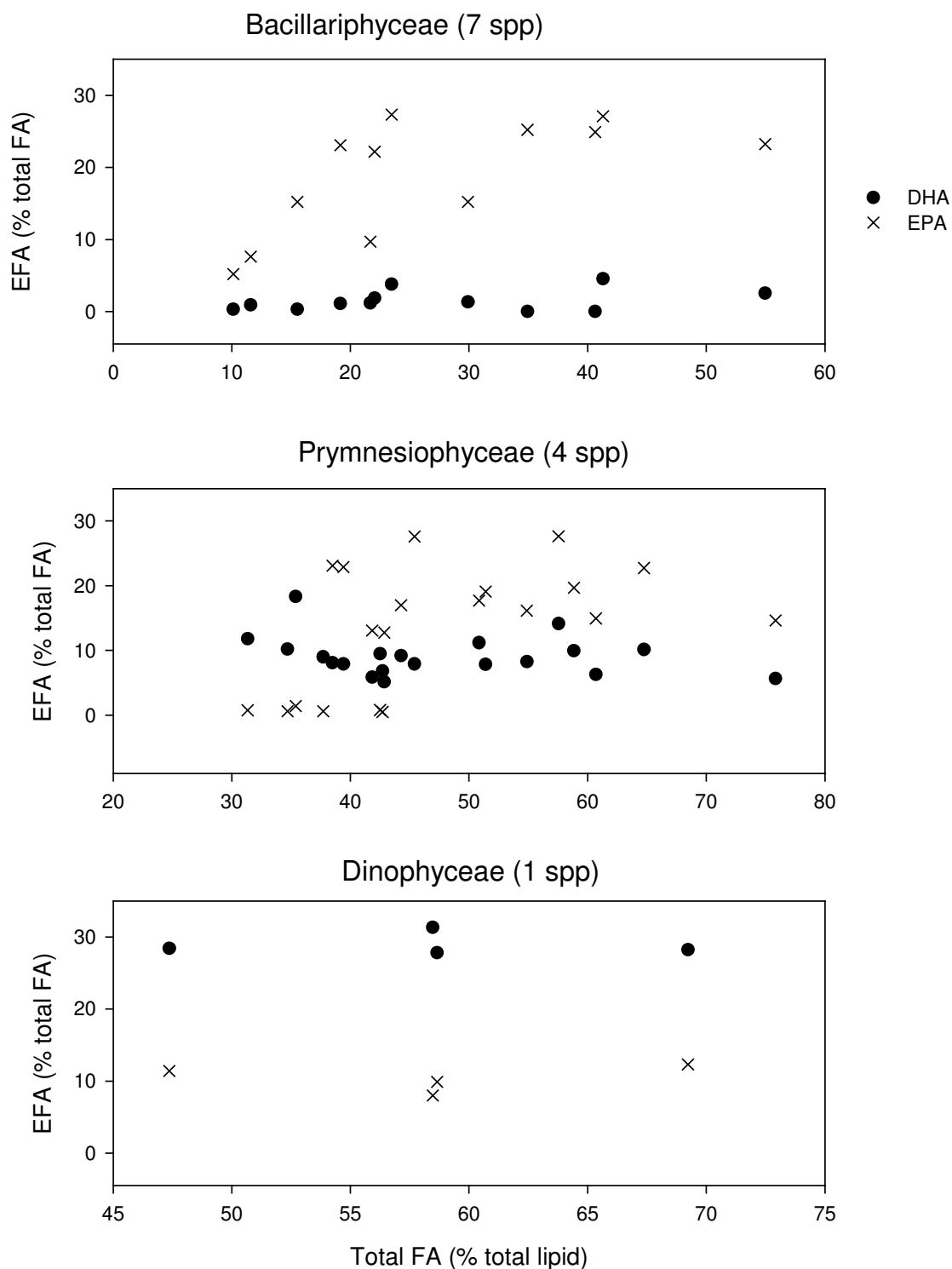


Figure 5. Individual essential fatty acid (EFA) composition of algae grouped into taxonomic classes. The fatty acids (FA) are eicosapentaenoic acid (EPA) and docohexanoic acid (DHA). SPP represents number of species

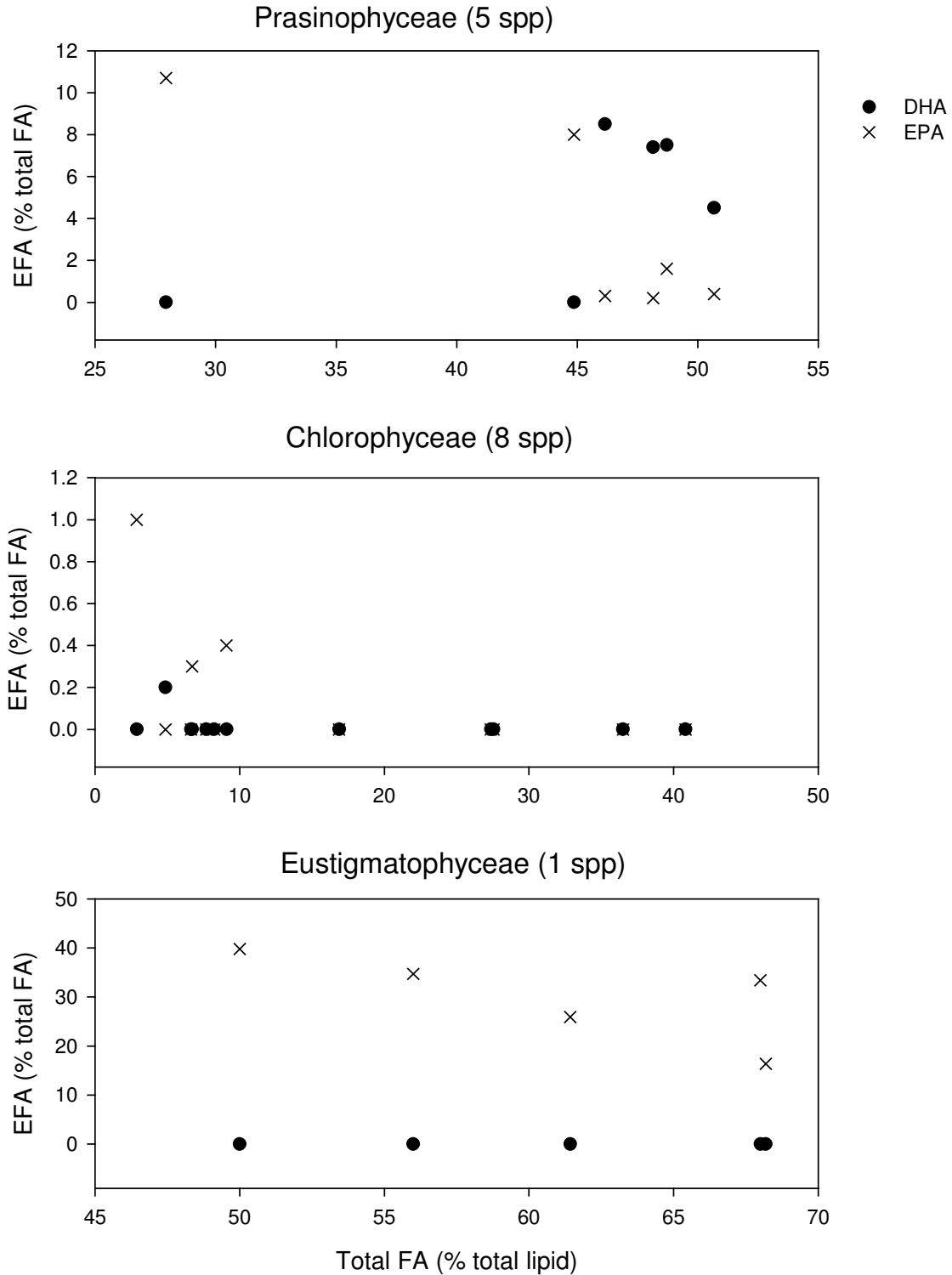


Figure 5 continued.

This is comparable with what others have reported from field studies on protein and carbohydrate accumulation by phytoplankton (e.g. Fernandez et al. 1991; Laws 1991). However, the results show a C-quota threshold (of ~4 pg) below which algal cells may contain

more protein than lipid, and above which lipid content of algae may exceed that of protein (Figure 2). The elemental compositions of biochemical substances are fixed or vary within a very narrow range (e.g. Laws 1991; Fernandez et al. 1996). Hence, the results here support recent argument against the idea of a fixed (Redfield) elemental ratio in phytoplankton (e.g. Broecker and Henderson 1998; Geider and Roche 2002; Flynn 2010).

Successful reproduction among aquatic herbivores hinges on the simultaneous and optimum supply of several chemical substances by algae (e.g. Guisande et al. 1999). Furthermore, herbivores differ in their demand for chemical substances due potentially to differences in ontogeny, physiology, and several other factors (e.g. Evjemo et al. 2003; Ventura and Catalan 2005). Hence, it can be argued based on the results in Figure 2 that differences in algal C-quota could cause trophic niche separation among herbivores (Guisande 2006; Behmer and Joern 2008), with individuals requiring less lipid (e.g. CI-CIII copepodids of *Calanus spp.*, Evjemo et al. 2003) potentially grazing on low C, lipid-poor algal cells, while those requiring more lipid (e.g. CIV-CV of *Calanus spp.*, Evjemo et al. 2003) may graze on high C, lipid-rich algal cells. This could potentially limit or reduce overlap in prey preference among herbivores, thus mitigating competition between herbivores and ensuring co-existence of different species or subpopulations of herbivores within the same habitat.

The nutritional quality of phytoplankton could be limited if algae are deficient in essential amino and fatty acids (Jónasdóttir 1994; Guisande et al. 2000; Shin et al. 2003). In terms of essential amino acid composition, the results here show general similarities between algal taxonomic groups (Figure 4, Table 3), which is consistent with field data from different ecosystems (Lee and Cronin 1984; Cowie and Hedges 1992; Kalachova et al. 2004). Only HIS, METH, ILE and VAL composition of algae differed significantly between phytoplankton taxonomic groups. How this may impact secondary production could be determined by comparing HIS, METH, ILE and VAL composition of algae with that of herbivores (Conceição et al. 2003; Anderson et al. 2004). HIS, METH, ILE and VAL respectively constituted on average 1.5 – 2.5%, 2.2 – 2.75%, 3.5 – 5.5% and 5.5 – 6.5% of total algal amino acids. These values are equal to or higher than the composition of the same compounds reported for different stages (including eggs) of major planktonic herbivores (e.g. Cowey and Corner 1963; Brucet et al. 2005). Moreover, aquatic herbivores are very efficient at assimilating protein and by extension amino acids from the algae they ingest, with reported protein assimilation efficiency being close to 90% (Anderson, 1994). Hence, algae may generally contain adequate essential amino acids for planktonic herbivores. Further research on this issue is needed.

Table 3. ANOVA table for the variability in the essential biochemical composition among algal taxonomic classes. A summary and description of the units of each compound are given in Table 4

Variable	df	MS	F	<i>p</i>
EPA				
Taxon	5	856.8356	19.1009	2.32E-12
Error	78	44.8585		
Total	83			
DHA				
Taxon	5	797.3567	69.6634	0.0000
Error	78	11.4458		
Total	83			
HIS				
Taxon	5	2.0353	3.5012	0.01019
Error	40	0.58131		
Total	45			
ARG				
Taxon	5	40.4147	1.1827	0.33475
Error	40	17.2608		
Total	45			
THR				
Taxon	5	1.8039	1.8894	0.11779
Error	40	0.95473		
Total	45			
VAL				
Taxon	5	3.3638	3.8194	0.006374
Error	40	0.88071		
Total	45			
LYS				
Taxon	5	1.3595	0.63182	0.67653
Error	40	2.1517		
Total	45			
ILE				
Taxon	5	9.871	17.6213	3.37E-9
Error	40	0.56018		
Total	45			
LEU				
Taxon	5	3.921	1.0772	0.38758
Error	40	3.6401		
Total	45			
PHE				
Taxon	5	1.3524	0.7293	0.60564
Error	40	1.8545		
Total	45			
METH				
Taxon	5	2.6771	3.5081	0.010085
Error	40	0.76311		
Total	45			

Table 4. Essential amino acid (EAA) and essential fatty acid (EFA) composition (mean \pm STD) of different classes of phytoplankton. EAA in % of total amino acids, and EFA in % of total fatty acids. Abbreviated names of compounds are defined in figures 4 and 5, as well as in the text. Different letters within rows indicates significant difference at $p < 0.05$. n = number of species used

EAA n	Taxonomic class													
	Bacillariophyceae		Prymnesiophyceae		Chlorophyceae		Cryptophyceae		Prasinophyceae		Eustigmatophyceae		Dinophyceae	
	11	4	4	4	2	5	1	1	14					
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
HIS	2.1 \pm 0.3 ^a	2.4 – 1.6	2.4 \pm 0.5 ^a	3.5 – 1.5	2.1 \pm 0.5 ^{ab}	3.0 – 1.6	1.9 \pm 0.2 ^a	2.1 – 1.8	1.8 \pm 0.2 ^{ac}	2.2 – 1.5	2.6 \pm 0.3 ^{ab}	2.8 – 2.1		
ARG	7.0 \pm 1.2 ^a	10.1 – 5.8	7.0 \pm 0.8 ^a	8.4 – 5.6	7.4 \pm 2.5 ^a	13.4 – 5.9	7.0 \pm 0.4 ^a	7.2 – 6.7	9.1 \pm 4.3 ^a	15.0 – 4.7	6.7 \pm 0.5 ^a	7.3 – 6.2		
THR	5.2 \pm 0.4 ^a	5.9 – 4.5	4.9 \pm 0.4 ^a	5.8 – 4.3	5.1 \pm 0.6 ^a	5.9 – 4.0	5.6 \pm 0.3 ^a	5.9 – 5.4	4.9 \pm 0.5 ^a	5.5 – 4.1 ^a	5.0 \pm 0.4 ^a	5.5 – 4.5		
VAL	5.7 \pm 0.6 ^a	6.3 – 4.4	6.0 \pm 0.4 ^a	6.7 – 5.4	5.8 \pm 0.5 ^a	6.6 – 5.2	6.5 \pm 0.5 ^{ab}	6.8 – 6.1	5.3 \pm 0.5 ^{ac}	5.8 – 4.4	5.9 \pm 0.4 ^a	6.5 – 5.6		
LYS	6.3 \pm 1.0 ^a	7.8 – 5.0	6.1 \pm 0.3 ^a	6.7 – 5.6	6.0 \pm 0.8 ^a	7.4 – 4.9	6.6 \pm 0.7 ^a	7.1 – 6.1	6.2 \pm 0.7 ^a	7.3 – 5.3	6.6 \pm 0.5 ^a	7.2 – 6.1		
ILE	5.2 \pm 0.5 ^a	5.9 – 4.2	4.8 \pm 0.3 ^b	5.3 – 4.3	4.1 \pm 0.5 ^{bc}	4.8 – 3.4	4.3 \pm 0.3 ^{bc}	4.6 – 4.1	3.7 \pm 0.3 ^c	4.0 – 3.4	4.8 \pm 0.1 ^{ab}	5.0 – 4.7		
LEU	8.0 \pm 0.7 ^a	9.1 – 7.0	8.4 \pm 0.6 ^a	9.7 – 7.4	7.6 \pm 1.2 ^a	9.7 – 5.6	7.8 \pm 0.0 ^a	7.8 – 7.82	8.7 \pm 1.4 ^a	10.1 – 6.6	7.6 \pm 0.3 ^a	7.9 – 7.3		
PHE	6.0 \pm 0.7 ^a	7.0 – 5.0	6.2 \pm 0.3 ^a	6.7 – 5.4	5.8 \pm 0.5 ^a	6.7 – 5.1	5.3 \pm 0.3 ^a	5.5 – 5.1	6.3 \pm 1.0 ^a	7.6 – 4.9	6.0 \pm 0.3 ^a	6.2 – 5.6		
METH	2.2 \pm 0.4 ^a	2.6 – 1.3	2.6 \pm 0.5 ^{ab}	3.2 – 1.5	1.9 \pm 0.3 ^{ac}	2.3 – 1.4	2.6 \pm 0.3 ^a	2.7 – 2.4	2.0 \pm 0.2 ^a	2.3 – 1.6	2.6 \pm 0.7 ^a	3.2 – 1.6		
EFA n	9		5		10		3		6		1		4	
EPA	20.4 \pm 7.3 ^a	27.8 – 5.2	12.0 \pm 9.9 ^{bg}	27.7 – 0.5	0.2 \pm 0.3 ^c	1.0 – 0.0	15.4 \pm 3.9 ^{abg}	20.0 – 8.4	2.9 \pm 3.2 ^{ce}	10.7 – 0.2	30 \pm 9.1 ^{fg}	39.8 – 16.4	8.5 \pm 3.9 ^{bd}	12.6 – 1.8
DHA	2.9 \pm 2.3 ^a	0.0 – 6.4	9.4 \pm 3.8 ^b	19.5 – 4.7	0.0 \pm 0.1 ^{ac}	0.2 – 0.0	8.9 \pm 2.8 ^b	12.6 – 3.8	2.3 \pm 3.6 ^a	8.5 – 0.0	0.0 ^a	0.0	24.0 \pm 6.1 ^d	31.3 – 13.0
C ₁₈ - PUFA	4.9 \pm 2.4 ^a	9.6 – 1.2	19.3 \pm 10 ^b	40.7 – 6.5	41.5 \pm 6.5 ^c	51.1 – 30.2	49.4 \pm 5.1 ^{cf}	58.5 – 43.0	27.6 \pm 11.6 ^{be}	52.2 – 13.7	2.2 \pm 0.6 ^a	2.8 – 1.3	25 \pm 21 ^{bd}	55.5 – 1.7

The results show that EFA composition may differ between algal taxonomic groups (Figure 5 and Table 3). EPA constituted 10% or more of total fatty acids in most of the algae. On the other hand, DHA proportion of the total fatty acids exceeded 10% only among dinophytes. EFA content of most of the algae in this study is too low when compared with the composition of the same in herbivorous zooplankton (Lacoste et al. 2001; Shin et al. 2003; Graeve et al. 2005; Rajkumar and Vasagam 2006; Kreibich et al. 2008). Hence, planktonic herbivores may be limited by EFA content of algae. However, the considerable composition of C₁₈-polyunsaturated fatty acid (C₁₈-PUFA) within most of the algae (Table 4) may moderate the degree of limitation by EPA and DHA as some major planktonic herbivores are capable of converting C₁₈-PUFA into EFA (though less efficiently) when they feed on EFA-deficient algae (Von Elert 2002; Bec et al. 2003; Veloza et al. 2006).

In conclusion, it can be said that this study affirms already established understanding that there is a simple log-log linear relationship between algal carbon-quota and bulk biochemical (protein, lipid, and carbohydrate) composition (e.g. Montagnes et al. 1994). Here, biochemical data on algae cultured with different growth media, under diverse degrees of nutrient richness, light intensity and duration, as well as temperature, and harvested at different stages of growth were used. Hence, the parameters determined here (Table 2), unlike those from studies, are not restricted to any species of algae or environment. They could therefore be employed (by modelers) to determine algal biochemical composition under variable habitat/growth conditions provide the carbon content of the algae is known under such conditions. It has been shown that DHA and EPA composition of algae differs significantly between algal taxonomic groups, which may have consequences for food consumption and processing by aquatic herbivores. Conversely, essential amino acid composition of algae is mostly similar and probably may not be major controlling factor for planktonic herbivory.

Appendix 3

Food availability effect on reproductive strategy: the case of *Acartia tonsa* (Copepoda: Calanoida)

This manuscript has been published in Marine Ecology Progress Series (2011: volume 428, pages 151 – 159). I developed the concept of the study. Experiment and chemical analysis were conducted with the help of Diekmann A. B. S. (Universität Hamburg, Germany). I did lipid and data analyses, as well as wrote the manuscript with scientific and editorial advice from Campbell R. W. (Prince William Sound Science Center, Cordova, AK, 99574, USA) and St. John M. A. (Universität Hamburg, Germany).

A3.1 ABSTRACT

Food availability has been linked to changes in the biochemical composition of zooplankton eggs. However a number of species are capable of resource storage and are thereby able to use accumulated reserves for reproduction during periods of poor food conditions. Conversely in species such as *Acartia tonsa* with limited storage capacities, there can be a strong dependence of egg composition on ambient food conditions. The aim of this study was to determine the effect of food availability on the carbohydrate, protein and fatty acid composition of *A. tonsa* females and their eggs after being fed with different concentrations of the cryptophyte *Rhodomonas baltica*. No significant differences in the biochemical composition of females were observed, however egg protein composition was higher in food-limited females. We propose that the production of protein-rich eggs by food-limited copepods is a reproductive strategy for ensuring the survival of offspring during poor feeding conditions. In terms of their relative biochemical content, there were no significant differences between both adult and egg stages of *A. tonsa* and *R. baltica*. However, these biochemical similarities did not influence egg production. Rather, higher biochemical similarities were observed between *R. baltica* and eggs when females were food-limited. These suggest that food-limited females may moderate the cost of reproduction by producing eggs without much modification to the substrates they ingest.

A3.2 INTRODUCTION

Copepods occupy a key trophic position in marine food webs, and are responsible for the transfer of a large proportion of the energy between primary producers and tertiary and higher trophic levels (Cushing 1990). Vital for the persistence of copepod populations is the production of viable eggs. Among other factors, normal egg development and subsequent hatching depends on their chemical constituents. For example, carbohydrates are mainly used as energy source during hatching (Guisande & Harris 1995), proteins modulate cellular events such as gene expression and growth while fatty acids (specifically polyunsaturated fatty acids) are involved in the metabolism of chemicals responsible for regulating cell differentiation, and hatching (Xu et al. 1994; Sessler & Ntambi 1998). Often these roles are specific to individual biochemical constituents, and are typically not interchangeable. Several studies have demonstrated the effect of prey biochemical contents on egg viability (e.g. Jónasdóttir & Kjørboe 1996; Guisande et al. 2000). It is however not clear whether egg viability is also affected by food availability (see Tang et al. 1998; Guisande & Harris 1995).

Food availability influences egg size, which influences the amount of chemical substances required for embryogenesis and subsequent hatching (Guisande & Gliwicz 1992; Guisande 1993; Galindo et al. 1993; Guisande & Harris 1995; Auel 2004). Furthermore, the extent to which ambient food levels influence egg biochemical characteristics may depend on reproductive strategy. During poor food conditions, species capable of storing lipids rely mostly on accumulated reserves for reproduction (Lee et al. 2006; Kattner et al. 2007). This adaptive strategy, where trophic history plays a key role, frees lipid requirement for egg production from the immediate nutritional needs of females, thus limiting the dependence of egg lipid composition on the prevailing ambient food conditions, as seen in *Paraeuchaeta antarctica* (Alonzo et al. 2000). On the other hand, in species with smaller lipid stores, the dependence of egg lipid composition on ambient food conditions can be marked (see Ederington et al. 1995). Also, the effect of ambient food variability on compounds such as proteins and carbohydrates that are not stored in large amounts by copepods could be significant and possibly independent of species' reproductive strategy. However, this has been investigated only in eggs produced by species capable of accumulating resources (e.g. Alonzo et al. 2000).

Acartia tonsa is a common euryhaline copepod in estuarine and coastal waters, and does not store appreciable amounts of reserve biomass (Sargent & Falk-Petersen 1988). Several studies have demonstrated the effect of food availability on the biochemical composition of the species (Mayzaud 1976; Roman 1991; Ederington et al. 1995). Most

investigations have however considered the extremes of the food availability spectrum, with copepods either starved or feed at saturating levels (e.g. Mayzaud 1976; Ederington et al. 1995), and rarely has the effect of food availability on egg biochemical composition been considered (Ederington et al. 1995). As a result, the biochemical composition of the eggs *A. tonsa* produces under non-saturation food levels are not known. As has been done for other copepod species (e.g. Guisande & Harris 1995), such an investigation is needed because low food conditions are a feature of the natural environments of *A. tonsa* (Durbin et al. 1983; Beckman & Peterson 1986; Bellantoni & Peterson 1987).

The aim of this study was to determine whether or not the biochemical content (i.e. protein, carbohydrate and fatty acid) of *A. tonsa* varies in relation to the quantity of food available to it, and whether such variation could affect the chemical composition of its eggs. Huntley & Boyd (1984) suggest that zooplankton have a minimum maintenance food concentration, which is required to balance respiratory losses. As well, growth limitation by chemical substances vary with food availability (Sterner 1997). Hence, the assumption here is that sub-optimal amounts of food (i.e. food quantity limitation) would cause females to produce eggs with a chemical composition dissimilar to those produced by individuals provided with adequate food concentration, due to the energy demand for maintenance.

A3.3 MATERIALS AND METHODS

A3.3.1 Algal culture

The cryptophyte *Rhodomonas baltica* (clone RCC 350) was used as food source. The algae were maintained in a continuous culture in 33 psu seawater at $20 \pm 1.5^\circ\text{C}$ under 11:13 light:dark cycles with a photo-saturating light intensity ($\sim 100 \mu\text{E}/\text{m}^2\text{s}$). Light intensity was monitored daily using a TriOS ACC VIS spectral irradiance meter. The algae were maintained in Walne's medium (Mc Vej 1993) and kept in exponential growth phase by replacing approximately $\frac{1}{2}$ of the culture with fresh media daily. The cultures were continually aerated to prevent self-shading and ensure mixing. Prior to being fed to the copepods, daily triplicate samples of the algae were filtered onto a pre-combusted Whatmann GF/F filter, washed with isosmotic ammonium formate to remove excess salts, and immediately stored at -80°C for later determination of biochemical constituents. Each filter contained at least 3×10^6 cells, an amount determined to be sufficient from preliminary experiments.

A3.3.2 Copepod cultures

Batches of *Acartia tonsa* eggs were placed in filtered seawater and incubated in a 50 L polyethylene tank. After hatching, the cultures were fed a daily ration of the experimental diet at concentrations $>50,000$ cells ml^{-1} , which allows unlimited growth (Kiørboe et al. 1985, Støttrup & Jensen 1990). Algae cell counts were made daily with a Beckman Multisizer 3. Cultures were maintained on the same light, salinity and temperature as the algae and received gentle aeration for mixing. Only young adults (about three weeks old) from the same batch of eggs were used for the experiments. Copepods were conditioned to the experimental prey concentration for 24 h.

After the conditioning period, groups of 50 individuals (40 females and 10 males) were placed in mesh-bottom sieves (130 μm mesh size) and suspended in 1 L beakers at three different food concentrations (110, 550 and 1100 $\mu\text{g C L}^{-1}$). A carbon content of 36.7 pg per cell (Kiørboe et al. 1985) was assumed in estimating the number of *Rhodomonas baltica* cells needed to meet the carbon requirement at each food concentration. Nine setups were established for each food concentration, with three replicates for each biochemical considered.

Every 24 h, the adult copepods were placed into new beakers with fresh food (at the same experimental level) by carefully transferring the sieves. The contents of the old beakers were sieved through a 35 μm sieve, followed by several washings with double filtered seawater and rinsed into a Bogorov counting tray. The number of eggs was counted under a binocular microscope at 16x magnification. Using forceps and pins, individual eggs were freed of fecal pellets and detritus before transferring them into a new beaker using a small pipette. 7 to 10 eggs were sub-sampled from each beaker for egg size measurement.

Egg size measurements were made by photographing eggs with a digital camera (Leica® DC300) connected to an image analysis system (IMAGEPRO plus 4.5.1) mounted on an inverted microscope at 400x magnification. Diameters of circles drawn to cover the outline of the images on the screen were used to represent egg diameter. Three circles were generally drawn for each egg and the mean of the three measurements was used to represent egg diameter.

The remaining eggs (between 120 to 250) were filtered onto a pre-combusted Whatmann GF/F filter, washed with isosmotic ammonium formate, and immediately stored at -80°C for later analysis of biochemical composition. Using these methods, samples were collected from each replicate beaker every day for 3 days. At the end of the trials, copepods were checked for mortality and all remaining females (between 20 to 32) were filtered onto a pre-combusted Whatman GF/C filter, washed with isosmotic ammonium formate, and

immediately stored at -80°C . The number of copepods and eggs filtered for biochemical analysis are comparable with those used in similar studies (Ederington et al. 1995; Shin et al. 2003).

A3.3.3 Biochemical analysis

Protein content was measured with the Bicinchoninic acid assay of Smith et al. (1985). Absorbance was measured at 562 nm and calibrated with a Bovine Serum Albumin (BSA) standard.

Total carbohydrate was measured following the sulphuric acid-phenol method of Herbert et al. (1971) and Dubois et al. (1956), and expressed as glucose equivalents. Absorbance was read at 490 nm.

Lipids were extracted following the method of Folch et al. (1957) in 8:4:3 dichloromethane: methanol:water; a known concentration of nonadecanol was added as an internal standard. Samples were vortexed, placed on ice in a sonication bath for 30 minutes, and stored at -20°C over-night. Transesterification of fatty acids was performed following the method of Kattner & Fricke (1986) and analysed by capillary gas chromatography using an Agilent Technologies 6890N Network GC System with flame ionisation detection. Separations were performed with a DB-WAX capillary column (30 m long and of 0,32 mm inner diameter) with helium as the carrier gas at 68.9 kPa. The temperature program was 40°C to 150°C at a rate of 10°C per minute followed by 20°C per minute to 220°C . Fatty acid methyl esters were detected at 250°C . Chromatographic data were collected with a data system (Chemstation - Hewlett-Packard) and quantified by comparison of peak areas with the internal standard. The fatty acid content of an organism was calculated as the sum of all the individual fatty acids detected.

A3.3.4 Data analysis

Only samples collected on the last day of the experiment were analysed. Analysis of variance (ANOVA) tests were done using the ANOVAN function of MATLAB[®] software for windows (version 7.0.4). ANOVAN allows for the multi-way analysis of variance for variable observations with respect to any number of different factors. Unless stated, the critical p-value was taken to be 0.05. Whenever differences were found between any set of data, the Tukey HSD (alpha of 0.05) procedure was used as a post hoc test to determine which pairs of means are significantly different.

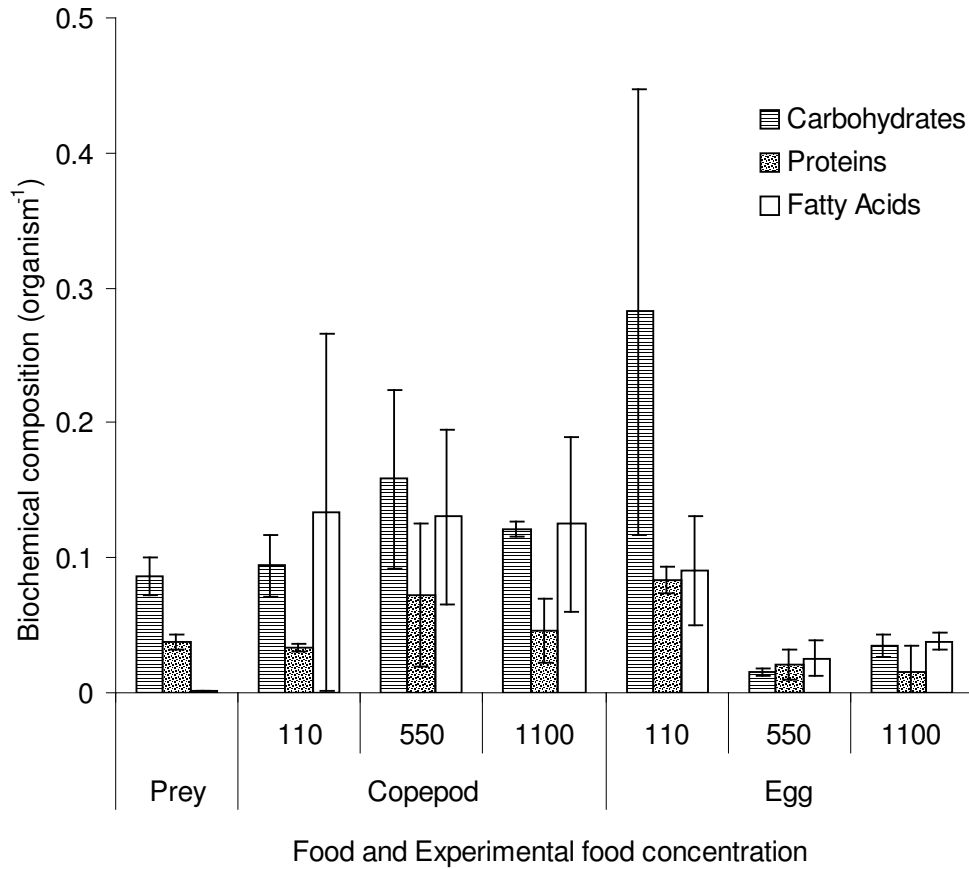


Fig. 1. Biochemical constituents (mean \pm standard deviation, sample size = 3) of experimental food (ng), *Acartia tonsa* females ($\mu\text{g} \times 10$) and eggs (μg) at 110, 550 and 1100 $\mu\text{g C L}^{-1}$ food concentrations.

Within-group tests (e.g. biochemical composition of female *Acartia tonsa* or eggs in the different treatments) were done on measured compositions for each component (i.e. mass per individual). One-way ANOVA was used to compare the composition of individual biochemicals between food treatments. Whenever two dissimilar groups were compared (females vs. eggs vs. food), all biochemical constituents were converted into proportions and arcsine transformed prior to analysis. When multiple 1-way ANOVAs were done, the critical p-value was Bonferroni corrected by dividing 0.05 by the number of tests. Where no significant differences in relative chemical compositions were found between experimental organisms, a dissimilarity index was used to determine how the biochemical similarities between egg, food and copepod relate to egg production rate.

A pairwise Euclidean distance (D_{jk}) was used to estimate dissimilarity between the constituents of females, their eggs and the food:

$$D_{jk} = \frac{\sqrt{\sum_{i=1}^n (X_{ij} - X_{ik})^2}}{n}$$

Where X_{ij} and X_{ik} are the proportions of the biochemical i of the females or eggs (j) and the food (k), and n is the number of biochemical constituents. In this study, D_{jk} indicated the distance between organisms in a fatty acid – protein – carbohydrate space, so the value of D_{jk} would be larger if organisms being compared are less similar and vice versa. Prior to calculating D_{jk} , the data were standardized to have zero mean and unit standard deviation to remove the effect of differences in magnitude of the different constituents.

A3.4 RESULTS

A3.4.1 Biochemical contents of the algae, copepods and eggs

The different biochemical substances had differing contributions to total measured organic content in the experimental *Rhodomonas baltica* diet (Fig. 1). Carbohydrates were the major biochemical constituent (about 69 - 82% on average), followed by proteins (14 - 30%) and fatty acids (1- 4%). Testing for differences in the food between days is difficult, because the amounts can be expected to be autocorrelated, and the short-term nature of the experiments (samples from only three days) makes detection of a trend unlikely. Given that the cultures were grown semi-continuously (i.e. nutrients added daily), we will proceed with the assumption that the composition of the food did not change over time.

Differences between individual biochemical constituents of females held at different food concentrations were tested with one-way ANOVA. There were no significant differences in the biochemical composition of the females among the three food treatments (Fig. 1, carbohydrate: $F_2 = 1.07$, $p = 0.4105$; proteins: $F_2 = 0.64$, $p = 0.5648$ and fatty acid: $F_2 = 0.47$, $p = 0.6577$). Fatty acid content of females was $1.31 \pm 1.33 \mu\text{g individual}^{-1}$ (Mean \pm STD), which compares well with the range of $0.07 - 0.1 \mu\text{g individual}^{-1}$ reported by Ederington et al. (1995) for females of *Acartia tonsa* cultured with different prey items. Published protein and carbohydrate composition of adult *Acartia* ranges from $3.40 - 4.49$ and $0.12 - 0.77 \mu\text{g individual}^{-1}$ respectively (Ikeda & Skjoldal 1980, Kapiri et al. 1997, Rajkumer & Vasagam 2006). Carbohydrate composition (mean \pm standard deviation = $1.25 \pm 1.58 \mu\text{g}$) observed per female in this study is comparable with these data. The protein composition ($0.51 \pm 0.70 \mu\text{g}$) observed here was relatively low for *Acartia* but comparable with data on adults of similar sized copepods such as *Euterpina acutifrons* (protein content: $0.81 - 2.88 \mu\text{g individual}^{-1}$; Guisande et al. 2000).

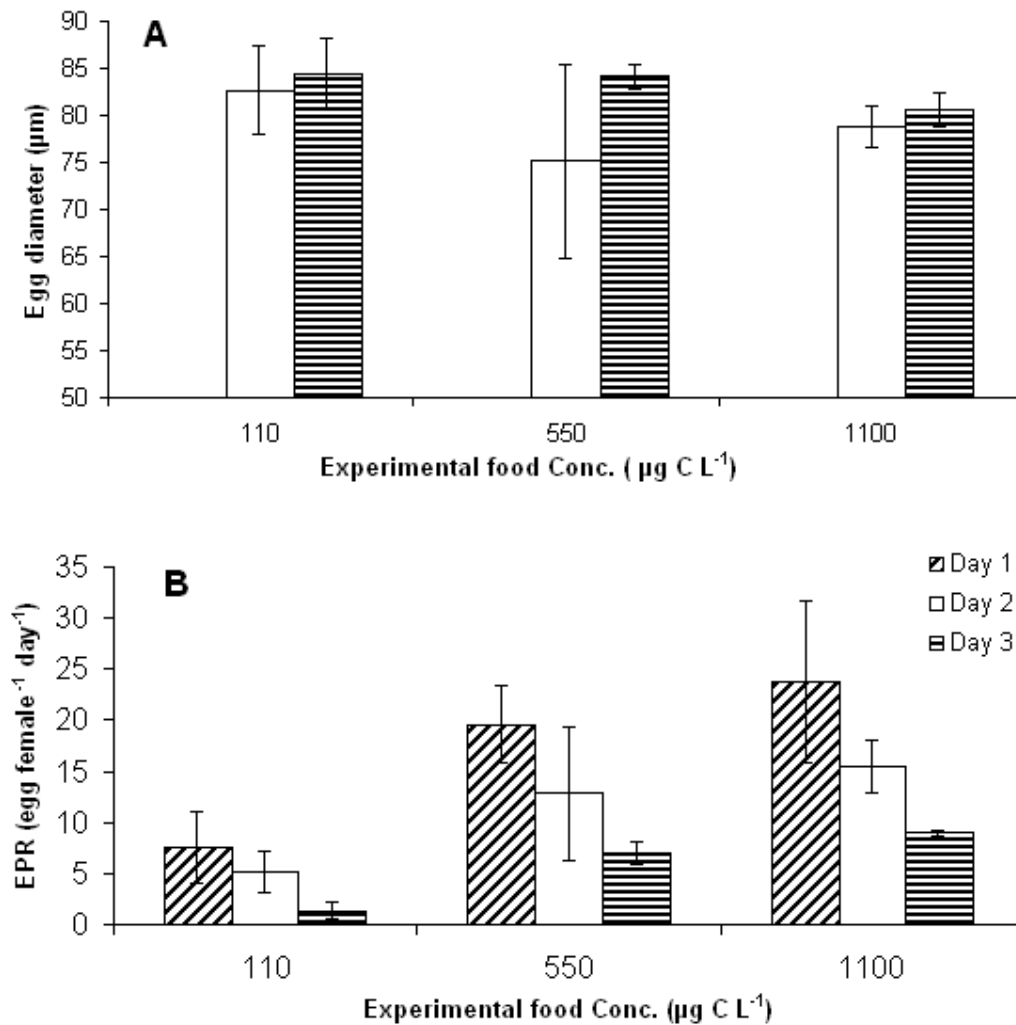


Fig. 2. Egg diameter (A) and egg production rate, EPR (B) of *Acartia tonsa* kept at different food concentrations over a period of three days. Bar height = mean \pm standard deviation.

Sample size equals 9 for each day and food concentration.

Differences between individual biochemical constituents of eggs produced at different food concentrations were tested with one-way ANOVA. Egg carbohydrate ($F_2 = 6.86$, $p = 0.0509$) and FA ($F_2 = 5.22$, $p = 0.0767$) contents did not change significantly with food concentration. Respectively, they constituted 0.11 ± 0.28 and $0.05 \pm 0.09 \mu\text{g egg}^{-1}$. These are comparable with data from other studies (Drillet et al. 2006, Wang et al. 2005). Only the protein content of eggs differed significantly between treatments ($F_2 = 12.54$, $p = 0.0189$). It was significantly higher in egg produced by food-limited ($110 \mu\text{g C L}^{-1}$) than food-saturated (550 and $1100 \mu\text{g C L}^{-1}$) females (Tukey HSD post hoc test, $p < 0.05$). On average, protein composition per eggs decreased from 0.08 to $0.02 \mu\text{g}$ with increasing food availability, which

is comparable with the total amino acid composition of *Acartia tonsa* eggs (Drillet et al. 2006, 2008), assuming amino acids mainly occur in proteins.

To determine whether the individual biochemical constituents of *Acartia tonsa* and their eggs reflect that of their prey, the relative biochemical composition of all the treatment groups (*Rhodomonas baltica*, the female *Acartia tonsa* under the three feeding levels, and the eggs produced under the three feeding levels; see Fig. 1) were compared using a 1-way ANOVA. There were no significant differences between food, eggs and females for any of the biochemical substances (carbohydrate: $F_6 = 3.02$, $p = 0.059$; protein: $F_6 = 1.39$, $p = 0.34$; fatty acids: $F_6 = 2.99$, $p = 0.061$). Furthermore, there were no significant differences between females and eggs at any of the feeding levels, for any of the biochemical substances (Table II).

Table I. Two-way ANOVA test for the effect of treatment day and food concentration (three treatments: 110, 550 and 1100 $\mu\text{g C L}^{-1}$) on egg production rate by *Acartia tonsa*.

Source	SS	df	MS	F-test	p-value
Day	1312.8	2	656.4	5.36	0.0068
Food Conc.	1857.9	2	928.9	7.59	0.001
Day x Food Conc.	322.9	4	80.724	0.66	0.62
Error	8691.2	71	122.4		
Total	12239.3	79			

A3.4.2 Egg size, production rate and biochemical dissimilarities between experimental organisms

Fig. 2 shows the effect of food concentration on egg size and production rate. There were no significant differences in egg size at the three food concentrations on day 2 (1-way ANOVA, $F_2 = 0.76$, $p = 0.49$) or day 3 (1-way ANOVA, $F_2 = 0.45$, $p = 0.64$). Egg production rate was however different between both days and the feeding treatments (Table I). Again, the inclusion of a “day” term leaves open the possibility of temporal autocorrelation, although the danger with an autocorrelation would be a type-II error (finding no difference when one did exist), so the significant result can be considered to be conservative. A post-hoc Tukey test found that egg production during day 1 was significantly higher than during day 3, but that day 2 was not significantly different from either day. A post-hoc Tukey test found that

egg production rate in the lowest feeding treatment ($110 \mu\text{g C L}^{-1}$) was significantly lower than in the two highest treatments (550 and $1100 \mu\text{g C L}^{-1}$), but that there were no significant differences between those two highest treatments. These suggest that egg production by females held at the lowest food level was limited by food availability.

Egg production rate was correlated with the dissimilarity between the biochemical composition of the prey and both *A. tonsa* (Fig. 3A; $r^2 = 0.59$, $p = 0.0447$) and eggs (Fig. 3B; $r^2 = 0.68$, $p = 0.0117$). Shortest dissimilarity distances (with one outlier) were observed in the low food treatments where egg production rates were low. Egg production rate was however independent of the biochemical similarities between eggs and females (Fig. 3C; $r^2 = 0.01$, $p = 0.1857$), and females and eggs were more similar in terms of their relative composition of fatty acids, protein and carbohydrate.

Table II. Two way ANOVAs testing for difference between stage of *Acartia tonsa* (female or egg) and feeding level (110 , 550 and $1100 \mu\text{g C L}^{-1}$) in the feeding experiments, by biochemical

Biochemical	Source	SS	df	MS	F	p-value
Carbohydrate	Stage	0.01116	1	0.01116	0.49	0.5014
	Level	0.01058	1	0.01058	0.46	0.5124
	Stage*Level	0.08213	1	0.08213	3.58	0.0877
	Error	0.22934	10	0.02293		
	Total	0.32241	13			
Protein	Stage	0.0305	1	0.0305	2.46	0.148
	Level	0.02218	1	0.02218	1.79	0.2109
	Stage*Level	0.01957	1	0.01957	1.58	0.2377
	Error	0.1241	10	0.01241		
	Total	0.18197	13			
Fatty Acids	Stage	0.00476	1	0.00476	0.13	0.7225
	Level	0.00212	1	0.00212	0.06	0.8123
	Stage*Level	0.02152	1	0.02152	0.6	0.4555
	Error	0.35694	10	0.03569		
	Total	0.40188	13			

A3.5 DISCUSSION

A3.5.1 Egg production and biochemical similarities between experimental organisms

The goal of this study was to investigate the effect of food quantity on the biochemical composition of female copepods and their eggs. *Acartia tonsa* was chosen for the study because it has been demonstrated that carbon ingested by adult females of the species appears

rapidly in their eggs (< 10 h: Tester & Turner 1990), therefore making it a good candidate for short-term laboratory experiments regarding the possible changes in the biochemical composition of eggs as well as adults.

Egg production of *Acartia* spp. has been the subject of much research (e.g. Kiørboe et al. 1985; Støttrup & Jensen 1990; Jónasdóttir & Kiørboe 1996), and egg production rate has been reported to be 10 – 50 eggs female⁻¹ day⁻¹, which compares well with the rates observed in this study (2 – 33 eggs female⁻¹ day⁻¹). At the 110 µg C L⁻¹ food concentration, egg production rate was lower (by about 2.5 times) than that observed at higher food levels, suggesting food quantity limitation. Over the study period, there was a general decline in egg production irrespective of the food concentration at which females were held (Fig. 2B). This could be due to loss of female reproductive ability with age, as observed in other studies (e.g. Durbin et al. 1992; Ianora et al. 1995). No variation in egg size was observed between the different food treatments in this study (Fig. 2A), contrary to what has been reported for other copepod species (e.g. *Calanus helgolandicus*: Guisande and Harris 1995). Hence, it is plausible that the size of an *Acartia* egg is independent of the quantity of food available to females.

Acartia tonsa is omnivorous (Anraku & Omori 1963) and capable of food selection (Cowles et al. 1988). It can therefore feed to satisfy its optimum requirement for specific substances, given the broad mixture of *in situ* prey species, trophic types and biochemical composition. This has been observed in field studies involving other copepod species (Pond et al. 1996). For this study, we raised *Acartia* on a single prey with the same biochemical composition. This may force feeding to be non-selective, and as a consequence limit the diversity of biochemical resources the copepod might otherwise acquire for biosynthesis. Moreover, *Acartia tonsa* does not store appreciable amounts of reserve biomass (Sargent & Falk-Petersen 1988) that could serve as a buffer against biochemical limitations of a uni-algal diet. We therefore did not observe significant chemical composition difference (on relative basis) between *Acartia* females, their eggs and the prey. Others have made similar observations in studies involving single prey species (Lee et al. 1971) or copepods that feed non-selectively (Peters et al. 2007).

Copepods exposed to uni-algal diet can however satisfy their specific requirement for chemical substances by increasing food intake, and investing acquired biochemical excesses into biosynthesis. This could be realistically achieved when ambient food availability is high or saturating. Low food availability would however limit animals' capacity to "overfeed" for excesses resources.

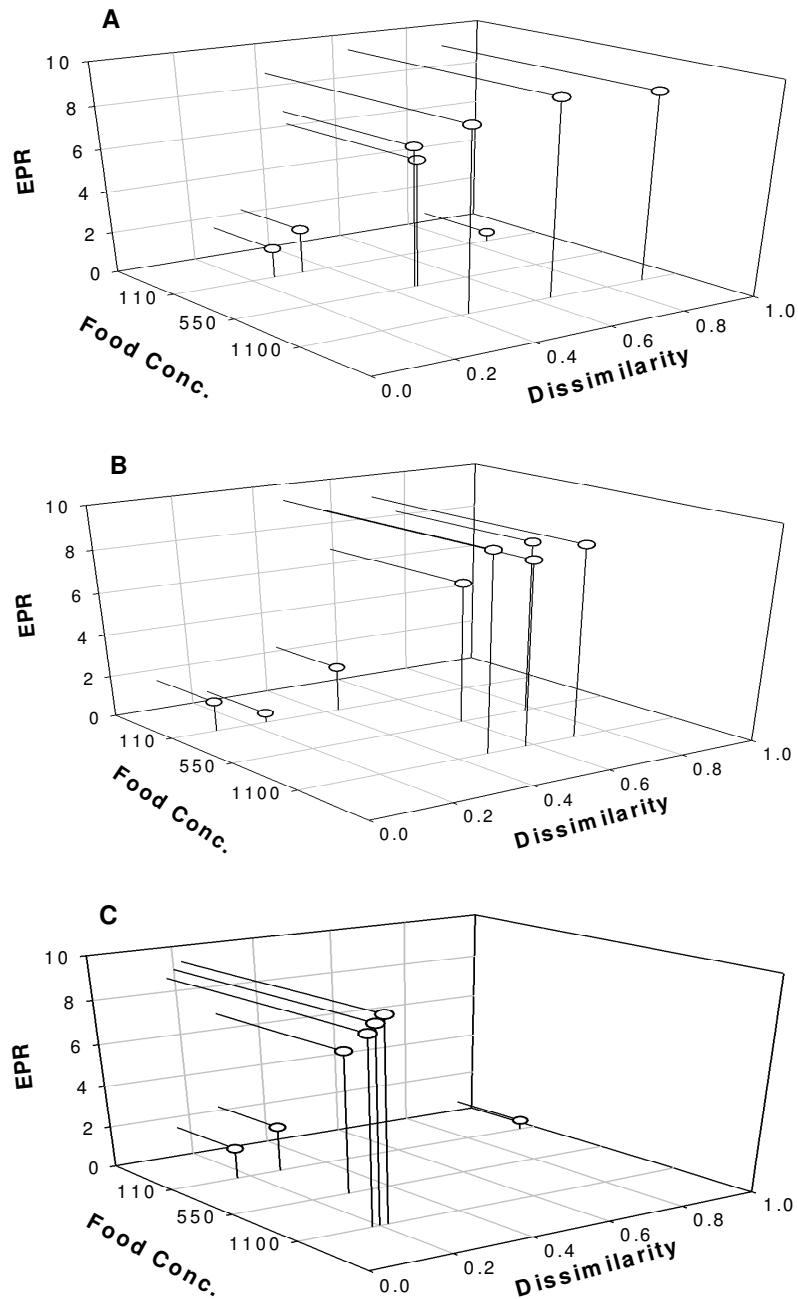


Fig. 3. Food availability effect on the relationship between egg production rate, EPR (mean, sample size = 3) and the biochemical similarity between (A) *Acartia tonsa* females and their food ($y = 11.47x + 0.49$; $r^2 = 0.59$, $p = 0.0447$), (B) females' food and eggs ($y = 13.60x - 0.94$; $r^2 = 0.68$, $p = 0.0117$) and (C) eggs and females ($y = -6.71x + 7.70$; $r^2 = 0.01$, $p = 0.1857$). A smaller dissimilarity indicates organisms being compared are more similar

Hence, food-limited females may lack the energy to markedly modify the substrates they ingest for biosynthesis. This may explain why biochemical similarities between *Rhodomonas baltica* and both stages of *A. tonsa* were mostly higher when food availability was low, and decreased with increasing food concentration (Figure 3A, B).

A3.5.2 Biochemical constituents of females and eggs

Data on chemical composition of zooplankton is inconclusive and at times difficult to interpret (Tang & Dam 1999). Typically, studies have investigated the elemental composition of zooplankton and the data show variations in the C:N ratio of zooplankton (e.g. Tande, 1982), in some cases by as much as 30% (Groenik & Hopkins 1984). In contrast, reports of zooplankton maintaining a constant chemical composition independent of ambient conditions (i.e. chemical homeostasis) also exist (Sterner and Schulz 1998; Hessen 1990). In this study, individual chemical constituents of females did not change with food concentration (Fig. 1), despite the fact that prey density influences grazing rate (e.g. Kiørboe et al. 1985) as well as the efficiency with which the ingested prey chemical constituents are assimilated (e.g. Urabe & Watanabe 1991; Besiktepe & Dam 2002). Hence, *Acartia* can be said to maintain homeostasis at the biochemical level as reported for other copepod species (Guisande et al. 1999; Guisande & Harris 1995). This occurs as many copepods can regulate their post ingestion processes (e.g. assimilation, metabolism) to meet their specific requirement for chemical substances (Lehane et al. 1995; Ivanovic et al. 2002; Mitra & Flynn 2005).

Contrary to the observation made in females, the biochemical composition of eggs changed with food concentration (Fig. 1). This has been observed in other copepod species (Guisande & Harris 1995). Consumers feed first to meet their maintenance requirements with subsequent egg production possible only when there are excess resources to be invested into egg production. In the presence of adequate but not abundant (i.e. not saturating) food for egg production, it would be necessary for consumers to regulate how they use the substances they acquire in order for egg production to be possible. Consequently, this may lead to a decrease in egg production in favour of a composition that meets the minimum requirements for successful egg development (Anderson & Pond 2000). However, if the level of food is below the regulatory capacity of the animal, then the animal will be constrained to accept surpluses of some biochemicals and/or deficits of others in the eggs. We observed significant differences in the protein content of eggs produced at different food concentrations. Egg protein content decreased with increasing food availability. As a result, total biochemical composition (μg) per egg produced by females held at higher food concentration was

significantly less than those produced by food-limited females. To the extent that inadequate food availability is a feature of the natural environments of copepods (Durbin et al. 1983, 1992; Beckman & Peterson 1986; Bellantoni & Peterson 1987), it may be that this was a consequence of *Acartia*'s reproductive strategy under food-limited conditions.

Interestingly, it has been hypothesized that copepods produce heavier eggs as an adaptation to food-limited environments (e.g. Guisande et al. 1996; Auel 2004). This is because heavy energy-rich eggs may provide adequate internal energy reserves for early ontogenetic lecithotrophic development, thus making nauplii relatively independent of the prevailing feeding conditions. Even for species not known for lecithotrophic development, having energy-rich early ontogenetic stage in a food-limited environment may be advantageous for surviving the relatively longer time nauplii may spend before encountering food. However, this strategy may not entirely apply to *Acartia*. Our results show protein, an unlikely substrate for energy reserve due to its comparatively low energy content (Cauffopé & Heymans 2005), as the substance responsible for the weight differences between eggs produced at different food concentrations.

Results from several studies indicate that *Acartia tonsa* produces normal, subitaneous and diapause eggs (e.g. Sullivan & McManus 1986; Madhupratap et al. 1996). Normal eggs hatch rapidly within the water column. Conversely, both subitaneous and diapause eggs have been described as “resting eggs” with suppressed development (Marcus 1996). Subitaneous eggs are quiescent eggs that may forego hatching in unfavourable conditions but can hatch as soon as improved environmental conditions are experienced. Diapause however is a more profound interruption that routes the metabolic programme of an organism away from direct developmental pathways and into a much more clearly organized break in development (Dank 1987). Thus, diapause forces the development of eggs into a refractory phase, which may last from few months to years, during which development does not resume, even if conditions are suitable for (Grice & Marcus 1981; Marcus 1996).

When copepods are food limited or starved, egg hatching success decreases (Ederington et al. 1995; Lacoste et al. 2001). Ephippial (i.e., diapause egg) formation in *Daphnia* has been shown to be caused by extreme starvation (Slobodkin 1954). In *Onychodiptomus birgei*, food availability affects the timing as well as the rate of diapause eggs production (Walton 1985). Moreover, production of both subitaneous and diapause eggs has been observed in crowded cultures of copepods such as *Eurytemora affinis* (Ban 1992), *Acartia latisetosa* (Belmonte 1992) and *Diaptomus clavipes* (Gehrs & Martin 1973). These

suggest that stressed conditions such as inadequate resource supply may play a role in resting egg production.

Based on these observations and our results, we hypothesize that food limitation is one mechanism triggering resting egg production in *Acartia* and that the role of the relatively high egg protein content observed in the low food treatments could be to provide sufficient storage product to maintain metabolism during diapause. This hypothesis is based on the observation that resting copepod eggs contain relatively higher proteins than lipids and carbohydrates (Wang et al. 2005). Also, resting eggs have reduced metabolic activity (Romano et al. 1996) and thus may require less energy that could be met by respiring protein.

Furthermore, resting eggs sometimes sink onto the seabed where they get buried into anoxic sediment (Marcus et al. 1994). Under such conditions, protein may be the appropriate substrate for respiration because less oxygen is required for the catabolism of protein than lipids and carbohydrates (Marcus 1996). Conversely, production of lipid-rich eggs under low food conditions may be physiologically advantageous for species that do not produce resting eggs (see Auel 2004). Here lipids could provide nauplii with adequate energy reserves to survive the relatively longer time animals may require before encountering food in low food environment.

A major, but yet unanswered question is if diapause and subitaneous eggs are those with a high protein content. We did not measure hatching success in this study due to the need for high quantities of eggs for the biochemical analysis. As a consequence, we cannot currently tell whether the protein-rich eggs produced under food limited females were subitaneous or diapause eggs. We are currently investigating this as part of an experiment to test the hypothesis suggested here. Hence we will discuss why it may be advantageous for *Acartia* to produce resting eggs when confronted with poor feeding conditions without alluding to the type of egg.

Producing resting egg under food-limited conditions could be crucial for the persistence of the species for two major reasons. First, resting eggs, if not preyed upon, could serve as nauplii recruitment source when food conditions improve or diapause is broken (Marcus 1996). The biochemical weight of the eggs produced in food-limited environment was significantly higher than those produced at relatively higher food treatments (Fig. 1). However, eggs size remained the same among females held at different food concentrations (Fig. 2A). Hence, eggs produced in food-limited treatment may be denser than those produced by females provided with saturated food levels. This could increase the velocity with which eggs may sink (Marcus & Fuller 1986; Wang et al. 2005) for eventual burial into

the seabed if they are resting eggs. Since resting eggs can survive many years of burial, their slow accumulation in the seabed could provide copepods with a pool of genetic information – egg bank according to Marcus (1996) – that may slow down the rate of evolutionary change (Hairston & De Stasio 1988). So the second benefit *Acartia* may derive for producing resting eggs under food-limited conditions may be that the perpetuation of the species would be ensured in the habitat they are adapted to. During adequate food conditions, this reproductive strategy against resource limitation may not be necessary since hatchlings can benefit from the readily available prey.

Our results demonstrate that food quantity limitation does not affect the biochemical composition of adult *Acartia tonsa*, but it does affect production rate and the biochemical composition of eggs. The major assumption in this study was that differences in the availability of food to females would result in dissimilar biochemical composition of eggs due to the demand for maintenance by females. We observed significant effect of food availability on the biochemical compositions of eggs produced by females held at different food concentrations (Fig. 1). We argue that food availability influences the reproductive strategy of *Acartia* females *vis-à-vis* the production of resting eggs under food-limited conditions.

APPENDIX 4

Published data on the effect of temperature (T, °C) on respiration rate (V_o , $\mu\text{l O}_2 \cdot \text{individual}^{-1} \cdot \text{d}^{-1}$) used for determining the activation energy for metabolism in female zooplankton. Dw = Dry weight (μg); V_m = mass normalised respiration rate ($\mu\text{l O}_2 \cdot \mu\text{gDw}^{-1} \cdot \text{d}^{-1}$)

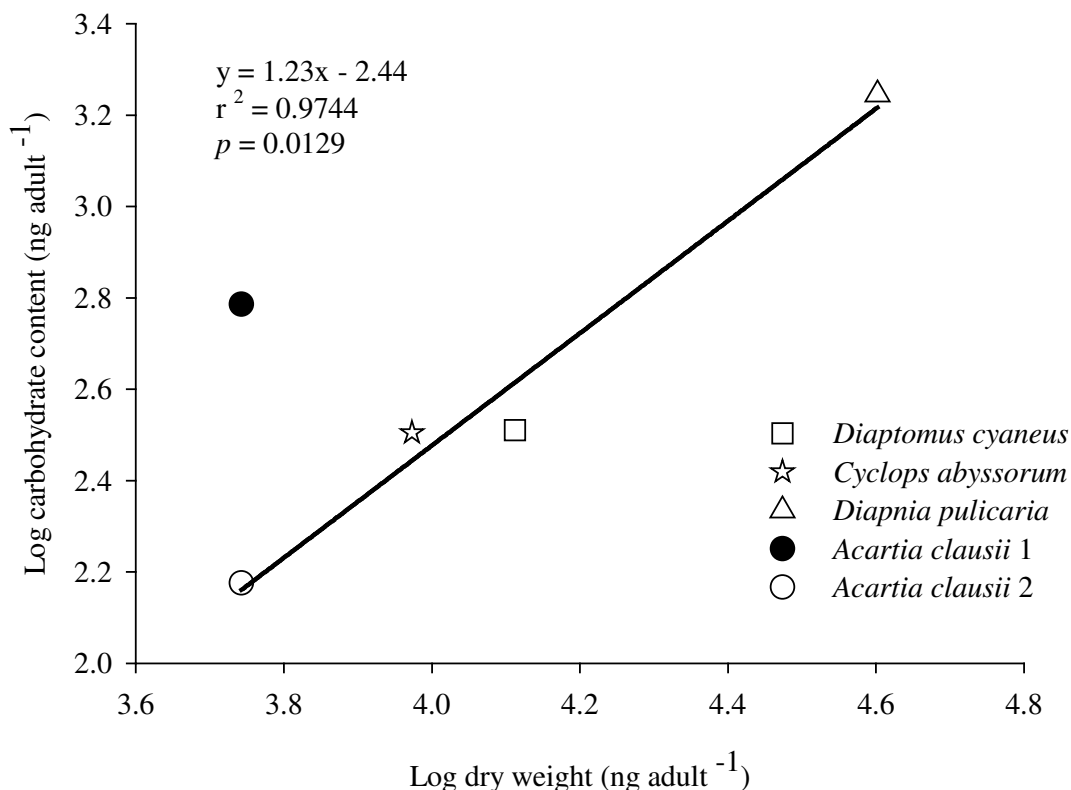
Species	T	Dw	V_o	V_m	Source
<i>Acanthocyclops viridis</i>	5			0.044	Laybourn-Parry & Tinson, 1985
	7			0.029	
	10			0.042	
	12.5			0.052	
	15			0.060	
	20			0.055	
<i>Acartia clausi</i>	10	10	0.16	0.016	Gaudy et al., 2000
	10	10	0.13	0.013	
	10	10	0.14	0.014	
	15	10	0.19	0.019	
	15	10	0.15	0.015	
	15	10	0.15	0.015	
	20	10	0.30	0.030	
	20	10	0.29	0.029	
	20	10	0.27	0.027	
<i>A. tonsa</i>	10	5	0.13	0.026	
	10	5	0.11	0.023	
	10	5	0.15	0.031	
	15	5	0.09	0.018	
	15	5	0.09	0.018	
	15	5	0.18	0.036	
	20	5	0.20	0.039	
	20	5	0.23	0.046	
	20	5	0.34	0.068	
	<i>Boeckella delicata</i>	10	10.1	0.90	
15		10.1	1.19	0.118	
20		10.1	2.06	0.204	
25		10.1	3.34	0.331	
10		4.46	0.41	0.091	
15		4.46	1.18	0.264	
20		4.46	1.97	0.441	
25		4.46	1.54	0.344	
<i>B. symmetrica</i>	10	18.21	0.92	0.050	
	15	18.21	1.23	0.068	
	20	18.21	1.76	0.096	
	25	18.21	2.90	0.159	
<i>Calamoecia lucasi</i>	10	1.23	0.20	0.166	
	15	1.23	0.24	0.191	
	20	1.23	0.41	0.334	
	25	1.23	0.64	0.521	

Appendix 4 continued

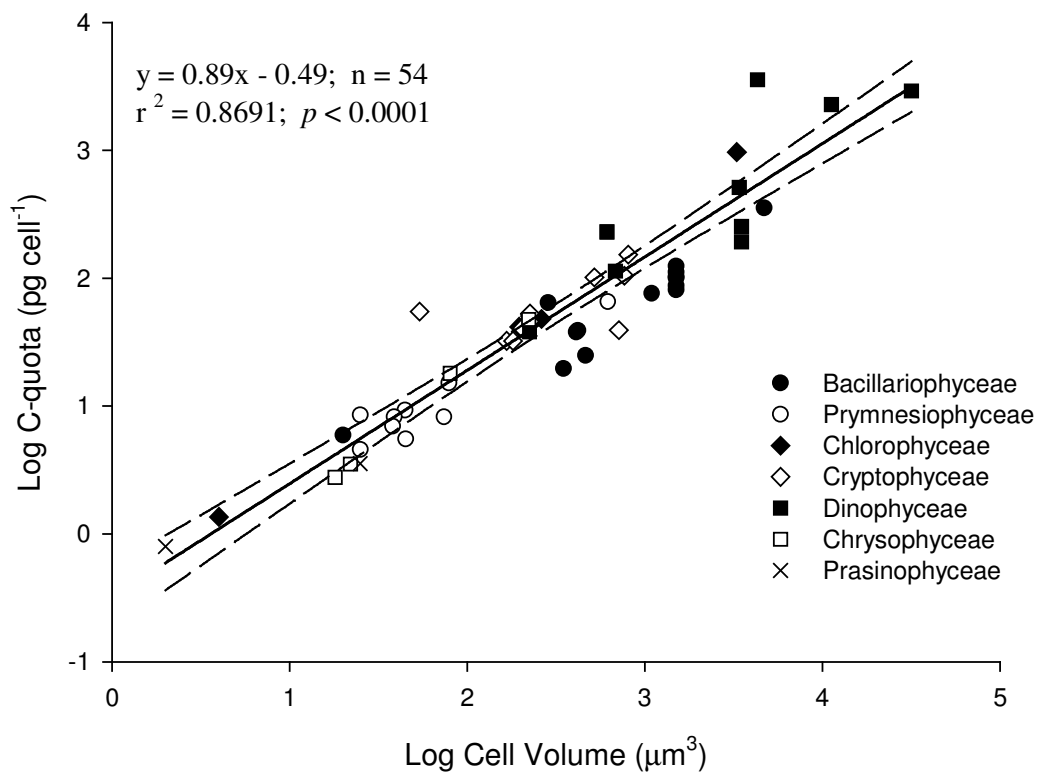
Species	T	Dw	V _o	V _m	Source
<i>Calanopia elliptica</i>	26	56	6.72	0.120	Ikeda et al., 2001
<i>Calanus finmarchicus</i>	0.1	387	7.87	0.020	
<i>C. glacialis</i>	2.3	474	14.78	0.031	
<i>C. hyperboreus</i>	1.3	3950	35.64	0.009	
<i>Cytherissa lacustris</i>	5			0.001	Newrkla, 1985
	10			0.002	
<i>Cytherissa lacustris</i>	13			0.003	
	15			0.003	
	20			0.004	
<i>Eucalanus bungii</i>	6	1010	19.08	0.019	Ikeda et al., 2001
<i>E. subcrassus</i>	24	104	15.72	0.151	
<i>Eucyclops agilis</i>	5			0.120	Parry & Tinson, 1985
	7			0.066	
	10			0.132	
	12.5			0.141	
	15			0.228	
	20			0.140	
<i>Metridia longa</i>	0.1	353	9.74	0.028	Ikeda et al., 2001
<i>Pseudodiaptomus hessei</i>	10	11.8	0.53	0.045	Isla & Perissinotto, 2004
	15	11.8	0.67	0.057	
	20	11.8	1.38	0.117	
	25	11.8	2.36	0.200	
	30	11.8	3.36	0.284	
<i>Tigriopus brevicornis</i>	0			0.018	McAllen et al., 1999
	5			0.114	
	10			0.126	
	15			0.226	
	20			0.385	
	25			0.409	
	30			1.848	
	35			1.170	

APPENDIX 5

Appendix 5.1: Relationship between dry weight and carbohydrate content of zooplanktonic crustacean adults



Appendix 5.1. Relationship between dry weight and total carbohydrate content of zooplanktonic crustacean adults. Solid line through data represents the least-square regression, fitted without shaded data point. Data are from *Rajkumar and Vasagem (2006; *Acartia clausii* 1), Kapiris et al. (1997; *Acartia clausii* 2), Ventura and Catalan (2005; *Diaptomus cyaneus*, *Cyclops abyssorum*, *Daphnia pulicaria*). *Paper contains data on other species but could not be used because dry weights were neither reported nor could be found from other sources.

Appendix 5.2: The relationship between algal size and carbon content

Appendix 5.2a. Log-log relationship between algal size and carbon content. Solid line through data represents the least-square regression and broken lines are the 95% confidence intervals. Data on 33 different phytoplankton species belonging to 7 taxonomic classes that were cultured in diverse degrees of nutrient richness under different conditions of temperature (15-16 °C), photoperiod (10- 14 hours of light), and light intensity (20-90 $\mu\text{mol photon}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) were used in order to ensure that the new parameters could be applied under diverse habitat conditions. See appendix 5.2b for source of data.

Appendix 5.2b. Studies from which data on phytoplankton species were extracted for appendix 1a. GM = growth medium; T = temperature in °C; LI = light intensity in $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; L:D = light:dark cycle in hours; GP represents the growth phase at which cells were harvested where L = logarithmic, S = stationary, LL = late logarithmic, E = early, M = mid; * refers Freshwater diatoms. Blank spaces mean data not reported.

Reference	Phytoplankton		Culture condition				GP	Volume $\mu\text{m}^{-3}\text{cell}^{-1}$	Carbon pg cell ⁻¹
	Class	Species	GM	T	LI	L:D			
Montagnes et al., 1994	Bacillariophyceae	<i>Detonula pumilla</i>	EASW	16	20-60	14:10	M-LL	4697.0	355.2
Arendt et al., 2005	Bacillariophyceae	<i>Thalassiosira weissflogii</i>	B1	15	90		L	1092.6	76.1
Jónasdóttir, 1994	Bacillariophyceae	<i>T. weissflogii</i>	f/2	16		10:10	EL	1500.0	100.5
Jónasdóttir, 1994	Bacillariophyceae	<i>T. weissflogii</i>	f/2	16		10:10	ML	1500.0	111.7
Jónasdóttir, 1994	Bacillariophyceae	<i>T. weissflogii</i>	f/2	16		10:10	LL	1500.0	124.0
Jónasdóttir, 1994	Bacillariophyceae	<i>T. weissflogii</i>	f/2	16		10:10	S	1500.0	102.8
Jónasdóttir, 1994	Bacillariophyceae	<i>T. weissflogii</i>	f/2	16		10:10	EL	1500.0	87.2
Jónasdóttir, 1994	Bacillariophyceae	<i>T. weissflogii</i>	f/2	16		10:10	S	1500.0	80.8
Montagnes et al., 1994	Bacillariophyceae	<i>T. weissflogii</i>	EASW	16	20-60	14:10	M-LL	286.0	64.4
Montagnes et al., 1994	Bacillariophyceae	<i>T. pseudonana</i>	EASW	16	20-60	14:10	M-LL	20.0	5.9
Lynn et al., 2000	Bacillariophyceae	<i>Stephanodiscus minutulus</i> *	normal COMBO	16	80	14:10	S	463.0	24.9
Lynn et al., 2000	Bacillariophyceae	<i>S. minutulus</i> *	Low Si	16	80	14:10	S	421.0	39.0
Lynn et al., 2000	Bacillariophyceae	<i>S. minutulus</i> *	Low N	16	80	14:10	S	349.0	19.7
Lynn et al., 2000	Bacillariophyceae	<i>S. minutulus</i> *	Low-P	16	80	14:10	S	411.0	38.0
Montagnes et al., 1994	Chlorophyceae	<i>Chlamydomonas sp.</i>	EASW	16	20-60	14:10	M-LL	3300.0	969.7
Arendt et al., 2005	Chlorophyceae	<i>Dunaliella tertiolecta</i>	B1	15	90		L	262.1	48.2
Montagnes et al., 1994	Chlorophyceae	<i>D. tertiolecta</i>	EASW	16	20-60	14:10	M-LL	196.0	41.7
Montagnes et al., 1994	Chlorophyceae	<i>Nannochloropsis oculata</i>	EASW	16	20-60	14:10	M-LL	4.0	1.4
Montagnes et al., 1994	Chrysophyceae	<i>Apendinella spinifera</i>	EASW	16	20-60	14:10	M-LL	222.0	47.6
Montagnes et al., 1994	Chrysophyceae	<i>Pelagococcus sp.</i>	EASW	16	20-60	14:10	M-LL	22.0	3.5
Montagnes et al., 1994	Chrysophyceae	<i>Pelagococcus sp.</i>	EASW	16	20-60	14:10	M-LL	18.0	2.8
Montagnes et al., 1994	Chrysophyceae	<i>Pseudopedinella pyiformis</i>	EASW	16	20-60	14:10	M-LL	80.0	18.0
Montagnes et al., 1994	Cryptophyceae	<i>Chroomonas salina</i>	EASW	16	20-60	14:10	M-LL	167.0	32.4
Montagnes et al., 1994	Cryptophyceae	<i>chroomonas sp.</i>	EASW	16	20-60	14:10	M-LL	714.0	39.3
Montagnes et al., 1994	Cryptophyceae	<i>Cryptomonas profunda</i>	EASW	16	20-60	14:10	M-LL	765.0	104.7
Montagnes et al., 1994	Cryptophyceae	<i>C. profunda</i>	EASW	16	20-60	14:10	M-LL	521.0	101.4
Montagnes et al., 1994	Cryptophyceae	<i>Cryptomonas sp.</i>	EASW	16	20-60	14:10	M-LL	807.0	152.8
Montagnes et al., 1994	Cryptophyceae	<i>Pyrenomonas salina</i>	EASW	16	20-60	14:10	M-LL	181.0	32.4

Appendix 5.2b continued

Reference	Phytoplankton		Culture condition				GP	Volume $\mu\text{m}^{-3} \text{ cell}^{-1}$	Carbon pg cell^{-1}
	Class	Species	GM	T	LI	L:D			
Montagnes et al., 1994	Cryptophyceae	<i>Rhodomonas lens</i>	EASW	16	20-60	14:10	M-LL	203.0	40.7
Jónasdóttir, 1994	Cryptophyceae	<i>R. lens</i>	f/2	16		10:10	EL	226.0	50.6
Jónasdóttir, 1994	Cryptophyceae	<i>R. lens</i>	f/2	16		10:10	LL	226.0	43.5
Jónasdóttir, 1994	Cryptophyceae	<i>R. lens</i>	f/2	16		10:10	S	226.0	52.3
Jónasdóttir, 1994	Cryptophyceae	<i>R. lens</i>	f/2	16		10:10	EL	226.0	46.0
Jónasdóttir, 1994	Cryptophyceae	<i>R. lens</i>	f/2	16		10:10	S	226.0	53.0
Broglio et al., 2003	Cryptophyceae	<i>R. salina</i>	L/1 or f/2			12:12		53.9	55.0
Broglio et al., 2003	Dinophyceae	<i>Gymnodinium sanguineum</i>	L/1 or f/2			12:12		4310.3	3560.0
Montagnes et al., 1994	Dinophyceae	<i>G. sanguineum</i>	EASW	16	20-60	14:10	M-LL	31761.0	2913.2
Montagnes et al., 1994	Dinophyceae	<i>G. simplex</i>	EASW	16	20-60	14:10	M-LL	224.0	38.0
Montagnes et al., 1994	Dinophyceae	<i>G. vitiligo</i>	EASW	16	20-60	14:10	M-LL	683.0	113.8
Montagnes et al., 1994	Dinophyceae	<i>Gyrodinium aurealum</i>	EASW	16	20-60	14:10	M-LL	3399.0	513.9
Montagnes et al., 1994	Dinophyceae	<i>G. uncatenum</i>	EASW	16	20-60	14:10	M-LL	11246.0	2275.2
Jónasdóttir, 1994	Dinophyceae	<i>Prorocentrum minimum</i>	f/2	16		10:10	EL	3500.0	253.7
Jónasdóttir, 1994	Dinophyceae	<i>P. minimum</i>	f/2	16		10:10	LL	3500.0	192.6
Montagnes et al., 1994	Prasinophyceae	<i>Mantoniella squamata</i>	EASW	16	20-60	14:10	M-LL	25.0	3.6
Montagnes et al., 1994	Prasinophyceae	<i>Micromonas pusilla</i>	EASW	16	20-60	14:10	M-LL	2.0	0.8
Montagnes et al., 1994	Prymnesiophyceae	<i>Chrysochromulina herdlensis</i>	EASW	16	20-60	14:10	M-LL	74.0	8.2
Montagnes et al., 1994	Prymnesiophyceae	<i>Coccolithus pelagicus</i>	EASW	16	20-60	14:10	M-LL	619.0	65.5
Montagnes et al., 1994	Prymnesiophyceae	<i>Emiliana huxleyi</i>	EASW	16	20-60	14:10	M-LL	25.0	4.6
Montagnes et al., 1994	Prymnesiophyceae	<i>Isochrysis galbana</i>	EASW	16	20-60	14:10	M-LL	38.0	7.0
Arendt et al., 2005	Prymnesiophyceae	<i>Isochrysis sp</i>	B1	15	90		L	38.8	8.2
Montagnes et al., 1994	Prymnesiophyceae	<i>Pavlova lutheri</i>	EASW	16	20-60	14:10	M-LL	25.0	8.6
Arendt et al., 2005	Prymnesiophyceae	<i>Phaeocystis globosa</i>	B1	15	90		L	44.6	9.3
Montagnes et al., 1994	Prymnesiophyceae	<i>P. pouchetii</i>	EASW	16	20-60	14:10	M-LL	45.0	5.5
Montagnes et al., 1994	Prymnesiophyceae	<i>Prymnesium parvum</i>	EASW	16	20-60	14:10	M-LL	79.0	15.1

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