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You eat what you need

food quality and trophic interactions in planktonic food webs

Von der Fakultät für Mathematik und Naturwissenschaften
der Carl - von - Ossietzky - Universität Oldenburg zur
Erlangung eines Doktortitels (Dr. rer. nat.)
angenommene Dissertation von

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Tag der Disputation: *13.06.2012*

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Chapter 1: General introduction

Ecological stoichiometry

The concept of stoichiometry was first developed in the field of chemistry and corresponds to the calculation of the quantitative relations between reactants and products during a chemical reaction. In Richter's (1792) words stoichiometry is the *"art of chemical measurements, which has to deal with the laws according to which substances unite to form chemical compounds"*. Chemical stoichiometry involves a complex of limitations controlling the interactions between chemical elements. Also in biology, there are boundaries to the assemblages of chemical elements, especially as they have to function as a living cell, and these constraints to interacting biological systems are described and integrated by ecological stoichiometry. To function and grow all living cells need inputs of elements and nutrients that are amalgamated into cellular components. Autotrophs take up their energy and materials from different sources (solar light and uptake of inorganic nutrients) and, since the supply of light and inorganic elements is not coupled in the environment, they have developed strategies to obtain and store these resources whenever and wherever they are made available (Mohr and Schopfer 1994). As a result, autotrophs' elemental composition varies substantially among ecosystems due to the relative balance of key resources such as CO_2, solar energy and mineral nutrients in the environment (Sterner and Elser 2002). This variability in biochemical composition affects the quality of primary producers as food for herbivores which, as a result, are often eating prey not matching their requirements, as they typically take up food in packages and not as single nutrients. This can have substantial implications for herbivores performance and subsequent trophic dynamics (Urabe et al. 1997; Elser et al. 2000; Pimentel-Rodrigues and Oliva-Teles 2001; Frost and Elser 2002) and reproduction of the consumer (Gulati and DeMott 1997). Hence, grazers needed to acquire ways to deal with these nutrient imbalances of their prey, one of which is homeostatic regulation. Kooijman (1995) described stoichiometric homeostasis as *"The ability of organisms to keep the chemical composition of their body constant, despite changes in the chemical composition of the environment, including their food"*. Maintaining stoichiometric homeostasis probably comes with costs, but must generally provides benefits higher than these costs for it to have evolved. Although

homeostasis dampens the effect of low food quality on general fitness of metazoans and allows them to work efficiently in a broad range of food qualities, there is still considerable debate under which circumstances homeostasis is the best strategy (Persson et al. 2010). For instance, Droop (1973) recorded that protists replenished their nutrient stores after a period of nutrient limitation by raising the uptake of the previously limiting nutrient once it becomes available again, but also that feeding went beyond this simple replenishment. Luxury consumption results in considerable flexibility in body stoichiometry and allows these organisms to store a particular element in order to be prepared for future nutrient limitation. Not surprisingly, different degrees of homeostasis can be observed. Unlike planktonic metazoans, which have close to strict homeostasis (Andersen and Hessen 1991; DeMott and Pape 2005), protozoans have a weak homeostasis and lie, in terms of homeostatic ability, between autotrophs and metazoans (Grover and Chrzanowski 2006; Hantzsche and Boersma 2010). Moreover, as stated by Sterner and Elser (2002) *"Without homeostasis, ecological stoichiometry would be a dull subject"*, stoichiometric homeostasis must therefore be defined very precisely. Indeed, the critical evaluation of stoichiometric homeostasis contributes to a better understanding of food webs, which are generally driven by elemental imbalances between consumers and their resources (Persson et al. 2010).

Stoichiometric homeostasis

Sterner and Elser (2002) described graphical as well as mathematical ways to analyze different levels of homeostatic regulation. They explain that on a plot of consumer versus resource stoichiometry, homeostatic regulation of nutrient content can be diagnosed as a slope lower than the slope from a constant proportional response (Fig 1.1). They developed a conceptual model to interpret the homeostatic strength of an organism using the homeostasis coefficient H (eta):

$$H = \frac{log_{10}(x)}{log_{10}(y) - log_{10}(c)},$$

where *x* is the resource nutrient stoichiometry, *y* is the organism's nutrient stoichiometry and *c* is a constant. H is a regulation coefficient greater than 1 and, as H approaches infinity, the slope of consumer versus resource stoichiometry approaches zero indicating strict homeostasis. Although this tool has not been extensively used, it allowed identifying

important patterns enhancing our understanding of a species' or taxon's role in population dynamics, food webs, and nutrient cycles. For instance, aquatic macro-invertebrates seem to be significantly more homeostatic than terrestrial ones and heterotrophs are significantly more homeostatic than autotrophs (Persson et al. 2010).

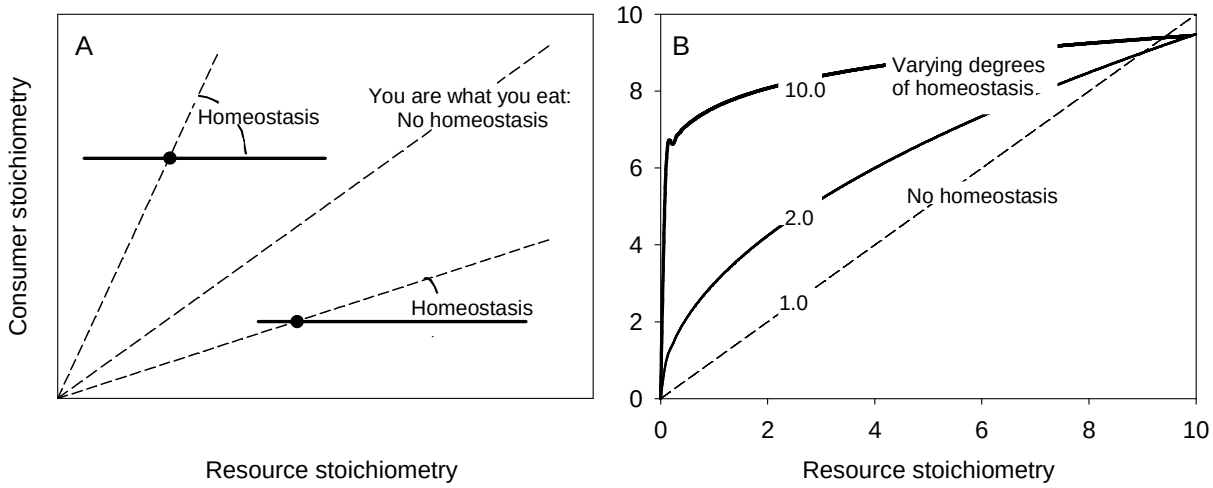

Fig 1.1 Generalized stoichiometric patterns relating consumer stoichiometry to resource stoichiometry (Sterner and Elser 2002, modified). (A) Horizontal and vertical axes are any single stoichiometric measure, such as N:P ratio. Elser and Sterner (2002) defined homeostasis graphically as a slope between 0 and y/x. (B) Degrees of homeostatic regulation based on Sterner and Elser's model (H=1, 2 and 10).

However, the homeostasis coefficient does not distinguish between strict homeostasis, when consumer stoichiometry is tightly constrained in spite of wide variation in resource stoichiometry, and cases where consumer stoichiometry is highly variable yet independent of resource stoichiometry. This parameter also simplifies the intrinsic physiology and biochemistry of homeostasis and this may mislead our interpretation of a species role in population dynamics, food webs, and nutrient cycles (Persson et al. 2010). Interestingly, this approach is also very different from the rest of the huge body of literature existing about regulation of an organism's internal state such as temperature or osmotic regulation (homeostasis in general).

In the nineteenth century, Claude Bernard showed the importance of homeostasis in animal physiology when he reported the ability of mammals to regulate the condition of their internal environment within rather narrow limits (Bernard 1865). Physiologists typically cluster organisms in two categories, conformers and regulators (Péqueux 1995). On the one hand, regulators are able to maintain their internal environment at a constant level over possibly wide ambient environmental variations. On the other hand, conformers allow the environment to determine their internal condition. For instance, endothermic animals (mammals and birds) keep a constant body temperature, while ectothermic animals (almost all other organisms) exhibit wide body temperature variation (Eckert 1978). The main difference between Sterner and Elser's approach and the one used by physiologists lies in the graphical interpretation of organisms' response to environmental conditions.

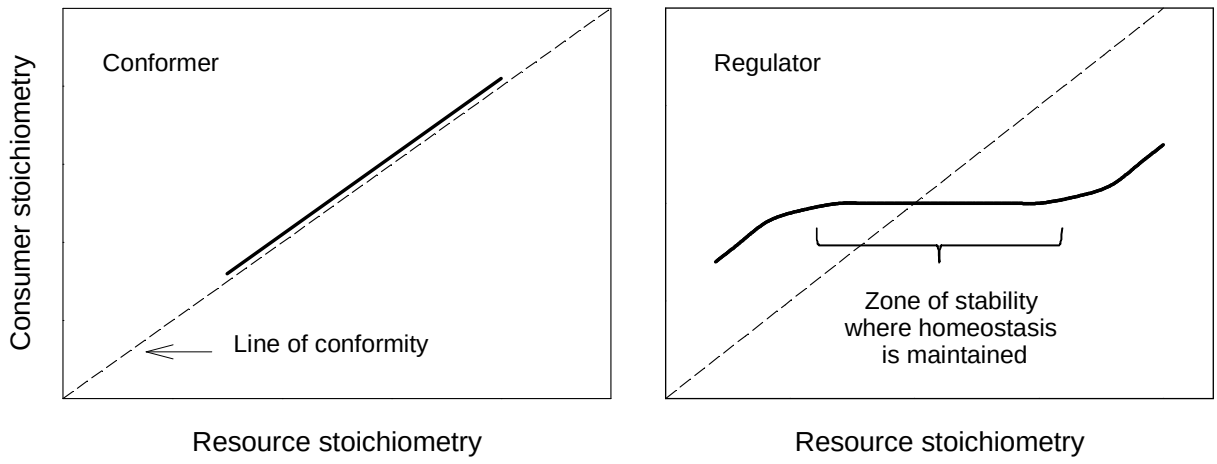

Fig 1.2 Generalized stoichiometric patterns relating consumer stoichiometry to resource stoichiometry (Eckert and Randall 1978, modified). While the stoichiometry of conformers is determined by the stoichiometry of their resource, regulators maintain their stoichiometry for a certain range of resource stoichiometry. The homeostasis strength of an organism is defined by the magnitude of the range of resource stoichiometry over which the stoichiometry of the consumer remains stable. As already observed for osmo- and thermoregulation (Eckert and Randall 1978), we expect breaking points on each side of the plateau after which the organism cannot regulate anymore.

While the ecological stoichiometric view concentrates only on the slope of consumer versus resource stoichiometry, physiologists consider two distinct parameters. The first one is the homeostatic strength and can be characterized as the range of environmental conditions

over which an organism maintains its internal environment constant. The second one is the homeostatic capacity and can be defined as the slope of consumer versus resource stoichiometry. As in ecological stoichiometry, the closer the slope is to 0, the stronger the homeostatic capacity is. Moreover, Sterner and Elser's model does not consider the breaking points at which an organism cannot keep its stoichiometry stable anymore and the subsequent (sub)lethal points. Hence, we suggest that a new approach of stoichiometric homeostasis, more representative of the underlying physiological mechanisms, is needed. This concept is inspired by the way physiologists define homeostasis and can be described graphically (Fig 1.2). Elemental homeostasis and the homeostasis strength of an organism is the range of variations in food stoichiometry over which the consumer stoichiometry parameter is kept constant.

Stoichiometry dynamics

While both limnologists and marine biologists intensively applied and investigated the principles of ecological stoichiometry for mesozooplankton, microzooplankton received less attention, although it is an essential component in planktonic ecosystems. Indeed, protists are often the major predators in microbial food webs (Sherr and Sherr 2002; Landry and Calbet 2004), and microzooplankters, by consuming pico-, nano-, and microplankton and being consumed by grazers of higher trophic levels such as copepods, act as a trophic link (Johansson et al. 2004; Sommer et al. 2005). Classically, models of trophic interactions focused on direct transfer of carbon from phytoplankton to mesozooplankton (Cushing 1989), but we know now that this simplification is incorrect since microzooplankton can contribute substantially to mesozooplankters' diets (Kleppel 1993). In fact, many mesozooplankters select actively for microzooplanktonic prey (Wiadnyana and Rassoulzadegan 1989; Gasparini et al. 2000; Löder et al. 2011). Although these observations were initially mainly attributed to prey size and motility (Levinsen et al. 2000), trophic upgrading exerted by protists, both in terms of nutrients (Malzahn et al. 2010) as well as in terms of biochemistry (Klein et al. 1986), might result in a higher quality of microzooplankton as food for metazoans relative to the algae that they consumed. Several studies have shown that protozoans regulate their elemental body composition (Hantzsche and Boersma 2010; Malzahn et al. 2010). Yet, Grover and Chrzanowski (2006) hypothesized that protozoans lie, in term of homeostasis ability, between metazoans and autotrophs. However, little is known about

protozoans' ecological stoichiometry and we know nothing about the rate with which homeostatic regulation processes take place, as previous work was always in a static context. Hence, the second chapter of this thesis focuses on the impact of food quality and quantity on the stoichiometric dynamics of a dinoflagellate species, *Oxyrrhis marina*. Since microzooplankters are fast growing organisms and have a relatively short life span they need to react quickly to environmental changes. I hypothesized that the processes involved in stoichiometric regulation are rapid and sensitive to food nutrient composition.

Stoichiometric regulations

Stoichiometric regulations involve several pre- (Kleppel 1993) and post-ingestion (Cowie and Hedges 1996; Mitra and Flynn 2005; Mayor et al. 2011) mechanisms allowing grazers to handle nutrient imbalances. On the one hand, the consumer may lower the ingestion rate of unfavourable food to use more time for extracting the limiting element efficiently or increase its food uptake to shorten the handling time and extract only the easily available parts of the limiting nutrient. On the other hand, consumer might show selective feeding behaviour. Studies on predators' selective grazing behaviour have substantially contributed to our understanding of food webs. Both metazoans and protozoans are often faced with a large variety of potential food items of different qualities. Prey size (Andersson et al. 1986; Paffenhöfer 1988; Chrzanowski and Šimek 1990; Jakobsen and Hansen 1997) and prey motility (Gonzalez et al. 1993; Jakobsen et al. 2005; Jakobsen et al. 2006) are some of the factors which may affect food uptake. Food quality, however, is not only determined by the physical properties of the prey, but also by its elemental and biochemical composition. Since selective feeding is the basic mechanism to ensure a balanced diet when facing an imbalanced food supply (Anderson and Pond 2000), and to prevent an organism from being intoxicated by toxic food (Huntley et al. 1986; DeMott and Moxter 1991), it is a feature of utmost importance for any heterotroph (MacArthur and Pianka 1966). Nevertheless, only little is known about the ability of planktonic grazers to select for food quality (Paffenhöfer and Van Sant 1985; Irigoien et al. 2005). Hence, the third chapter assesses different feeding mechanisms, selective and compensatory feeding, used by a protozoan species, *O. marina*. In the fourth chapter, I studied the stoichiometric fluctuations occurring during copepod ontogeny and their consequences for the feeding strategy of different copepod life stages. I

hypothesized that grazers affect nutrients cycling not only by the excretion of abundant elements but also by the selective removal of scarce ones.

Stoichiometry, motility and trophic interactions

As I already stated, besides food quality of the prey, several parameters affect trophic interactions. For instance, motility increases encounter rates between predators and prey (Visser and Kiørboe 2006). Motility is the basic mechanism allowing consumers to actively search and move towards patches of prey and capture food items, while it allows prey to escape or reduce predation pressure (Buskey 1997; Tillmann and Reckermann 2002; Matz and Jürgens 2005). Besides protozoan grazers, numerous phytoplankton species are also motile and thus able to move towards light or nutrients (e.g. Eppley et al. 1968; Cullen and Horrigan 1981; MacIntyre et al. 1997), but the role of algal motility in predator-prey interactions has received less attention. Further, the impact of nutrient limitation on organisms' motility has not been yet investigated. Hence, the aim of the fifth chapter of the thesis is to investigate the impact of nutrient status on swimming speed of predators and prey as well as the effect of prey motility on trophic interactions.

The main goal of my thesis is to investigate pre-ingestion mechanisms zooplankters use to regulate their stoichiometry. I tested the ability of various herbivorous zooplankton species to select for food quality exemplified by nutrient content. This information is crucial to understand how zooplankton responds to differences in phytoplankton quality and affects nutrient cycles. I also studied the dynamic of stoichiometric changes in plankters in response to food quality and quantity fluctuations. Finally, I investigated the effect of resource quality on the motility of phyto- and zooplankton and the consequences for trophic interactions. The results presented in this thesis enable to mechanistically understand the importance of nutrient supply rates and ratios on trophic interactions in general and to predict consequences of altered marine biogeochemistry on coastal food webs.

Chapter 2

Dynamic stoichiometric response to food quality fluctuations in the heterotrophic dinoflagellate *Oxyrrhis marina*

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Meunier CL[1], Haafke J[1], Oppermann B[1], Schnell S[1], Boersma M[1], Malzahn AM[1,2]: Dynamic stoichiometric response to food quality fluctuations in the heterotrophic dinoflagellate *Oxyrrhis marina.* Marine Biology in review

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2.1 Abstract

With respect to nutrients, plants are rather non-homeostatic while most metazoans have much more confined ranges of nutrient ratios. It was recently highlighted that the homeostatic ability of microzooplankters is in between these two extremes. Nevertheless, we know very little on the dynamics of stoichiometric changes. Hence, we investigated how the stoichiometry of the heterotrophic dinoflagellate *Oxyrrhis marina* is affected 1) during a starvation period and 2) when fed nutrient deplete *Rhodomonas salina* after having been pre-conditioned on nutrient replete algae and *vice versa*. We observed that the dinoflagellate was able to maintain a constant relaxed homeostasis for 78h of starvation. We identified that under starvation nitrogen limited *O. marina* mainly used fat as energy source while nitrogen rich individuals also used proteins as fuel in cellular respiration. Further, we showed that *O. marina* presents resistance to nutrient limitation, with stronger regulation against P-limitation as against N-limitation. General high resilience in microzooplankton stoichiometry after food quality stress would have great implications for both top-down (nutrient remineralisation) and bottom-up controls (quality as food).

2.2 Introduction

Homeostasis is the ability of an organism to regulate its internal milieu, thus maintaining constant internal conditions. The main advantage of homeostatic regulation is that it allows organisms to work efficiently in a wide range of environmental conditions. Stoichiometric or elemental homeostasis refers to the internal elemental composition (e.g. C, N and P) of organisms. Whereas phytoplankton typically exhibits a wide range of plasticity in elemental composition and thus exhibits relatively weak stoichiometric homeostasis (Hillebrand and Sommer 1999a; Quigg et al. 2003; Klausmeier et al. 2004), planktonic metazoans have close to strict homeostasis (Andersen and Hessen 1991; DeMott and Pape 2005). Thus, there are boundaries in elemental body stoichiometry between which organisms can fluctuate, and these are broad for planktonic autotrophs and narrower for many metazoans. Maintaining stoichiometric homeostasis probably comes with costs, but must provide benefits higher than these costs for it to have evolved. Homeostasis buffers the impact of bad food quality on general fitness of metazoans and allows them to work efficiently in a broad range of food qualities, but there is still considerable debate under which circumstances homeostasis is the best strategy (Persson et al. 2010). For example, Droop (1973) observed that after a period of nutrient limitation, protozoans increased uptake of the previously limiting nutrient once it became available again. This of course, replenished the nutrient stores, but feeding went beyond that. Droop argued that this luxury consumption, and thus considerable flexibility in body stoichiometry, allows protists to store a particular element in order to be prepared for future nutrient limitation.

Microzooplankton is an essential component in planktonic ecosystems. Indeed, it often comprises the major predatory group in microbial food webs (Sherr and Sherr 2002; Landry and Calbet 2004), and microzooplankters form a trophic link between pico-, nano-, and microplankton on the one hand and higher trophic levels such as copepods on the other hand (Johansson et al. 2004; Sommer et al. 2005). Traditional food-web models mainly focused on direct transfer of carbon from phytoplankton to mesozooplankton (Cushing 1989), but we know now that microzooplankton can be an important part of the diet of mesozooplankters (Kleppel 1993). In fact, many mesozooplankters actually prefer microzooplanktonic prey (Wiadnyana and Rassoulzadegan 1989; Gasparini et al. 2000; Löder et al. 2011). Although this has mainly been attributed to the size and motility of the prey (Levinsen et al. 2000),

differences in food quality through trophic upgrading both in terms of nutrients (Malzahn et al. 2010) as well as in terms of biochemistry (Klein et al. 1986) might also play a role. Further, protists' excretions of nitrogen and phosphorus compounds, and also of trace metals such as iron, are a major source of regenerated nutrients in aquatic systems (Sherr and Sherr 2002). Here, we focus on the elemental stoichiometry of microzooplankton and investigate the dynamic of stoichiometric fluctuations in response to food quality and quantity and discuss their implications for nutrient remineralisation as well as protists' quality as food for mesozooplankters.

Several studies have shown that *Oxyrrhis marina*'s elemental stoichiometry is significantly different from the nutrient ratios of the ingested food (Hantzsche and Boersma 2010; Malzahn et al. 2010), and that indeed *O. marina* regulates its body composition. Yet, Grover and Chrzanowski (2006) hypothesized that protozoans lie, in term of homeostasis ability, between metazoans and autotrophs. However, little is known about protozoans' ecological stoichiometry and we know nothing about the speed with which homeostatic regulation processes take place, as previous work was always in a static context. Hence, since it is reasonable to suspect low-prey conditions over short-term time frames in certain marine habitats (e.g. within rock pools or microscale aggregations of *O. marina*, Martel 2010), here we investigated the impact of starvation on *O. marina*'s biochemical composition and energetic metabolism, and followed body composition of *O. marina* previously fed with nitrogen and phosphorus limited *Rhodomonas salina* during a starvation period. Further, in order to identify how fast *O. marina* recovers after a limitation period and how fast it gets nutrient-limited, we offered several concentrations of phosphorus and nitrogen limited *R. salina* to differently pre-conditioned *O. marina* and measured how food quality and quantity impact the protozoan's stoichiometry.

2.3 Material and Methods

Experimental cultures

The dinoflagellate *Oxyrrhis marina* is the most commonly used marine protozoan grazer in laboratory experiments and, as it is a filter feeder as well as a raptorial grazer (Jeong et al. 2008), it is representative of many planktonic protists. Furthermore, it is relatively simple to culture, has a broad distribution, and tolerance to a wide range of environmental conditions (Lowe et al. 2010).

Oxyrrhis marina Dujardin was obtained from the Göttingen culture collection (Strain B21.89) and fed on *Rhodomonas salina* (Wislouch) Hill et Wetherbee (Cryptophyceae) grown in batch cultures at 18.5°C in an 18:6h light : dark cycle using sterile filtered F/2 medium (Guillard and Ryther 1962). *R. salina* and *O. marina* were cultured following Meunier et al. (2012). For the different limitations, one set of *R. salina* was grown three days in F/2 medium without phosphorus and the other set in F/2 medium without nitrogen. This treatment resulted in significant differences in algal stoichiometry in the direction expected from the nutrient conditions of the media (Tab 2.1).

Tab 2.1 Stoichiometric measures C:N, C:P and N:P of nitrogen depleted and phosphorus depleted *Rhodomonas salina* given as food to *Oxyrrhis marina* during the experiments. All differences between treatments were significant at $p<0.05$ (Tukey's honest significant difference test) after a One-Way ANOVA, n=4 replicates for each treatment.

	C:N mean (±Sd)	C:P mean (±Sd)	N:P mean (±Sd)
Nitrogen depleted	14.1 (0.9)	327.1 (104.7)	23.1 (7.0)
Phosphorus depleted	7.9 (0.4)	970.2 (158.1)	140.5 (19.6)

Cell density and mean biovolume of *O. marina* and *R. salina* cultures were determined using a CASY particle counter (SCHÄRFE SYSTEMS, Reutlingen, Germany). Prior to the

experiments we starved the *O. marina* culture for one week in order to eradicate any effects of pre-culture conditions. This culture was split into two cultures, one culture was fed nitrogen depleted and the other one phosphorus depleted *R. salina* for four days. Food pulses were given every 24h. Since there is no way to separate *O. marina* from *R. salina*, we adjusted the quantity of *R. salina* at the last preconditioning day in such a way that all algal cells had been consumed at the start of the experiments.

Starvation experiment

As we were interested in short-term responses that are ecologically relevant (Kimmance et al. 2006; Martel 2010), and wanted to avoid potential grazing on bacteria (Schumann et al. 1994; Jeong et al. 2008) or cannibalism (Flynn and Davidson 1993) in our cultures, the starvation experiment focused on the first three days of starvation. In this short-term starvation experiment, we investigated changes in body stoichiometry of *O. marina* in order to assess the level of homeostasis under the probably most stressful of feeding environments. During the first 48h, we also measured respiration rates to infer, which energy source (carbohydrates, protein or fat) was predominantly used during starvation. At the end of the preconditioning period, the two *O. marina* pre-cultures, one with high P and low N ("Oxy_NP") and the other one with high N and low P ("$OxyN_P$"), were used to create four 1L replicates, the cell concentration of which was adjusted to 20,000 cell mL^{-1}. These experimental cultures were starved for 78h, during which we measured a number of parameters. We followed cell densities, and measured oxygen consumption during starvation at 0h, 1h, 2h, 3h, 6h, 12h, 24h and 48h. At the same time, we measured the body stoichiometry (C:N:P) and the total lipid content of *O. marina*. The dinoflagellate's C:N, C:P and N:P; C, N and P fluctuations; density and respiration rate variations were analyzed using repeated measures ANOVA, using precondition treatment as the independent factor and nutrient ratio, nutrient content, cell density or oxygen consumption as the dependent variables. Tukey's HSD test was used as posthoc test in all cases.

The oxygen consumption was measured using Oxygen Micro-optods (Needle-Type PSt1, PreSens) according to Gatti (2002). Aliquots of the cultures were placed in gas tight respiration chambers (Hamilton-injection T II without stop). Control measurements without *O. marina* were performed in order to correct the results for microbial respiration which was

low at all times. The respiration chambers were placed in a darkened and temperature controlled (18.5°C) water bath (cold circulating pump FP40, Julabo), and incubated for 10 minutes.

O. marina C, N and P measurements were carried out by filtering an estimated amount of 200 µg carbon onto precombusted Whatman GF/F filters. The particulate carbon and nitrogen content of *R. salina* and *O. marina* were measured with a Vario Micro Cube elemental analyzer (Elementar). Particulate phosphorus was analyzed as orthophosphate after acidic oxidative hydrolysis with 5% H_2S (Grasshoff et al. 1999). In order to investigate whether differently preconditioned *O. marina* use varying energy sources, total lipid content was determined as described by Urzúa and Anger (2011) and was analyzed using 2-way ANOVA with *O. marina* precondition and time as independent variables and lipid content as dependent variables. Tukey's HSD test was used as posthoc test.

Food change experiment

The aim of this experiment was to investigate how fast *O. marina* changes from one limitation to another limitation, by switching animals preconditioned on nitrogen limited algae to phosphorus limited ones and *vice versa*. Thus, N- and P-deplete *O. marina* were fed *R. salina* reared in phosphorus depleted medium and in nitrogen depleted medium respectively in six different concentrations in quadruplicates. As a control, we also supplied a food pulse without food quality change to the preconditioned *O. marina*. In order to measure *O. marina* N:P, we estimated the time when all algal cells were eaten by CASY counting and waited at least 2h more, to allow *O. marina* to digest the ingested food (Gaines and Taylor 1984; Klein et al. 1986) before sampling. Based on the results of the starvation experiment we knew that *O. marina* C:N:P remains stable for at least 3h of starvation, thus a 2 hours wait after food depletion was a good compromise.

2.4 Results

Starvation experiment

During the starvation experiment we followed changes in cell density and stoichiometry of *O. marina* over a 78 hours starvation period. The cell densities remained stable around 21,000 cell mL^{-1} in the first three hours, increased in the next nine hours to reach 31,000 cells mL^{-1} for N-deplete *O. marina* and reaching the same density for P-deplete *O. marina* after 24 hours (Fig 2.1). The respiration (oxygen consumption) was not significantly different between the preconditioning, although there was a marginally significant (p=0.06) tendency for nitrogen limited *O. marina* to show higher respiration rates per cell (Fig 2.1). Especially between 3 and 12 hours of starvation, the respiration rate of the N-limited organisms was higher, exactly in the period when these organisms were dividing, leading to a significant interaction term between treatment and time (p=0.02). The oxygen consumption remained stable around 0.05 $\mu gO_2\ \mu gC^{-1}\ h^{-1}$ in the first 4 hours, then increased until 12h to reach 0.07 for P-limited *O. marina* and 0.11 $\mu gO_2\ \mu gC^{-1}\ h^{-1}$ for N-limited *O. marina*, and finally decreased to 0.04 and 0.02 $\mu gO_2\ \mu gC^{-1}\ h^{-1}$ for N- and P-limited *O. marina*.

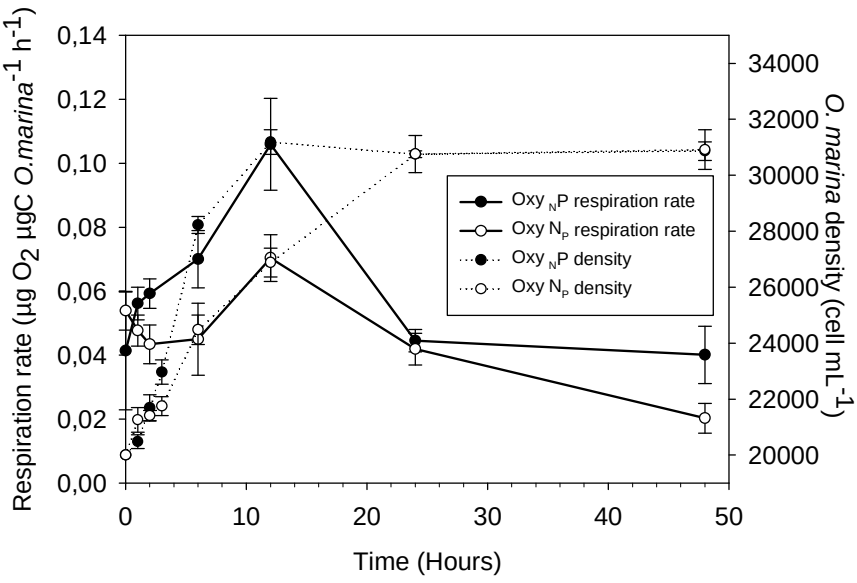

Fig 2.1 Oxygen consumption (left Y axis) and cell density (right Y axis) during starvation experiment of *O. marina* previously fed with nitrogen depleted (Oxy_NP; black dots) and phosphorus depleted ($OxyN_P$; white dots) *Rhodomonas salina*. n=4 replicates for each value. For clarity reasons bars represent standard errors.

To measure whether differently preconditioned *O. marina* used varying biomolecules as energy source, we measured total lipid content at the beginning and after 24h of starvation. We observed that N-limited *O. marina* total lipid concentration decreased (p=0.02) during starvation (Fig 2.2) while it remained stable for P-limited *O. marina* (p=0.42).

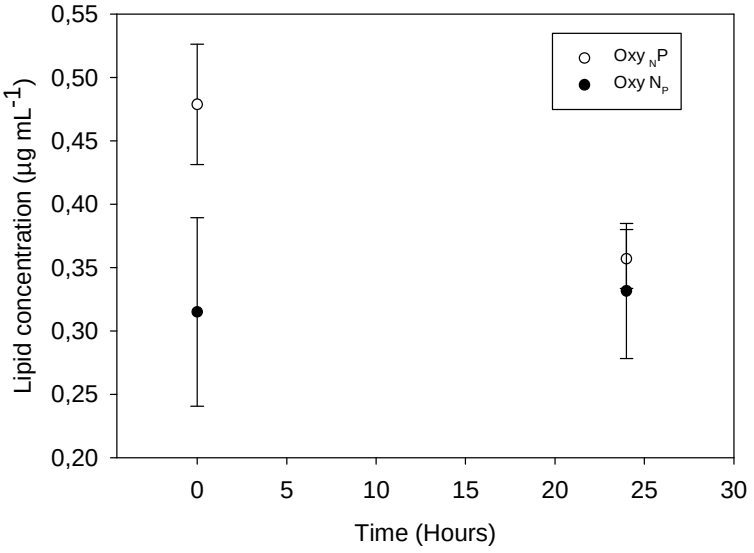

Fig 2.2 Lipid concentration in *O. marina* P-limited (Oxy N_P, white circles) and N-limited (Oxy $_NP$, black circles) at 0h and 24h in the starvation experiment. Values are significantly different (p=0.02) for N-limited cells while there is no difference for P-limited ones (p=0.42).

Both preconditioned *O. marina* cultures showed significant decreases (p<0.001) in the particular carbon content per mL (Fig 2.3A) indicating that *O. marina* was truly starving and if feeding on e.g. bacteria this did not represent a significant carbon supply. The concentration of particular N in the N-rich *O. marina* (P-limited) decreased consistently (p<0.001) to reach the same level as the low N organisms after 78 hours (Fig 2.3B). This suggests that in the high N treatments, *O. marina* used a considerable part of its proteins for energy, whereas this was not the case for the low N *O. marina*, where particulate organic nitrogen was obviously lower, and did not show a consistent pattern over time, but was more or less constant. Phosphorus concentrations were much higher in the high P *O. marina* than in the low P treatments (p<0.001). Surprisingly, the P content of low P *O. marina* started to decrease after 24h while it remained stable for high P *O. marina* (Fig 2.3C).

Both N- and P-limited *O. marina* exhibited similar trends for carbon (p=0.23) on a per-cell basis; stable during the first 4 hours at 77 pmolC *O. marina*$^{-1}$, the carbon cell content then decreased until 78h to reach values around 32 pmolC *O. marina*$^{-1}$ (Fig 2.3D). The nitrogen

content of P-limited cells (Fig 2.3E) dropped from 11 and stabilized at 5.9 pmolN *O. marina*$^{-1}$ at 24h. The nitrogen content of N-limited cells (Fig 2.3E) remained stable the first two hours at 8.5 pmolN *O. marina*$^{-1}$, decreased to 5.9 pmolN *O. marina*$^{-1}$ at 12h and remained stable until the end. The phosphorus content of both N- and P-limited *O. marina* (Fig 2.3F) reacted in a similar way as the nitrogen content with initial contents of 0.39 and 0.23 pmolP *O. marina*$^{-1}$ for N- and P-limited *O. marina* respectively, and final of 0.29 and 0.08 pmolP *O. marina*$^{-1}$.

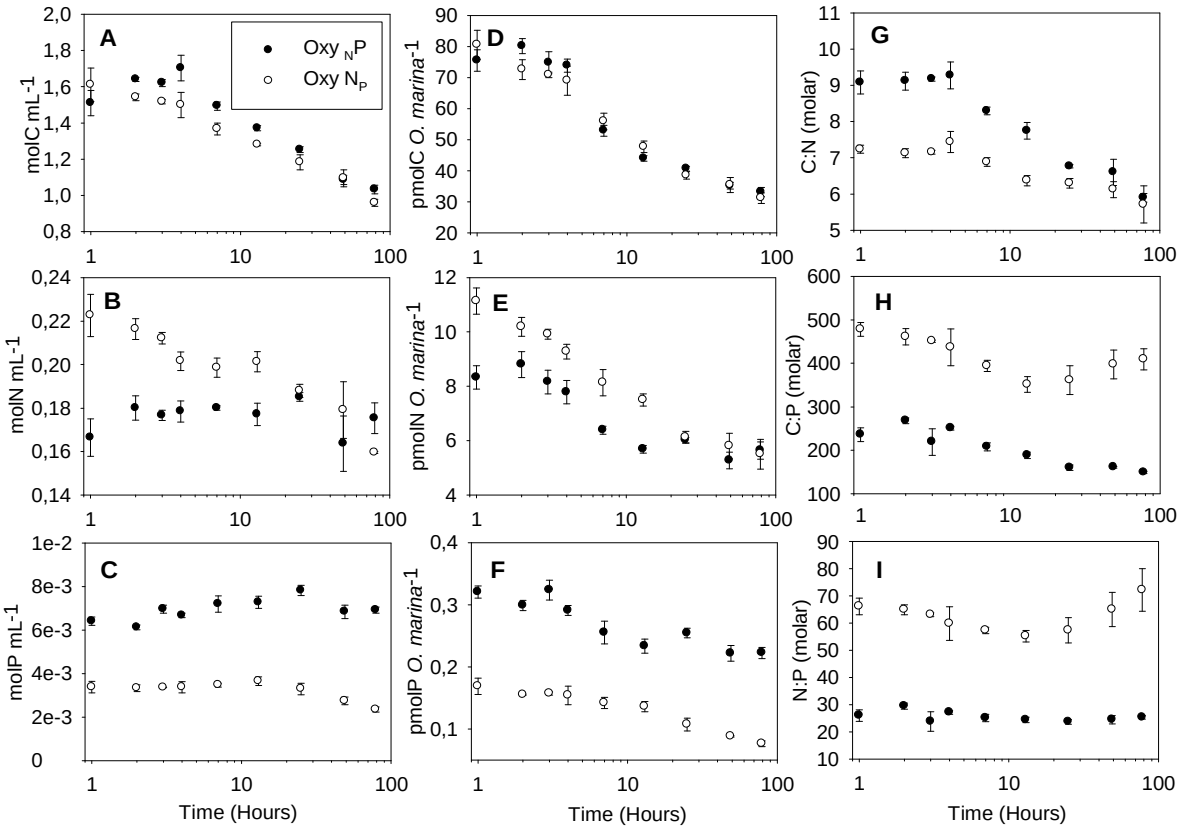

Fig 2.3 Elemental Carbon, Nitrogen and Phosphorus per mL (A, B and C) and per *O. marina* cell (D, E and F) and stoichiometric C:N, C:P and N:P (G, H and I) fluctuations during starvation (mean+Sd) of *O. marina* previously fed with nitrogen depleted (Oxy $_N$P; black dots) and phosphorus depleted (Oxy N$_P$; white dots) *R. salina*. n=4 replicates for each value. For plot requirement the X-axis is shifted of one hour, thus t0h appears as t1h on the graph.

The stoichiometry of both N- and P-limited *O. marina* followed similar trends during the starvation process (Fig 2.3). C:N (Fig 2.3G) and C:P (Fig 2.3H) remained stable for at least 5h

at values around 9 and 250 for N-limited cells and 7.2 and 425 for P-limited ones. This stationary phase was followed by a decrease and nutrient ratios stabilized between 12h and 24h at values around 6.5 for C:N of both *O. marina* preconditions; and 340 for C:P of P-limited cells and 150 for N-limited ones. The N:P of P-limited *O. marina* decreased in the first 12h (Fig 2.3I) due to decrease in N content (Fig 2.3B) and increased afterwards due to decrease in P content (Fig 2.3C). Both *O. marina* preconditions were always significantly different in terms of C:N, C:P and N:P ($p<0.001$) and these nutrient ratios varied significantly over time ($p<0.001$).

Food change experiment

During the food change experiment we measured the dynamic response in *O. marina* stoichiometry to changes in food quality. P-limited *O. marina* exhibited a strong response (N:P decreased almost by a factor of 2) to the addition of the lowest quantity of N-limited *R. salina* (Fig 2.4) thus recovering rapidly from the P-limitation.

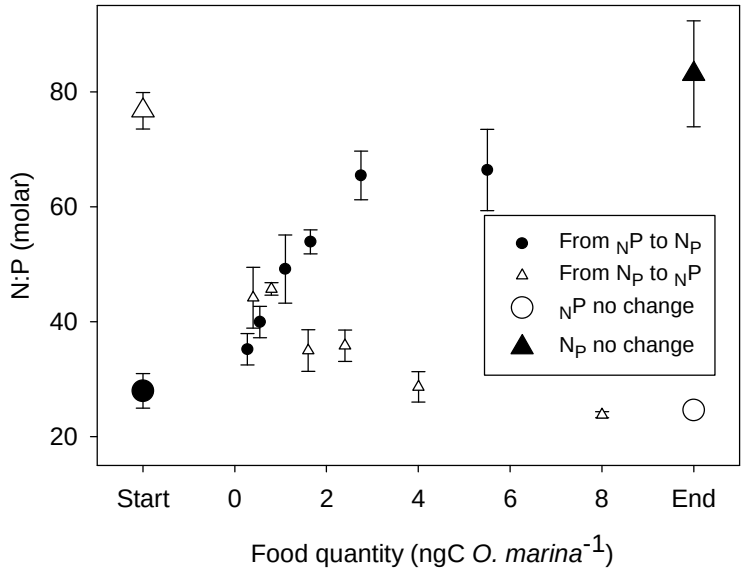

Fig 2.4 Stoichiometric measures after food change, new food was given in different quantities (X axis), N:P (mean+Sd) of *O. marina* previously fed with nitrogen depleted and fed with a pulse of phosphorus depleted (From $_N$P to N_P; black circles) and previously fed with phosphorus depleted and fed with a pulse of nitrogen depleted (From N_P to $_N$P; white triangles) *R. salina*. For control, we gave to each pre-conditioned *O. marina* a food pulse using the same algal quality on which they were prefed ($_N$P; white circle and N_P; black triangle). n=4 replicates for each value.

The N:P ratio of P-limited cells remained stable around 40 (typical ratio for nutrient replete *O. marina* cells) for intermediate food quantities (about 0.8 ngC *O. marina*$^{-1}$) and decreased progressively with increasing food quantities. Hence, *O. marina* showed a two step response when going from a P- to a N-limitation. At first, it recovered rapidly and reached its optimal N:P ratio with very low food quantities. Secondly, its N:P decreased slowly and progressively towards a N-limited N:P ratio of 25 with increasing quantities of N-limited food.

Unlike P-limited cells, N-limited ones displayed only a weak response for low food quantities. Their N:P ratio started to increase slowly and the rate of increase accelerated with higher food quantities until reaching a P-limited N:P ratio of 68 which was lower than the control. These observations are coherent and highlight a strong resistance and resilience to P-limitation.

2.5 Discussion

In this paper we investigated the processes that play a role in the stoichiometric regulation of macronutrients in *O. marina*. The body stoichiometry of *O. marina* is fairly flexible (see also Hantzsche and Boersma 2010), indicating that *O. marina* regulates its internal environment but is unable to maintain it stable and constant. As stated above, we knew very little still on the dynamics of these changes, a gap we wanted to close with the present study. In short, the response is rapid, especially when feeding. In most cases feeding on a different food source will immediately lead to changes in the body stoichiometry.

Starvation experiment

Total carbon in the experimental vessels started to decrease after three hours of starvation, and carbon loss rates were very similar in both preconditions. This shows that *O. marina* is able to withstand short phases of starvation without any problem or effect on body composition. Additionally P-limited *O. marina* lost a considerable amount of nitrogen from the particulate phase and, unexpectedly, they also lost P from the particulate phase in the second part of the starvation experiment. Thus, as N-rich molecules such as proteins can be used for energy

allocation (Sakami and Harrington 1963), these molecules were obviously metabolized and used as energy source. Proteins are digested in amino acids which are substrates in the citric acid cycle, itself coupled with the electron transport chain enabling the formation of ATP while ammonia is excreted at the end of the urea cycle. Proteins have a higher Respiratory Quotient (RQ: the amount of CO_2 eliminated per oxygen consumed) than fats, thus organisms burning proteins should show lower oxygen consumption than those metabolizing fats (Lusk 1924; De Weir 1949). Indeed, we observed that in the period after the initial starvation of three hours, P-limited (N-rich) *O. marina* showed consistently lower respiration rates than nitrogen limited ones, thus indicating that these individuals used more proteins. We observed that N-limited *O. marina* total lipid concentration decreased during starvation, confirming that they mainly metabolized their fat reserves for respiratory processes, while it remained stable for P-limited *O. marina*. Using different substrate for respiration will directly affect nutrient remineralisation and therefore impact phytoplankton communities and nutrient cycling.

The stable N:P of Oxy_NP is explained by constant N and P pool in the population while the decrease in N and P content per cell was due to cellular division. Since significant differences were observed in the C:N:P of Oxy_NP and $OxyN_P$ we can conclude that *O. marina*'s stoichiometry is flexible. This flexibility, in comparison to strict homeostasis of planktonic crustaceans (Andersen and Hessen 1991; DeMott and Pape 2005), provides an important advantage in times of food shortage since *O. marina* can store elements through luxury consumption. This makes particularly sense in the case of this protozoan, as it is often exposed to variations of food quality and quantity in its natural habitat (Kimmance et al. 2006; Martel 2010). Thus, it might be that such conditions are handled differently by *O. marina* and planktonic crustaceans such as copepods.

Food change experiment

The results from the food change experiment show more precisely that *O. marina* regulates its stoichiometry to a certain degree but also highlight a resistance to nutrient limitation. Such mechanisms are already known for metazoans such as daphnids and copepods (Hessen 1990; Sterner 1990; Andersen and Hessen 1991; Bossuyt and Janssen 2005; Laspoumaderes et al. 2010) but have never been shown for protozoans. Since no effect of food change could be observed on the C:N of *O. marina* (data not shown) we hypothesize that only exceptionally high algal C:N could impact the stoichiometry of this dinoflagellate. Furthermore, we

observed that *O. marina* opposes more resistance to P-limitation until a breaking point at which this protozoan cannot keep its N:P stable anymore. The P-limited N:P ratio after food change was lower than the control. *O. marina* might have accumulated this element during the precondition phase in order to be prepared for a limitation period. This mechanism has already been highlighted in a wide range of heterotrophs and autotrophs and expressed in two ways, luxury feeding (Sommer 1984; Sommer 1985; Elser et al. 1987; Sterner and Schwalbach 2001) and compensatory growth (Reche et al. 1997; Tian and Qin 2004; Sommer and Sommer 2006). Our results are coherent with the observation that *O. marina's* growth rate is affected by P- but not by N-limited food (Hantzsche and Boersma 2010). This is related to the high amount of phosphorus in nucleic acids and its importance for growth and is described by the growth rate hypothesis (Sterner and Elser 2002). When conditions are favourable for growth, rRNA genes (rDNA) are activated resulting in a large assembly of ribosomes allowing an extensive protein synthesis necessary for high growth rates. Moreover, it was recently highlighted that two dinoflagellate species, *Oxyrrhis marina* (Meunier et al. 2012) and *Gyrodinium dominans* (unpublished data), are selecting for prey items based on food quality differences. Both dinoflagellates, independently on their precondition, always selected for P-rich algal cells. Hence, microzooplankters developed behavioural and physiological adaptations to buffer the impact of limitation by the nutrient they need the most, namely phosphorus.

As we highlighted that *O. marina* has a weak homeostasis this dinoflagellate and maybe other protozoans lie, in term of homeostasis ability, between metazoans and autotrophs. This is congruent with the hypothesis presented by Grover and Chrzanowski (2006). Based on theoretical considerations, this would have implications for omnivorous grazers. Protozoan grazers are always of better food quality for herbivores compared to autotrophs but are, under normal conditions, very diluted. However, after periods of nutrient limitation (e.g. late spring bloom), protozoan grazers are highly abundant and, due to the physiological momentum of their homeostatic regulation, protozoan herbivores should represent a significant part of omnivores diets. Further, we observed that the processes involved in the regulation of their internal nutrient composition are rapid and very sensitive to food quality and quantity. General high resilience in microzooplankton stoichiometry after food quality stress would have great implications for both top-down and bottom-up controls, namely nutrient remineralisation and quality as food.

2.6 Acknowledgements

This study is a part of the PhD study conducted by C.L.M. at the Biologische Anstalt Helgoland, Alfred-Wegener-Institut Bremerhaven, Germany, financed by Deutsche Forschungsgemeinschaft (DFG) and complies with current german laws and regulations on animal studies.

Chapter 3

Intraspecific selectivity, compensatory feeding and flexible homeostasis in the phagotrophic flagellate *Oxyrrhis marina*: three ways to handle food quality fluctuations

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This manuscript is published in Hydrobiologia as:

Meunier CL[1], Hantzsche FM[1,2], Cunha-Dupont AÖ[1], Haafke J[1], Oppermann B[1], Malzahn AM[1,2], Boersma M[1] (2012) Intraspecific selectivity, compensatory feeding and flexible homeostasis in the phagotrophic flagellate *Oxyrrhis marina*: three ways to handle food quality fluctuations. Hydrobiologia 680: 53-62

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3.1 Abstract

The phagotrophic flagellate *Oxyrrhis marina* shows a strong stoichiometric plasticity when fed differently grown *Rhodomonas salina*. We tested whether differently pre-conditioned *O. marina* displayed selective feeding behaviour from a mixture of nitrogen and phosphorus depleted *R. salina*. We observed selective feeding of *O. marina*, always selecting phosphorus rich *R. salina* independent of the pre-conditioning of the protists. In a second experiment, *O. marina* was again pre-conditioned either with nitrogen- or phosphorus-depleted *R. salina* and was refed with either of the differently limited *R. salina* in single food treatments (not in a mixture). The phagotrophic flagellate displayed compensatory feeding which means that *O. marina* feeds more on the food source which it was not given before. Due to its stoichiometric plasticity, *O. marina* might handle bad quality food by following the stoichiometry of its prey and additionally by active selective feeding towards P-rich algae to enhance growth. Post-ingestion selection might as well be an important feature which means that ingested elements in excess are quickly excreted and scarce elements are ingested through accelerated food uptake.

3.2 Introduction

Studies on food selectivity between interacting trophic levels, such as selective grazing behaviour of predators, have contributed substantially to our understanding of food webs. Not only metazoans, but also other heterotrophic organisms such as phagotrophic protists are often faced with a huge variety of potential food items of different qualities. Therefore, it is not surprising that selective feeding behaviour of heterotrophic flagellates and ciliates has been widely documented (Montagnes et al. 2008). Prey size (Andersson et al. 1986; Chrzanowski and Šimek 1990; Jakobsen and Hansen 1997) and prey motility (Gonzalez et al. 1993; Jakobsen et al. 2006) are two of the factors which may affect food uptake. Food quality, however, is not only determined by the physical properties of the prey, but also by its elemental and biochemical composition. Lee (1980) defined food quality as the nutritional value of the food relative to the energetic and molecular needs of the consumer. Sterner & Elser (2002), in the framework of ecological stoichiometry, developed this definition further and defined food quality in elemental ratios (mostly in terms of C:N:P). In the framework of ecological stoichiometry, this translates as optimal food quality being defined as the food composition matching the consumer's elemental ratios most closely, thus resulting in the lowest amounts of excretion products (Martel 2009a). Flexible stoichiometry of the consumer as well as luxury consumption can make it difficult to determine these optimal ratios and, obviously, the ultimate response factor of interest of the quality of certain food for a consumer is growth and/or reproductive success. Besides studies which have investigated selective behaviour of protist grazers for specific food taxa (Buskey 1997; Stoecker et al. 1981; Stoecker et al. 1986), studies on the potential of protists to compensate for low quality food through consumption of better food, even if the low quality food is the dominant part of the available food, have helped to increase our knowledge of plankton dynamics. Jakobsen et al. (2001) showed that the tintinnid ciliate *Amphorides quadrilineata* did not select between food of lower and better qualities. Whether prey selection and compensatory feeding are contrasting behavioural features or different life strategies among protists in terms of generalist or specialist feeding is not known yet.

Two mechanisms might allow heterotrophs to achieve a balanced nutrition even when food quality is suboptimal (Mitra and Flynn 2005). The first is a change in the ingestion rate on unfavourable food. Alternatively, heterotrophs might show selective feeding behaviour

dependent on their own nutritional status. The latter requires that body regulation mechanisms are in place so that the organism 'knows' what to feed on. Furthermore, mechanisms are needed to identify suitable prey (physical and/or chemical cues emitted by the prey) in an accessible area (Buskey and Stoecker 1988; Buskey and Stoecker 1989; Verity 1991; Jakobsen et al. 2006), or to follow the (biochemical) trail of these potential food sources (Fenchel and Blackburn 1999). Our knowledge on the chemosensory-receptors in marine protists which allow these organisms to detect areas of elevated prey densities is poor (Buskey and Stoecker 1988; Verity 1988; Fenchel and Blackburn 1999; Menden-Deuer and Grünbaum 2006). Recently, Martel (2009b) and Wootton et al. (2007) found differences in cell surface structures and bio-chemicals released by the food which might be responsible for food identification. In feeding experiments with preconditioned *Oxyrrhis marina*, Martel (2009a, b) indeed showed a discrimination against nitrogen depleted cells of the alga *Isochrysis galbana* compared to nitrogen replete cells, but the mechanisms in this interaction remained unclear (Martel 2009a).

In this study, we used the phagotroph *O. marina* as consumer and the phototroph *Rhodomonas salina* as algal prey. In previous functional response experiments, Hantzsche and Boersma (2010) fed starved *O. marina* with nutrient replete, nitrogen- and phosphorus-depleted *R. salina* cultures. They found that food uptake (in terms of carbon) of starved *O. marina* was largely independent of food quality. Furthermore, only phosphorus-depleted *R. salina* had a significantly negative effect on the growth rate of *O. marina* compared to nitrogen depleted and nutrient replete *R. salina*, confirming that phosphorus is an important element for fast growing consumers (Boersma and Kreutzer 2002; Frost et al. 2006). Since there is a strong need for additional information on feeding and selectivity of heterotrophic protists, we set out to answer the following questions: (1) Does the heterotrophic protist *O. marina* actively select between nitrogen and phosphorus depleted *R. salina*? ; (2) Does its pre-condition impact its behaviour? and (3) Does it compensate for nutritional imbalances through compensatory feeding?

3.3 Material and Methods

Experimental cultures

Oxyrrhis marina Dujardin was obtained from the Göttingen culture collection (Strain B21.89) and maintained on exponential-phase *Rhodomonas salina* (Wislouch) Hill et Wetherbee as food. *R. salina* was reared in gently aerated batch cultures at 20°C in an 18:6 h light:dark cycle (185 µmol m^{-2} s^{-1}) using sterile filtered F/2 medium (Guillard and Ryther 1962).

For the different limitations, *R. salina* cultures were grown 3 days in 0.2 µm filtered seawater in 1L bottles under the conditions described above. One set of cultures was kept in F/2 medium without phosphorus (treatment ‘‘Rho-P’’) and the other set with maximum 20% of normal nitrogen addition (treatment ‘‘Rho-N’’). The start concentration for Rho-N cultures was 200,000 and 300,000 cells mL^{-1} for Rho-P. This treatment ensured that Rho-N and Rho-P were in the stationary phase at which a significant limitation of the desired nutrient was obtained (Hantzsche and Boersma 2010).

Pre-conditioning *O. marina*

Cell number and mean biovolume of *O. marina* and *R. salina* cultures were determined using a CASY particle counter (SCHÄRFE SYSTEMS, Reutlingen, Germany). To establish different pre-conditioning regimes, the original *O. marina* culture was split into two cultures. Each pre-conditioned culture was then refed with nitrogen depleted (‘‘Oxy-N’’) and phosphorus depleted *R. salina* (‘‘Oxy-P’’) for minimum 2.5 days in 12 h intervals. Both *O. marina* cultures were kept without mixing by aeration at 20°C in dim light (18:6 light:dark cycle; 5 µmol m^{-2} s^{-1}).

Since there is no means to separate *O. marina* from *R. salina*, we had to ensure that all *R. salina* in the preconditioned *O. marina* cultures had been eaten before the start of the experiments. In previous experiments, we found that *O. marina* feeds ≈6 *R. salina* cells h^{-1}. The amount of food required to ensure that all algae were eaten in 2.5 days without inducing severe starvation effects in *O. marina* was calculated based on these numbers. Due to significant cell density differences between the different *R. salina* cultures, resulting in

different volumes of algal suspension added to the different *O. marina* treatments, the differences in water volume (mL) between the cultures were compensated through addition of artificial, sterile and nutrient-free seawater (Aqua Marin). Each *R. salina* and pre-conditioned *O. marina* culture was filtered onto pre-combusted Whatman GF/F filters at the start of the experiment. The particulate carbon and nitrogen content of *R. salina* and *O. marina* were measured with a Vario Micro Cube/CN-analyzer (Elementar). Particulate phosphorus was analyzed as orthophosphate after acidic oxidative hydrolysis with 5% H_2SO_4 (Grasshoff et al. 1999).

Selection experiments with Rho-N and Rho-P

At the start of the experiment, nitrogen- and phosphorus-depleted *R. salina* were centrifuged (600x g; 5 min; viability of *R. salina* was tested before), the supernatant was discarded and the pellet resuspended in artificial (N- and P-free) seawater. This procedure was repeated twice to ensure that nutrient limited algae remained nutrient stressed since the algal medium of the one algal treatment contained the nutritional element which was limited in the second algal treatment. After cell density determination, a mixture of 50% nitrogen depleted and 50% phosphorus depleted *R. salina* was prepared in 100 mL plastic beakers (four replicates) and diluted with artificial seawater to a ratio predator:prey of 1:25. As we could not separate the preconditioned *O. marina* from its ambient medium through centrifugation as done with the *R. salina* treatments (viability test failed), we could not prevent that nutrients from the pre-conditioned cultures were transferred to the experimental plastic beakers. We tried to keep this as low as possible and the added volume of the pre-conditioned *O. marina* cultures never exceeded 10% of the experimental end volume. In order to prevent that the mixed *R. salina* treatments would change their elemental composition through the addition of the pre-conditioned *O. marina* cultures, the selection experiment was run only for 2 h.

The experiment was carried out twice, with two different analysis techniques to establish cell densities of differently limited cells before and after feeding. In the first experiment, we counted the algal cells using a FlowCAM in the fluorescence triggered image mode (Sieracki et al. 1998). As fluorescent properties of the algal cells depend on their nutrient status this provides the opportunity to differentiate between cells automatically (Da Silva et al. 2009). The FlowCAM was able to differentiate between Rho-N and Rho-P and produced reliable data within a short time (≈10 min for 400 pictures measurement^{-1}), using the 100 µm flow cell

and the 20x objective (pump rate 0.47 mL min^{-1}; camgain 700; fluorescent gain 4; fluorescent threshold 1). We used 0.2 mL of the algae mixture and diluted it (10 mL end volume) with artificial seawater to 1,000 cells mL^{-1} for the start measurement with the FlowCAM (equipped with a green laser beam—532 nm).

Tab 3.1 Summary of discriminant analysis between nitrogen (Rho-N) and phosphorus (Rho-P) depleted *R. salina* (n=609) in the selection experiment with the FlowCAM. Five of twenty potential FlowCAM parameters contributed significantly to discriminate between Rho-N and Rho-P (ch 2 peak, ch 1 peak, length, width and transparency) and used in the classification model.

Variable/ parameter	Wilks'Lambda	Part.Lambda	F	p-value	Tolerance	R^2
Ch 2 Peak	0.75	0.58	445.08	<0.0001	0.29	0.71
Ch 1 Peak	0.50	0.86	95.70	<0.0001	0.29	0.71
Length	0.44	0.99	9.02	0.0028	0.34	0.66
Width	0.44	0.99	6.47	0.0112	0.17	0.83
Transparency	0.44	0.99	5.31	0.0216	0.34	0.66

We were able to discriminate between nitrogen (Rho-N) and phosphorus depleted *R. salina* (Rho-P) and found that they were significantly different in five of twenty FlowCAM parameters (Tab 3.1). Best discriminating factors were the fluorescence signal in channel 2 for phycoerythrin, followed by the fluorescence signal in channel 1 for chlorophyll a, since Rho-P tended to have a higher fluorescence in both channels than Rho-N. Further parameters were the cell length and cell width of *R. salina*, which were greater under phosphorus limitation than under nitrogen limitation.

Lastly the cell transparency of nitrogen depleted *R. salina* was higher than the one of phosphorus depleted *R. salina*. We used these five parameters for the classification equation (Tab 3.2) and were able to correctly classify ≈79% of Rho-N and ≈96% of Rho-P (Tab 3.3) in the single cell treatments.

Tab 3.2 Summary of the five parameters which defined the classification equation (f(x) = constant+ax+bx+cx+dx+ex) for each algal treatment (Rho-N and Rho-P) in the selection experiment with the FlowCAM.

Parameter	Rho-N (p=0.45)	Rho-P (p=0.55)
A=Ch 2 Peak	-0.0075	-0.0035
B=Ch 1 Peak	0.017	0.013
C=Length	1.90	2.24
D=Width	6.22	5.81
E=Transparency	211.19	202.90
Constant	-70.38	-68.24

Tab 3.3 Counting methods' accuracy: Percentage and number of cases where the classification model or counting estimates the correct contribution to one of both groups (Rho-N or Rho-P).

	FlowCAM			Fluorescence microscopy		
Group	Rho-N	Rho-P	Percent correct	Rho-N	Rho-P	Percent correct
Rho-N	214	58	78.7	392	8	98.0
Rho-P	14	323	95.9	7	343	98.0
Total	228	381	88.2	399	351	98.0

Total densities after 2 h were measured again using the FlowCAM. Using the classification functions established, we could identify the algal cells, count them and selectivity could be calculated using Chesson's selectivity index.

The second selectivity experiment had a similar setup to the first experiment, we fixed the samples with formalin (formaldehyde 20% buffered with hexamine) and stored them cool and dark. In order to count and discriminate Rho-N and Rho-P cells, 2.973 mL of each sample were settled in sedimentation chambers (HYDROBIOS) for 24 h, and counted under a Zeiss Axiovert 135 inverted microscope using epifluorescence. Identification of Rho-N and Rho-P cells was possible by coupling to the inverted microscope a Zeiss HBO 50 lamp and a BP546 green filter allowing only red light and fluorescence to be observed. Thus, Rho-P red cells appeared fluorescent while Rho-N green cells remained grey. In addition, we could show that, with an accuracy of 98%, this second method is more precise than the FlowCAM (Tab 3.3).

Prey selectivity α was calculated according to Chesson (1978, 1983):

$$\alpha = \frac{r_i/n_i}{\sum_{j=1}^{m} r_j/n_j}$$

whereby r_i is the frequency of prey i in the diet and n_i is the frequency of prey in the environment, divided by the sum of all relationships between the frequency of prey in the diet and in the environment. Significance of the selectivity was tested against α=0.5 (Student's *t* test), using the different replicates of the selection experiment.

Food compensation experiment

In this third experiment, we prepared pre-conditioned Oxy-N and Oxy-P as described above (2,000 cells mL^{-1}) and refed them either with Rho-N or with Rho-P in four different cell concentrations (7,500; 15,000; 25,000 and 50,000 cells mL^{-1}) in five replicates for 1h. Ingestion rates were calculated following Frost (1972).

Cell ingestion was analyzed for the two differently pre-conditioned *O. marina* with respect to offered food source and food concentration (3-way ANOVA; factor A=pre-condition of *O. marina* (Oxy-N, Oxy-P), factor B=food treatment (Rho-N, Rho-P), factor C=offered cell density of *R. salina* [cells mL^{-1}]. Cell ingestion [*R. salina O. marina*$^{-1}$ h^{-1}] was the dependent variable).

To estimate the time after which *O. marina*'s elemental composition would change from its precondition to a balanced elemental composition, we used *O. marina* pre-conditioned with phosphorus depleted *R. salina* and refed them with nitrogen depleted *R. salina* in three replicates. As soon as *O. marina* had eaten all of its food (after 18 and 24 h), the cultures were filtered and the elemental composition of *O. marina* was analyzed as described above.

3.4 Results

Selection experiment

In terms of stoichiometry, significant differences ($p<0.05$) exist between the two experiments. The nutrient limitations (N and P) were stronger in the FlowCAM experiment than in the fluorescence microscopy one (Tab 3.4). This is indicated by the higher C:P of Rho-P and higher C:N of Rho-N in the FlowCAM experiment (1,177 and 16.20) than in the fluorescence microscopy experiment (759 and 14.29). Despite these differences, in both selection experiments the stoichiometry of the pre-conditioned *O. marina* reflected the stoichiometry of their food sources (Tab 3.4). In both experiments, Oxy-P had higher C:P (582 and 417) and N:P (71.85 and 52.64) than Oxy-N (238 and 144 for C:P; 19.96 and 16.46 for N:P). In addition, Oxy-P had lower C:N (8.09 and 7.94) than Oxy-N (11.94 and 8.81). Furthermore, in both experiments we observed selective feeding of *O. marina*. Irrespective of the different pre-conditioning, *O. marina* selected positively for phosphorus rich *R. salina* (Fig 3.1). In the FlowCAM experiment, Oxy-N Chesson's index for Rho-N was $\alpha=0.66$ (n.s.) and the one of Oxy-P was $\alpha=0.78$ (significantly different from $\alpha=0.5$, $p<0.05$). In the fluorescence microscopy experiment, both pre-conditioned *O. marina* had the same selectivity index $\alpha=0.59$ (significantly different from $\alpha=0.5$, $p<0.05$).

Tab 3.4 Selection experiment analysed with the FlowCAM and fluorescence microscopy: Mean carbon, nitrogen, phosphorus cell content (pg cell^{-1}) and mean biovolume (µm³ cell^{-1}) of used *R. salina* cultures and of preconditioned *O. marina* in paired t-tests (n=5; FG=8; $p<0.05$). Different letters (^{a},^{b},^{c},^{d}) indicate significant differences between the different *R. salina* and *O. marina* treatments. Different numbers (^{1,2}) indicate significant differences between the two experiments.

	Treatment	FlowCAM experiment		Fluorescence microscopy experiment	
		-N mean (±Sd)	-P mean (±Sd)	-N mean (±Sd)	-P mean (±Sd)
Rhodomonas salina	C [pg cell^{-1}]	92 (4)^{b,1}	83 (3)^{c,1}	70.32 (4.92)^{a,2}	71.88 (4.08)^{a,2}
	N [pg cell^{-1}]	6.59 (0.29)^{b,1}	11.74 (0.85)^{c,1}	5.74 (0.56)^{a,2}	11.9 (0.42)^{b,1}
	P [pg cell^{-1}]	0.69 (0.05)^{b,1}	0.19 (0.05)^{c,1}	1.24 (0.002)^{a,2}	0.25 (0.003)^{b,2}
	Vol [µm³ cell^{-1}]	531 (70)^{a,1}	583 (48)^{a,1}	532 (31)^{a,1}	588 (27)^{a,1}
	C:P (molar)	345 (34)^{a,1}	1,177 (285)^{b,1}	156 (5)^{a,2}	759 (35)^{b,2}
	C:N (molar)	16.20 (0.57)^{b,1}	8.29 (0.84)^{c,1}	14.29 (1.35)^{a,2}	7.08 (0.29)^{b,2}
	N:P (molar)	21 (2)^{b,1}	141(27)^{c,1}	11 (1)^{a,2}	107(3)^{b,2}
Oxyrrhis marina	C [pg cell^{-1}]	1,818 (85)^{a,1}	1,491 (109)^{a,1}	684 (23)^{a,2}	756 (10)^{a,2}
	N [pg cell^{-1}]	178 (8)^{b,1}	215 (18)^{c,1}	91 (7)^{a,2}	111 (6)^{b,2}
	P [pg cell^{-1}]	19.70 (0.59)^{a,1}	6.64 (0.76)^{b,1}	12.40 (0.62)^{a,2}	4.65 (0.12)^{b,2}
	Vol [µm³ cell^{-1}]	6,172 (1,017)^{a}	6,337 (1,051)^{a}		
	C:P (molar)	238 (14)^{b,1}	582 (40)^{c,1}	144 (6)^{a,2}	417 (4)^{b,2}
	C:N (molar)	11.94 (0.15)^{b,1}	8.09 (0.23)^{c,1}	8.81 (0.80)^{a,2}	7.94 (0.35)^{b,1}
	N:P (molar)	19.96 (1.26)^{b,1}	71.85 (2.94)^{c,1}	16.46 (2.00)^{a,2}	52.64 (2.10)^{b,2}

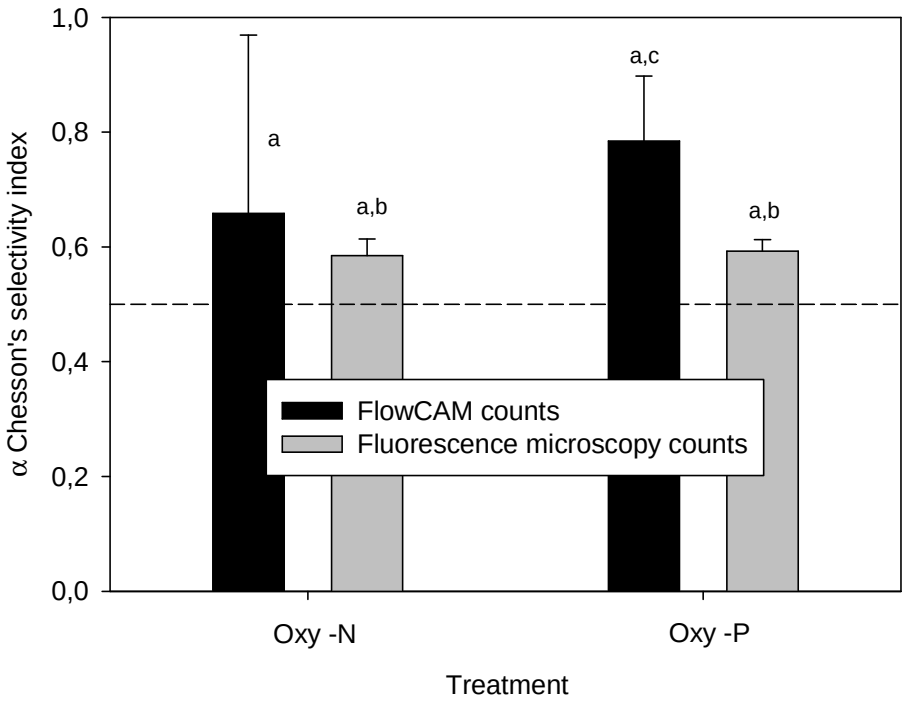

Fig 3.1 Selectivity of *O. marina* for nitrogen deplete (phosphorus rich) *R. salina* measured with α Chesson's selectivity index (mean+Sd). Black bars represents measurements obtained with the FlowCAM, white bars measurements obtained with fluorescence microscopy. Each bar represent mean of minimum 4 replicates. Dashed line represent selectivity threshold, values significantly higher than 0.5 indicates selectivity.

Food compensation experiment

We observed a slight but significant increase in food uptake of *O. marina* when refed with the *R. salina* treatment containing the element which *O. marina* failed during its pre-condition (Fig 3.2; Tab 3.5; ANOVA result interaction between pre-condition and food type; $F_{1,64=}4.004$; p=0.05). Thus, *O. marina* compensated its nutritional imbalance caused by the pre-conditioning by feeding more on the algae supplying the previously limiting nutrient.

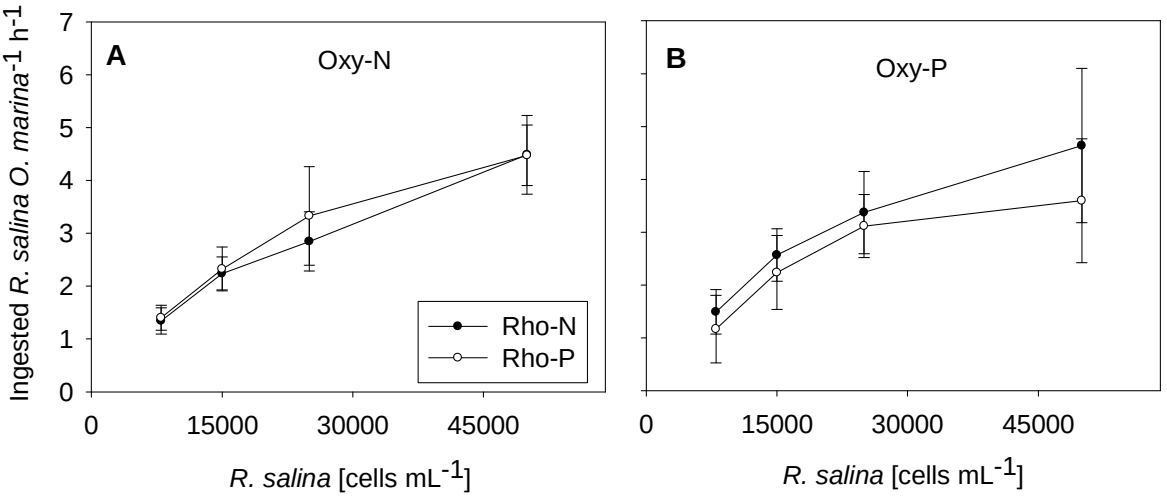

Fig 3.2 Compensation experiment: cell ingestion of *O. marina* h^{-1}, (A) preconditioned either on nitrogen depleted *R. salina* (Oxy-N) or (B) on phosphorus depleted *R. salina* (Oxy-P), refed either with nitrogen depleted (Rho-N) and phosphorus depleted *R. salina* (Rho-P). Error bars show the standard deviation of five measurements.

Tab 3.5 Summary of the "compensation experiment" of nitrogen and phosphorus stressed *O. marina* (Oxy-N and Oxy-P), refed with nitrogen and phosphorus depleted *R. salina* (3-way ANOVA; precondition of *O. marina*, food treatment of Rho-N or Rho-P, offered prey density as independent factors and ingestion rate [*R. salina* $Oxy^{-1}\ h^{-1}$] as dependent variable).

	FG	MQ	F	p-value
Precondition	1	0.02	0.04	0.85
Food	1	0.56	1.09	0.30
Prey density	3	31.26	60.63	0.000000
Precondition x Food	1	2.06	4.00	0.05
Precondition x Prey density	3	0.28	0.54	0.65
Food x Prey density	3	0.35	0.68	0.57
Precondition x Food x Prey density	3	0.12	0.23	0.88
Error	64	0.52		

On average, Oxy-N grazed ≈5% more on phosphorus depleted *R. salina* which were rich in nitrogen, whereas Oxy-P grazed ≈19% more on nitrogen depleted *R. salina* which were rich in phosphorus (Fig 3.3A). As a result of this feeding, the N:P ratio of *O. marina* adapted rapidly: within 18h the N:P ratio of P-limited *O. marina* (71) feeding on N-limited (P-rich) algae was identical to those individuals feeding on a F/2 diet (42) (Fig 3.3B). Thus, *O. marina* is very flexible and capable of compensating for the deficiency of elements through compensatory feeding.

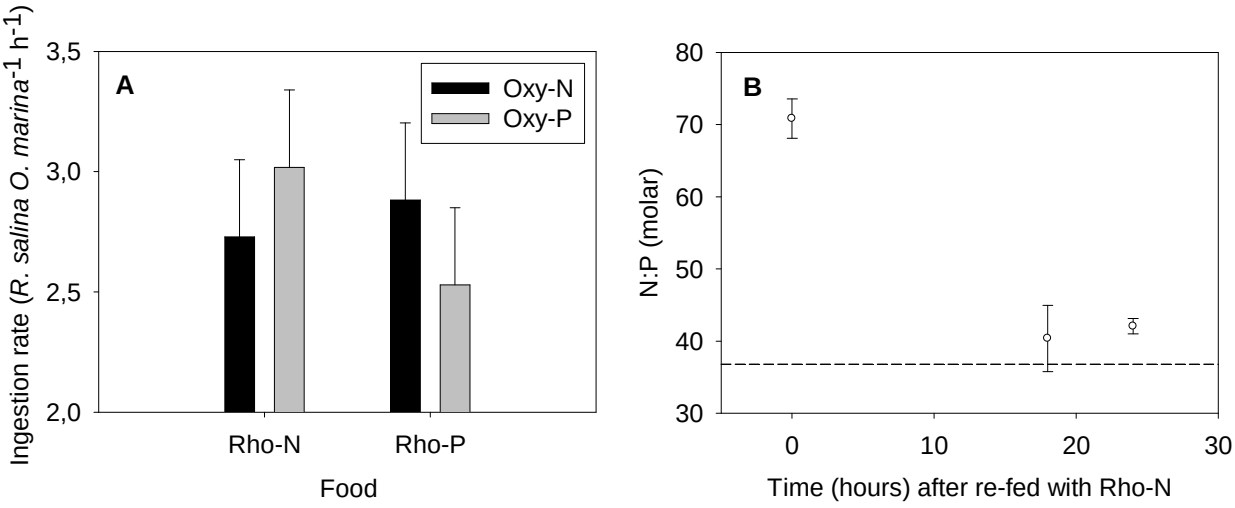

Fig 3.3 (A) Compensatory feeding of preconditioned *O. marina* (Oxy-N and Oxy-P) refed with nitrogen (Rho-N) and phosphorus depleted *R. salina* (Rho-P). Cell ingestion of *O. marina* h^{-1} derived from 3-way ANOVA (Tab 3.6) and the compensation experiment (Fig 3.3). Error bars show the standard error of five measurements. (B) Change of molar N:P ratio of Oxy-P after refed with nitrogen depleted *R. salina* (Rho-N). Error bars show the standard deviation of four measurements. Dotted line represent N:P ratio of *O. marina* nutrient replete, mean of 5 replicates. Data obtained from another experiment realised in our laboratory.

3.5 Discussion

The framework of ecological stoichiometry described by Sterner and Elser (2002) predicts that the elemental composition of photoautotrophic organisms is characterized by high plasticity whereas the carbon:nutrient (N and P) content of herbivores should be more constant. Consequently, herbivores are often faced with imbalanced food, which has effects on the somatic growth and reproduction of the consumer. The consumer, however, may modify its feeding behaviour either through lowering the ingestion rate of unfavourable food, thus using more time for extracting the limiting element efficiently or alternatively through an increase of food uptake, and a shortening of the handling time to extract only the easily available parts of the limiting nutrient. Furthermore, the consumer may show selective feeding behaviour to compensate for low nutritional quality of one food item through compensatory feeding on a second food item which contains the limiting element (Raubenheimer and Jones 2006). These pre-gut selection mechanisms are measurable as differences in food uptake. Alternatively, post-ingestion mechanisms, such as selective transfer efficiencies of ingested elements and excretion of excess elements might also be effective mechanisms to balance unbalanced food (Anderson et al. 2005; Frost et al. 2005).

In this study, we observed significant pre-gut selection of *O. marina* feeding on different resources, expressed as a selective feeding on P-rich prey, independent of the pre-conditioning of the consumers, and an increase in feeding on the limiting nutrient dependent on the pre-conditioning. This capacity of *O. marina* to identify the nutritional composition and potential deficiencies both inside its own cells as well as in its potential prey organisms provides a sophisticated mechanism which enables *O. marina* to compensate nutritional imbalance via compensatory and selective feeding. Recent studies were able to identify some of the biochemical mechanisms responsible for the ingestion or rejection of food particles (Nakamura et al. 1995). Roberts et al. (2006) and Wootton et al. (2007) found a variety of lectins, which are highly specific carbohydrate-binding proteins, along the cell surface of the phagotrophic protist *O. marina*. These lectins might be responsible for coupling with species-specific glycoconjugates of the prey and thus initiate phagocytosis (Martel 2009b). Probably, the nutritional status of algal cells modifies their structural or species-specific glycoconjugates on the cell surface. Therefore, the phagotrophic protist may be able to ‘‘taste’’ the nutritional status of the prey cells during cell-to-cell contact via the detection of

these glycoconjugates (Martel 2006; Martel 2009b). Depending on their nutritional status or growth stage, algae excrete certain substances (Wetz and Wheeler 2007) which are composed of different biochemical compounds such as carbohydrates and amino acids (Granum et al. 2002). Some of those compounds may serve as attractors, while others might be inhibitors for herbivores (Strom et al. 2003; Strom et al. 2007; Davidson et al. 2005; Martel 2006).

Even if the FlowCAM measurements showed more variation than the fluorescence microscopy method, both techniques showed that *O. marina* actively selects between different food qualities of the same food species presented to them in a mixture. *O. marina* is capable of dealing with different food qualities and selects P-rich preys. A mixed food uptake of nitrogen depleted and phosphorus depleted *R. salina* compensates for the lack of one element from the one algal treatment through food uptake of the other algal treatment. This feeding strategy is typical for omnivorous or opportunistic animals such as cockroaches (Raubenheimer and Jones 2006) and aquatic filter feeders such as *Daphnia sp.* (DeMott et al. 1998) which compensate the biochemical (carbohydrates vs. proteins) or elemental deficiencies (low P vs. high P food) through compensatory feeding. Thus, consumers have the ability to shape community composition by removing selectively certain preys (Löder et al. 2011; Svensson and Stenson 1991). It has also been documented that they strongly influence the nutrient composition of their environment by selective recycling of certain element (Elser and Urabe 1999; Vanni 2002). Our study gives evidence that grazers affect their environment not only by the selective retention of scarce elements and the excretion of abundant elements, but also by selective feeding, i.e. removal of scarce elements. Grazers high in, e.g. P such as *O. marina*, select for low N:P food, as phosphorus is the rare and limiting element. Thus, they indirectly increase the concentration of algae with a high N:P and vice versa by selective removal of the favoured algae.

In our compensation experiment with single food treatments, i.e. no choice (either nitrogen depleted or phosphorus depleted *R. salina*), we showed that *O. marina* grown on nitrogen depleted *R. salina* compensated its lack of elemental nitrogen by an increased uptake of phosphorus depleted *R. salina* which is rich in elemental nitrogen. *O. marina* which was grown on phosphorus depleted *R. salina* showed higher food uptake of nitrogen depleted *R. salina*, which are, in turn, rich in elemental phosphorus. Even though phosphorus depleted *R. salina* is poor quality food for *O. marina* (Hantzsche and Boersma, 2010, Malzahn et al. 2010), the dinoflagellates fed more on this food source when pre-conditioned on nitrogen depleted *R. salina* to compensate for the lack of nitrogen. *O. marina* suffering phosphorus

depletion compensated for the lack of elemental phosphorus with higher ingestion rates of nitrogen depleted *R. salina* which are phosphorus rich. Owed to this feeding strategy, *O. marina*'s elemental composition changed within a few hours. Relationships between selective feeding, compensatory feeding, and the consequences for consumers' fitness have rarely been described and quantified. Cruz-Rivera and Hay (2000) highlighted that some amphipod species are able to buffer the effect of low food quality via selective feeding while others completely circumvent the effects of low nutritional quality. There is still a lack of knowledge about the existence and functioning of such mechanisms in microzooplankton but our results indicate that *O. marina* buffers preys' nutrient imbalances through compensatory feeding and selective feeding.

The fact that *O. marina* actively selected towards P-rich *R. salina* shows, that food uptake of *O. marina* depends on food availability as well as on food quality, as Hantzsche and Boersma (2010) already described. Hence, we suggest that *O. marina* digests its prey very efficiently to extract the elements it needs at constant grazing rates rather than increasing food uptake to extract only the easily available parts of the limiting nutrient as described by Mitra and Flynn (2005). Thus, food quality-induced cell physiological processes in *O. marina* to handle unfavourable food and post-ingestion selection of elements might as well play an important role.

In conclusion, we have shown that the heterotrophic protist *O. marina* actively selects for phosphorus rich prey independently of its pre-condition. Furthermore, we highlighted that this species compensates for nutritional imbalances through compensatory feeding. Finally, we showed that *O. marina* has a weak homeostasis. This could be an adaptation allowing *O. marina* to handle fluctuations in food quality since the dinoflagellate can follow the stoichiometry of its prey instead of strongly regulating its own stoichiometry.

3.6 Acknowledgements

This study is a part of the PhD studies conducted by C.M. and F.M.H. at the Biologische Anstalt Helgoland, Alfred-Wegener-Institut Bremerhaven, Germany, financed by Deutsche Forschungsgemeinschaft (DFG) and GKSS Geesthacht, Germany, and complies with current

german laws and regulations on animal studies. We thank two anonymous reviewers, Martin Löder, Christina Gebühr, and Petra Brandt for useful discussions. Special thanks to Katherina Schoo whose linguistic suggestions improved the manuscript.

Chapter 4

You are what you eat, or you eat what you are: age specific selective feeding in the copepod *Acartia tonsa*

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Meunier CL[1], Boersma M[1], Wiltshire KH[1], Malzahn AM[1,2]. You are what you eat, or you eat what you are: age specific selective feeding in the copepod *Acartia tonsa*

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4.1 Abstract

The relative availability of light, CO_2 and nutrients causes specific and variable carbon (C), nitrogen (N) and phosphorus (P) contents in plants (Hillebrand and Sommer 1999a; Quigg et al. 2003; Klausmeier et al. 2004). The resulting ratios of the elements have ramifications for the quality of these primary producers as food for herbivores (Sterner and Elser 2002), and grazers should select those feeds that match their metabolic needs most closely (Kleppel 1993). Interestingly, the plankton literature almost exclusively deals with post-ingestion mechanisms to handle different quality food, and only little is known about the ability of grazers to select for food quality (Paffenhöfer and Van Sant 1985; Cowles et al. 1988; Irigoien et al. 2005). Hence, we performed two experiments investigating the ability of the most abundant metazoans on earth, copepods, to select for food quality differences and examined possible selective differences between two different copepod developmental stages (nauplii and copepodites). We show that nauplii select for P-rich food; as a consequence, or a reason, they are rich in phosphorus and have high potential growth rates. In contrast, the older stages, copepodites, are richer in nitrogen and select for N-rich food. Thus, copepods affect nutrient cycling not only by the selective retention of scarce elements and the excretion of abundant elements (Cowie and Hedges 1996), but also by selective feeding, i.e. removal of scarce elements. Since the cycling of nutrients is critical for the sustenance of ecosystems (Costanza et al. 1989; DeAngelis et al. 1989; DeAngelis 1992; Chapin Iii et al. 2000), understanding the biogeochemical cycles of both N and P is crucial to apprehend marine ecosystem dynamics.

4.2 Material and Methods

In a first experiment, we measured the fluctuations in N:P and growth rate occurring during ontogeny. We regularly sampled five replicate cohorts of *A. tonsa* fed with nutrient replete *R. salina* for their C, N and P content. The particulate carbon and nitrogen content of *A. tonsa* were measured with a Vario Micro Cube/CN- analyser (Elementar). Particulate phosphorus was analysed as orthophosphate after acidic oxidative hydrolysis with 5% H_2SO_4 (Grasshoff et al. 1999). N:P as well as carbon-specific growth rate were analyzed using repeated measures ANOVA.

Tab 4.1 Selection experiment for nauplii and copepodites: Mean carbon, nitrogen and phosphorus cell content (pg cell^{-1}) of *R. salina* cultures nitrogen ($_N$P) and phosphorus (N_P) limited used. Different letters (^{a},^{b}) indicate significant differences ($p<0.05$) between treatments and experiments.

	Nauplii experiment		Copepodites experiment	
	$_N$P mean (±Sd)	N_P mean (±Sd)	$_N$P mean (±Sd)	N_P mean (±Sd)
C [pg cell^{-1}]	49 (2)^a	52 (4)^a	46 (3)^a	48 (10)^a
N [pg cell^{-1}]	6.59 (0.26)^a	10.12 (0.44)^b	6.45 (0.67)^a	11.18 (0.52)^b
P [pg cell^{-1}]	0.96 (0.03)^a	0.22 (0.03)^b	1.01 (0.05)^a	0.20 (0.03)^b
C:P (molar)	132 (9)^a	652 (49)^b	118 (5)^a	620 (92)^b
C:N (molar)	8.73 (0.56)^a	5.68 (0.73)^b	8.37 (0.50)^a	5.01 (0.29)^b
N:P (molar)	15 (1)^a	110(21)^b	14 (1)^a	124(18)^b

In a second experiment, we allowed, in sextuplicates, naupliar and copepodite stages of the calanoid copepod *A. tonsa* to feed on 1:1 mixes of –N and –P algae in appropriately sized containers in artificial seawater for 10h, which was the duration needed to reduce the initial algal densities by 10-30%. Samples were then fixed and counted using the epifluorescence microscopy method described by Meunier et al. (2012). Prey selectivity α was calculated according to Chesson (Chesson 1978; Chesson 1983) and significance of the selectivity was

tested against α=0.5 (Student's *t* test). The particulate carbon, nitrogen and phosphorus content of *R. salina* were measured as described for the first experiment.

Nitrogen limited *R. salina* (-N) was 4 to 5 times richer in phosphorus ($p<0.001$) than phosphorus limited *R. salina* (-P) (Tab 3.1). On the other hand, -P *R. salina* was 1.5 times richer in nitrogen than –N *R. salina* ($p<0.001$). As a result, -N *R. salina* presented a N:P ratio 7 to 8 times higher ($p<0.001$). Additionally, no significant difference in algal stoichiometry could be observed between the nauplii and the copepodite experiments ($p=0.24$).

4.3 Results and discussion

Seasonal dynamics of phytoplankton production is closely linked with environmental fluctuations of abiotic parameters such as light and temperature but also nutrient availability which is a key parameter influencing phytoplankton growth. The long term monitoring realized at the Biologische Anstalt Helgoland (AWI, Germany) highlights large annual fluctuations of dissolved ratio of the nutrients nitrogen and phosphorus (Fig 4.1A) leading to varying degrees of nutrient limitation.

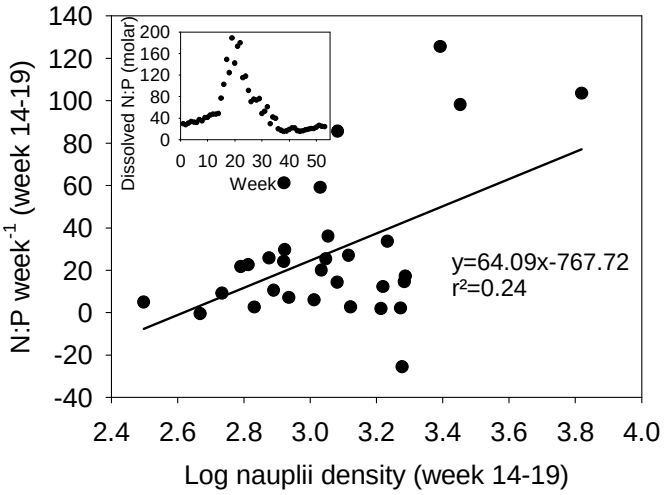

Fig 4.1 (A) Average from 1962 to 2005 of dissolved N:P over a year (B) Relationship between nauplii density and the change in dissolved N:P ratio between the weeks 14 to 19. These weeks were chosen since, as can be seen on the inlet (A), the strongest changes in N:P ratio over the year occurs at this period. Each point represents one year. Data are from 1962 to 2005 and are issued of the long-term monitoring realised at the Biologische Anstalt Helgoland (AWI, Germany).

While limitation of phytoplankton production is classically linked to external loading (Peňuelas et al. 2012), consumer-driven nutrient retention and recycling by mesozooplankton is an internal parameter influencing nutrient availability (Sterner et al. 1992; Johnson and Luecke 2012) and was shown to have substantial effects on both N (Elser et al. 1995) and P availability for primary production (Elser et al. 1988).

Thus, it can be hypothesized that that there should be a positive correlation between the speed of change in nutrient availability and the nutrient demands of the grazers within a system, or more concrete that the presence of high P-nauplii will lead to selective removal of P-rich cells as well as to the selective retention of phosphorus by nauplii resulting in an increase of the dissolved N:P (Elser et al. 1996; Villar-Argaiz et al. 2002). Indeed, we observed that speed of change in the N:P ratio of the dissolved nutrients at Helgoland Roads is correlated to the densities of the young copepod life stages, nauplii (Fig 4.1B).

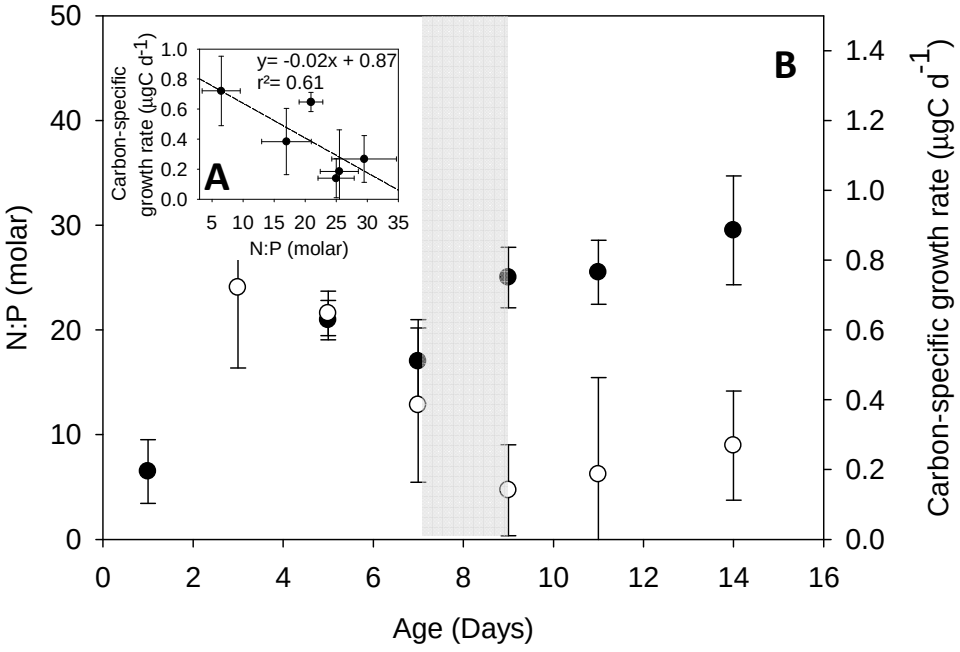

Fig 4.2 (A) Potential growth measured as carbon-specific growth rate versus molar N:P ratio of *A. tonsa* during ontogeny (mean+Sd). Each value represents the mean of 5 replicates. (B) Molar N:P ratio (full circles) and carbon-specific growth rate (open circles) of *A. tonsa* during ontogeny (mean+Sd). Each value represents the mean of 5 replicates. The grey zone indicates metamorphosis from nauplius to copepodite stage.

Nauplii and copepodites, the two major developmental stages of copepods, are our planet's most abundant metazoan grazers (Fryer 1986). They constitute an essential link in marine food webs, being major grazers of phytoplankton and microzooplankton, and prey for fish larvae and other pelagic carnivores, but are also an important component of the microbial loop (Turner 2004). The complex metamorphosis copepods undergo results in changes in elemental composition during ontogeny (Elser et al. 1996).

Furthermore, growth rates decrease strongly over the life time of a copepod (Dagg and Littlepage 1972; Båmstedt 1986). As substantial amounts of phosphorus-rich nucleic acids are needed to sustain high growth, the growth rate hypothesis (Sterner and Elser 2002) predicts a positive correlation between potential growth rate and P content of an organism. Originally this hypothesis was developed for between species comparisons, but the results of our experiment show that it also holds within species (Fig 4.2A). Furthermore, based on the growth rate decrease during ontogeny (Fig 4.2B, open circles), we predict that the N:P ratio in nauplii should be low and increase with age, which we observed (Fig 4.2B, full circles). These differences in nutrient composition between copepod life stages reflect varying nutrient requirements and should result in different feeding strategies.

The elemental composition of photoautotrophic organisms is characterized by high plasticity whereas the stoichiometry of herbivores is more constant (Sterner and Elser 2002). This is primarily due to autotrophs possessing the capability to adjust internal cellular pools of nutrient-rich biomolecules and to store nutrients in excess and consumers not (Frost et al. 2005). Therefore, there is a mismatch between grazers' metabolic requirements and their prey's nutrient content which results in reduced somatic growth and reproduction of the consumer (Gulati and DeMott 1997). There are several pre- (Kleppel 1993) and post-ingestion (Cowie and Hedges 1996; Mitra and Flynn 2005; Mayor et al. 2011) mechanisms allowing grazers to handle these imbalances. In order to reduce metabolic costs and avoid intoxication, pre-ingestion mechanisms such as selective feeding are of utmost importance. Grazers in fact do select for size (Paffenhöfer 1988) or taxa (Irigoien et al. 2000; Fileman et al. 2007) but it would be favorable to also select for the right concentrations of necessary elemental or biochemical building blocks of a prey item. Unfortunately only little is known about the ability of grazers to select for food quality (Paffenhöfer and Van Sant 1985; Cowles et al. 1988; Irigoien et al. 2005).

Copepods are able to feed selectively on food particles using different cues such as biochemical composition and chemoreception (Poulet and Marsot 1978; Cowles et al. 1988; Butler et al. 1989), but thus far this has really only been shown for between prey species comparisons (Paffenhöfer 1988; Irigoien et al. 2000; Fileman et al. 2007).

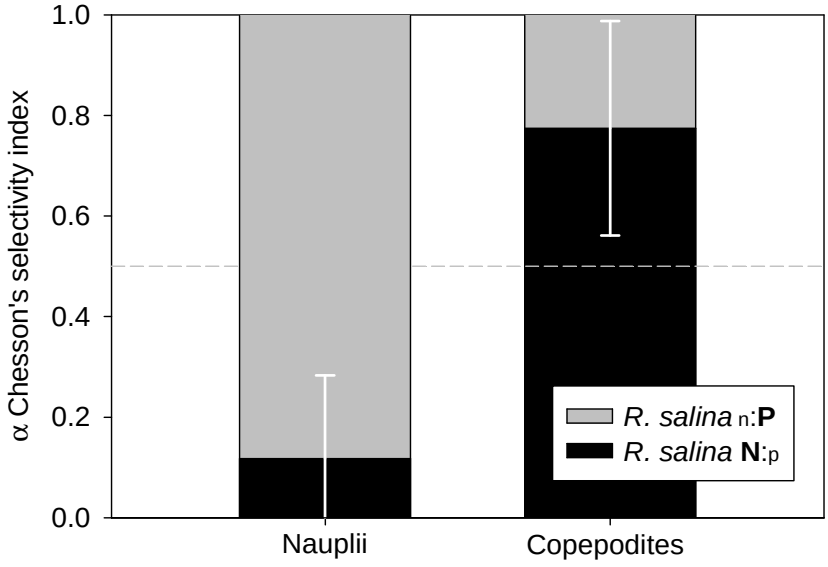

Fig 4.3 Selectivity of nauplii and copepodites of *A. tonsa* between nitrogen deplete (phosphorus rich) (grey bars) and phosphorus deplete (nitrogen rich) (black bars) *R. salina* calculated as α Chesson's selectivity index. Each bar represents mean of 6 replicates. Dashed line represents no selectivity, values were always significantly different than 0.5 ($p<0.01$) indicating selectivity.

Cowles et al. (1988) highlighted that adult copepods *A. tonsa* select for faster growing algal cells in order to maximize their nitrogenous ingestion. Moreover, based on the stoichiometric changes during ontogeny described above, we expect nauplii to be more dependent on phosphorus and copepodites to have higher nitrogen requirements. Hence, copepods' developmental stages should select for their food sources in contrasting ways. We performed two experiments investigating the ability of nauplii and copepodites to select for food quality differences and observed that different developmental stages of *A. tonsa* eat what they need, thus selecting for prey rich in the nutrient they need the most (P for nauplii and N for copepodites), irrespective of their own nutrient status (Fig 4.3).

Although stoichiometric fluctuations in coastal waters are also associated with varying nutrient inputs, zooplankton community structure plays an important role in nutrient cycling. Our results imply that the impact of individual copepods on relative nitrogen and phosphorus cycling in nature can vary strongly during development. Since the distribution of developmental stages in copepod populations fluctuates with depth and seasons (Razouls and Razouls 1988; Ozaki and Ikeda 1999; Takahashi and Uchiyama 2008), we can expect that nutrient cycling is strongly affected by the developmental stage dominating the mesozooplanktonic biomass, thus recycling more nitrogen in young (growing) populations and preferably phosphorus in older declining ones. This study highlights the potential importance of grazers on the growing conditions of their prey. Furthermore, the increasing N:P ratio of human influenced influx of nutrients into marine systems (Grizzetti et al. 2012) could lead to a decrease in copepod success as a result of the decreased relative availability of phosphorus for young stages.

3.4 Acknowledgements

This study is a part of the PhD study conducted by C.L.M. at the Biologische Anstalt Helgoland, Alfred-Wegener-Institut Bremerhaven, Germany, financed by Deutsche Forschungsgemeinschaft (DFG) and complies with current german laws and regulations on animal studies. This study was further partly supported by the y the German Federal Ministry of Education and Research (BMBF, FK2 03F0609A and 03F0603C).

Chapter 5

Linking swimming behaviour and predator-prey interactions in pelagic microbial food webs

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Meunier CL[1], Schulz K[1], Boersma M[1], Malzahn AM[1,2]. Linking swimming behaviour and predator-prey interactions in pelagic microbial food webs

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5.1 Abstract

Heterotrophic dinoflagellates are motile protozoans, important consumers of phytoplankton in aquatic environments. Motility is a main advantage for predators during grazing activities, but can also serve as defence mechanisms against being grazed. Thus, numerous microalgal species are also motile. We investigated the impact of nutrient (phosphorus) limitation on motility of two algal species, *Rhodomonas salina* and *Teleaulax sp.*, and the heterotrophic dinoflagellate *Oxyrrhis marina* and examined how differences in prey swimming speed affect grazing of *O. marina*. Furthermore, we investigated changes in swimming speed of algae and the dinoflagellate during predator-prey-interactions. We showed that algal swimming speed is species-specific and strongly influenced by nutrient limitation. These differences between species and nutrient treatments significantly influenced food uptake of *O. marina*. Finally, *Teleaulax sp.* presented an escape behaviour, which appeared to be an effective defence mechanism against grazing of *O. marina*. we show that applying successfully optimal foraging theory to motile prey requires knowledge about important parameters such as escape response that are needed to efficiently test the theory, and that with motile prey, differences between prey in vulnerability (encounter rate and capture success) are often more important than variation in predator active choice in determining predator diets.

5.2 Introduction

Heterotrophic dinoflagellates are important components of the planktonic community in aquatic ecosystems (Sherr and Sherr 2007). These protozoans can act both as predators (Sherr and Sherr 2002; Löder et al. 2011) as well as prey items (Stoecker and Capuzzo 1990; Lessard 1991; Suzuki et al. 1999), and as a result play a key role in carbon and nutrient cycling (Kirchman 2000).

Heterotrophic dinoflagellates are motile protozoans that actively swim with the help of two morphologically and functionally differentiated flagella allowing swimming behaviour to vary in speed and direction (Gaines and Taylor 1985; Fenchel 2001). Swimming speeds of heterotrophic dinoflagellates typically range from 10 to 1000 µm s^{-1} (Levandosky and Kaneta 1987; Crawford 1992), but exceptionally high swimming speeds of over 4000 µm s^{-1} by *Protoperidinium bipes* have also been reported (Jeong et al. 2004). The ability to move gives these organisms several advantages. It enables organisms to react to nutrient and chemical gradients in the environment and thus to position themselves in areas suited to their physiology (Thar et al. 2000). Furthermore, swimming inevitably influences interactions between species, particularly in predator-prey interactions. Motility enhances the rate at which food items are encountered but at the same time also increases the rate at which an organism encounters its predator (Visser and Kiørboe 2006). Moreover, motility allows predators to actively search and capture food items, while it allows prey to escape or reduce predation pressure (Buskey 1997; Tillmann and Reckermann 2002; Matz and Jürgens 2005). Besides protozoan grazers, numerous phytoplankton species are also motile. Algal motility is usually linked to resource availability, with movement towards light or nutrients (e.g. Eppley et al. 1968; Cullen and Horrigan 1981; MacIntyre et al. 1997), whereas the role of algal motility in predator-prey-interactions has received less attention, even though high motility of prey cells was shown to reduce dinoflagellate feeding rates (Buskey 1997).

Although it has been shown that light and nutrients play key roles in the determination of the swimming strategy of motile cells (e.g. Eppley et al. 1968; Cullen and Horrigan 1981; MacIntyre et al. 1997), the impact of nutrient limitation on the motility of algae and heterotrophs has not been yet investigated. Since nutrient limitation has a negative impact on phytoplankton metabolism (Hecky and Kilham 1988; Ridout and Morris 1988), we expect slower cell swimming activity under a nutrient stressed regime, unless high swimming

activity could potentially lead to a reduction of the stress (by finding patches of nutrients). A reduction in swimming speed decreases encounter rates, but at the same time it also increases escape rates. Thus, the effects of swimming speed changes are unpredictable, but should generally lead to an increase in capture success at initially very high rates, and an increase at initially lower rates (Fig 5.3A and B).

We performed three experiments using two phytoplankton species, the cryptophytes *Teleaulax sp.* and *Rhodomonas salina*, and the heterotrophic dinoflagellate *Oxyrrhis marina* as predator to investigate the impact of nutrient status on swimming speed of predators and prey as well as the effect of prey motility on trophic interactions.. We hypothesized that the nutrient status of an organism affects its swimming speed, and that motility of predator and prey influences feeding success of heterotrophic dinoflagellates.

5.3 Material and Methods

Experimental cultures

The dinoflagellate, *Oxyrrhis marina* is the most commonly used marine protozoan grazer in laboratory grazing experiments because of its simplicity of culture, broad distribution, and tolerance to a wide range of environmental conditions (Lowe et al. 2010). Although not usually found in open waters (Droop 1959), *O. marina*, being both a filter- and raptorial grazer (Jeong et al. 2008), is representative of many planktonic protists. As food for the heterotrophs we used *Rhodomonas salina* (Wislouch) Hill et Wetherbee (Cryptophyceae) and *Teleaulax* sp. (Cryptophyceae). Two different algae were used to investigate the effect of different swimming strategies on trophic interactions.

O. marina Dujardin was obtained from the Göttingen culture collection (Strain B21.89) and the culture was fed *R.* grown in batch cultures at 18.5°C in an 18:6h light : dark cycle using sterile filtered f/2 medium (Guillard and Ryther 1962). All three experimental organisms were cultured following Meunier et al. (2012), the only difference between the phytoplankton cultures being that *R. salina* cultures were aerated, whereas *Teleaulax sp.* cultures were placed on a KS 501 digital Shaker (IKA Labortechnik) since this species is more fragile and is sensitive to bubbling. Cell number and mean biovolume of *O. marina*, *R. salina* and

Teleaulax sp. cultures were determined using a CASY particle counter (Shärfe Systems, Reutlingen, Germany). Prior to the experiments we starved *O. marina* for one week in order to eradicate effects of pre-culture conditions. The starving *O. marina* culture was split into three cultures fed on phosphorus replete ("Oxy R+" and "Oxy T+") and phosphorus deplete ("Oxy R-" and "Oxy T-") *R. salina* ("R+" and "R-") and phosphorus deplete *Teleaulax sp.* ("T-"). This preconditioning lasted 4 days and was performed in triplicates. Food pulses were given every 24h, from independent algal cultures that were started on successive days of the experiment (Meunier et al. 2012). Since there is no way to separate *O. marina* from *R. salina* and *Teleaulax sp.*, we adjusted the food quantity at the last preconditioning day in order that all algal cells were eaten just in time when the experiment begun.

Tab 5.1 Elemental stoichiometry of *Rhodomonas salina* and *Teleaulax sp.* grown under P-replete (R+; T+) and P-deplete (R-; T-) conditions and *Oxyrrhis marina* fed with these algae (Oxy R+, Oxy R-, Oxy T-) and starved (Oxy St). Data are means and standard deviations of 5 measurements for algae and 3 measurements for dinoflagellates.

Treatment	C/N	C/P	N/P
R+	4.7 (±0.1)	212.0 (±32.4)	45.6 (±7.2)
R-	7.4 (±0.3)	967.5 (±101.7)	131.0 (±15.7)
T+	4.6 (±0.3)	177.9 (±29.2)	38.6 (±5.5)
T-	7.5 (±1.4)	902.4 (±398.7)	118.2 (±40.1)
Oxy R+	5.3 (±0.4)	180.7 (±5.6)	34.0 (±1.3)
Oxy R-	7.9 (±0.2)	637.9 (±14.6)	80.9 (±7.0)
Oxy T-	5.9 (±0.4)	217.3 (±44.0)	37.3 (±9.8)
Oxy St	5.7 (±1.0)	63.3 (±8.4)	11.5 (±3.1)

In order to measure organisms' C, N and P content, an estimated amount of 200 µg carbon was filtered onto precombusted Whatman GF/F filters. Filtration was performed during the preconditioning phase for *R. salina* and *Teleaulax sp.*, creating time replicates statistically independent by measuring C, N and P of algae given as food every day. Filtration was

performed at the end of the preconditioning for *O. marina*, thus taking only *O. marina* and not the algal cells. The particulate carbon and nitrogen were measured with a Vario Micro Cube elemental analyser (Elementar) while particulate phosphorus was analysed as orthophosphate after acidic oxidative hydrolysis with 5% H_2S (Grasshoff et al. 1999). C:N, C:P and N:P differed significantly between treatments in both algae (2-way ANOVA, Tukey's HSD post hoc test, FG=8, $p<0.001$), but algae showed similar nutrient stoichiometry under similar conditions (Tab 5.1, 2-way ANOVA, $p=0.80$), which was reflected in the stoichiometry of *O. marina* feeding on these algae (Tab 5.1), so that the prerequisites for the experiments described below were satisfied.

Swimming behaviour experiment

To examine species-specific and nutrient status specific swimming speeds, we filmed organisms alone and in predator-prey-interactions (*O. marina* with each of the four algae qualities). In order to investigate the effect of starvation on motility, we also measured starved *O. marina* from the initial culture. The movement of the organisms was videotaped using an Olympus DP71 camera attached to an Olympus SZX 16 microscope at a magnification of 10. Using the software cell'D, the record was started manually and stopped automatically after 3 seconds. Random videos with 12-14 frames per second were taken. Before the start of the experiment cell concentrations were measured using a CASY cell counter. Directly after this measurement, the algal cultures were put in the dark to prevent growth. Just before filming we prepared 1mL Sedgewick rafter counting cell slide with a concentration of 9000 cells mL^{-1} for *O. marina* and 60 000 cells mL^{-1} for the algae. After filming, the films were transformed into image sequences using the IrfanView 4.25 software. This step enabled us to use the ImageJ software to measure the swimming speed of the organisms. The software overlaid all the images of one video thus the swimming trace became visible. The length of each trace was determined before calculating the velocity. We additionally calculated the percentage of motile algae.

Since we observed that *Teleaulax sp.* displays escape behaviour during interaction with predators, we also investigated the influence of nutrient limitation on this escape behaviour, hypothesizing that nutrient depletion has negative effects on motility. To increase encounter rates between predator and prey we used a higher concentration of 20 000 cells mL^{-1} for *O. marina*. Furthermore we increased the time of recording to 6-10 seconds and videotaped

O. marina preconditioned on *R. salina* P-replete in interaction with *Teleaulax sp.* P-replete and P-deplete.

Grazing experiment

In order to investigate the resulting grazing of *O. marina* on *R. salina* and *Teleaulax sp.*, we conducted a grazing experiment. We offered each algal treatment ("R+, R-, T+ and T-") to *O. marina* that had been starved for 7 days, with 3 replicates for each treatment. We chose starved *O. marina* since pre-experiments have shown that they exhibit the highest ingestion rates, furthermore this approach avoids potential pre-condition effects caused by different food sources. The experiment was conducted in 100 mL plastic beakers under dim light at 19°C. Initially each beaker contained 25,000 algal cells and 2,000 *Oxyrrhis* cells in 80 mL artificial sea water with a salinity of 32 (hw Wiegandt, Marinemix professional). The experiment was stopped after 140 min by adding formalin (formaldehyde 20% buffered with hexamine) to each beaker. The content of the beakers was transferred into 100 mL brown glass bottles and stored cool and dark until further analysis. With an Axio Cam HRc camera and the software Axio Vision Rel 4.8, pictures of *O. marina* cells were made randomly. For each sample we measured the volume of 30 randomly chosen individual cells as well as the volume of their food vacuole. This allowed us to calculate the percentage of *O. marina* filled with food and use this as a proxy of ingestion.

The volumes of both the whole organisms as well as the vacuoles were calculated using the following formula:

$$V = \frac{\pi}{6} \times d^2 \times h$$

whereby V is volume, d width and h length of *O. marina* cells or food vacuoles (Hillebrand et al. 1999b). Food uptake was expressed as the percentage of the total *O. marina* volume that was taken up by food.

Selectivity experiment

Based on the grazing experiment's results, we calculated the outcome of *O. marina* selectivity between *R. salina* and *Teleaulax sp.* when offered together as prey. We plotted ingestion data versus prey swimming speed and fited a regression curve. This allowed to estimate the prey swimming speed for which *O. marina* ingestion is maximal. We then calculated the ingestion of any prey type as a percentage of the maximal ingestion of *O. marina* to estimate, when the dinoflagellate feeds on a mixture of prey, the frequency of each prey type in its diet as α Chesson's selecitivty index (Chesson 1978; Chesson 1983). To test the predicted outcome of selectivity, we performed a selectivity experiment in the same conditions as the grazing experiment and used a modified version of the experimental setup described by Meunier et al. (2012). We offered, 1:1 mixes of *Teleaulax sp.* F/2 and *R. salina* F/2 as well as *Teleaulax sp.* F/2 and *R. salina* -P in 100 mL plastic beakers filled with artificial seawater to *O. marina* in octuplicates. The experiment lasted 140 min which was the duration needed to reduce the initial algal densities by 10-30%, the samples were fixed and stored cool and dark until further analysis. An aliquot of each sample was counted with an AXIO Observer.A1 microscope (Zeiss) using 2.973mL centric counting chambers. Selectivity α was calculated according to Chesson (Chesson 1978; Chesson 1983) and significance of the selectivity was tested against α=0.5 (Student's one sample *t* test).

5.4 Results

Swimming experiment

Video microscopy was used to analyze the swimming speed and motility of algae and dinoflagellates as well as to investigate possible behavioural changes during predator-prey interactions. The analysis of the swimming speed showed significant differences between the two algal species as well as between the nutrient statuses.

There was a clear difference in the total motility of the different algal species and treatments (Fig 5.1A). Whereas in *R. salina* only a small percentage of the total number of cells was actively swimming, with no difference (2-way ANOVA, Tukey's HSD post hoc test, MQ=243.9, FG=36, p=0.99) between the nutrient treatments (R+: 20% and R-: 21%),

Teleaulax sp. cells were much more active (2-way ANOVA, $F_{1.36}$=46.04, p<0.001). Moreover, there was a significant difference (2-way ANOVA, Tukey's HSD post hoc test, MQ=243.9, FG=36, p=0.02) in the percentage of motile cells between nutrient treatments. 65% of P-replete *Teleaulax sp.* cells were swimming, whereas just 43% of P-deplete ones were active.

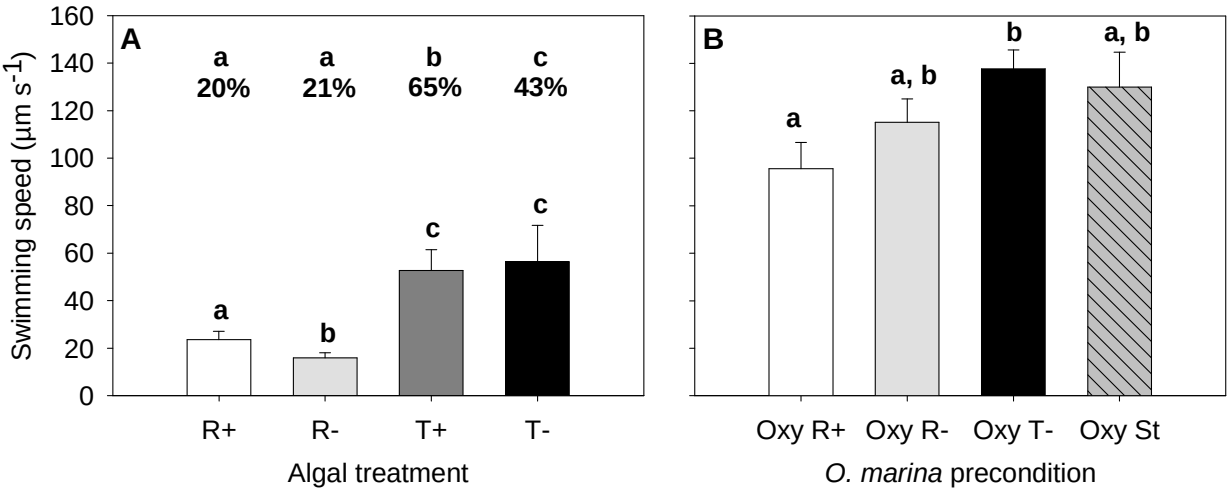


Fig 5.1 Swimming speed of *Rhodomonas salina* and *Teleaulax sp.* (A) grown under P-replete (R+; T+) and P-deplete (R-; T-) conditions and *Oxyrrhis marina* (B) fed with these algae (Oxy R+; Oxy R-; Oxy T-) and starved (Oxy St). Bars show the mean and error bars show the standard error of minimum 20 measurements. Different letters (a,b) indicate significant differences (three-factorial ANOVA, threshold p=0.05). Percentages indicate the proportion of motile cells.

Within the motile cells we also observed significant differences. In all cases *Teleaulax* swam significantly faster than *R. salina* (Fig 5.1A, 2-way ANOVA, $F_{1.12}$=211.78, p<0.001). Motile P-replete and P-deplete *Teleaulax sp.* swam at similar velocities (2-way ANOVA, Tukey's HSD post hoc test, MQ=1115, FG=458, p=0.93), respectively 53 µm s^{-1} and 56 µm s^{-1}. In contrast, swimming *R. salina* showed significantly different (2-way ANOVA, Tukey's HSD post hoc test, MQ=1115, FG=458, p=0.02) swimming speeds between the nutrient treatments. P-replete *R. salina* exhibited a swimming speed of 24 µm s^{-1}, while P-deplete ones had a significantly lower swimming speed of 16 µm s^{-1}.

With swimming speed ranging from 95 to 140 µm s^{-1}, *O. marina* didn't display large differences between the food treatments (Fig 5.1B). The only significant difference observed was between Oxy R+ and Oxy T- (1-way ANOVA, Tukey's HSD post hoc test, MQ=1630, FG=244, p=0.02).

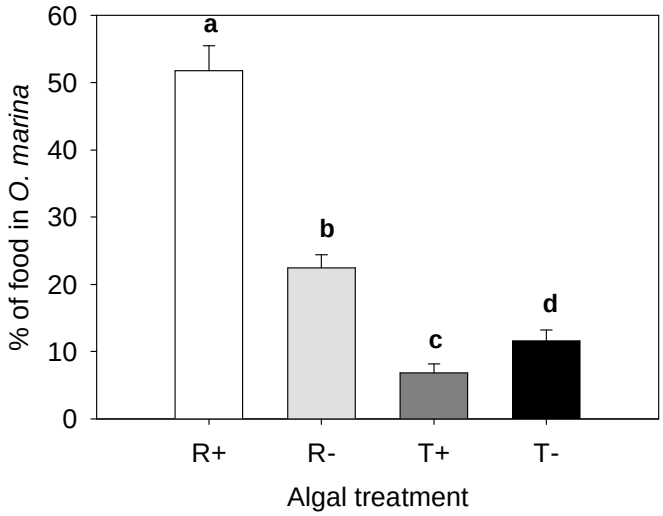


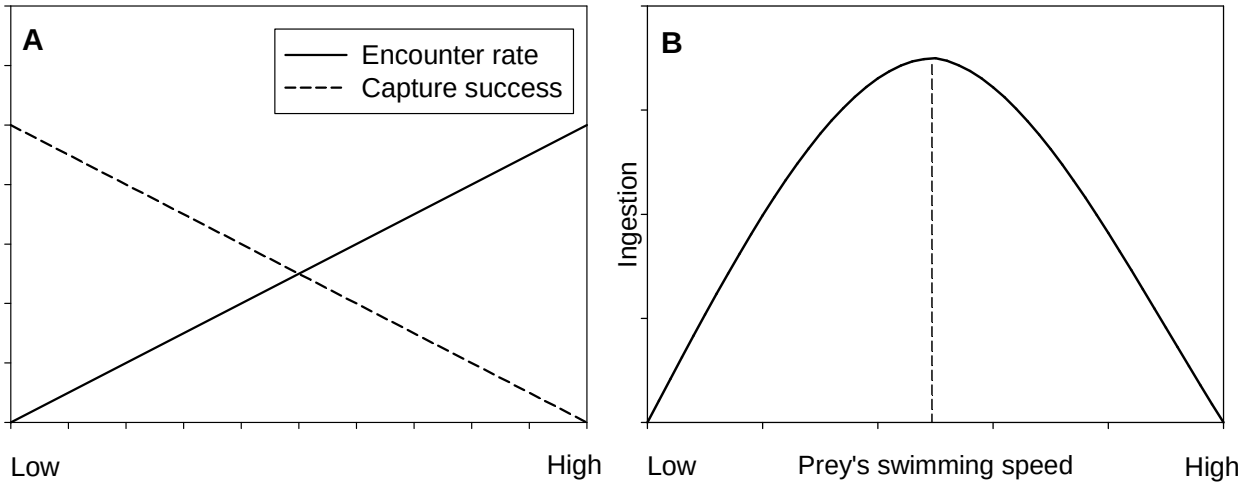
Fig 5.2 Food uptake of *O. marina* grazing on P-replete and P-deplete *R. salina* (R+; R-) and *Teleaulax sp.* (T+; T-). Values are expressed in % of *O. marina* filled with algal cells. Bars show the mean and error bars show the standard error of 30 measurements. Different letters (a,b,c,d) indicate significant differences (two-factorial ANOVA, threshold p=0.05).

Grazing experiment

The impact of algal motility on the ingestion of *O. marina* was studied in a grazing experiment using the 4 different algal treatments. *O. marina* grazed on all 4 different algal treatments but with significant differences in uptake depending on algal species and treatment (Fig 5.2). *O. marina* ingested significantly more *R. salina* than *Teleaulax sp.* cells (2-way ANOVA, $F_{1.116}$=141.85, p<0.001). Furthermore, P-limited *R. salina* was ingested significantly less (2-way ANOVA, Tukey's HSD post hoc test, MQ=164.68, FG=116, p<0.001) by *O. marina* (22% food volume of total volume) in comparison to P-replete *R. salina* (52%).

Sih and Christensen (2001) suggested that increasing prey' swimming speed leads to increasing encounter rate and decreasing capture success of the prey by the predator (Fig 5.3A). This shall result in low ingestion at low and high prey' swimming speed and maximal ingestion for intermediate prey' swimming speed (Fig 5.3B). As predicted, plotting ingestion versus algal swimming speed highlights that differences in algal motility influenced grazing activities of *O. marina* (Fig 5.3C). Surprisingly, although both P-replete and P-deplete *Teleaulax sp.* swam at the same speed, ingestion of P-replete algae was significantly lower

than of P deplete algae, (Student's *t* test, FG=58, p=0.03) indicating that in this case swimming speed differences is not the only parameter explaining variations in ingestion.



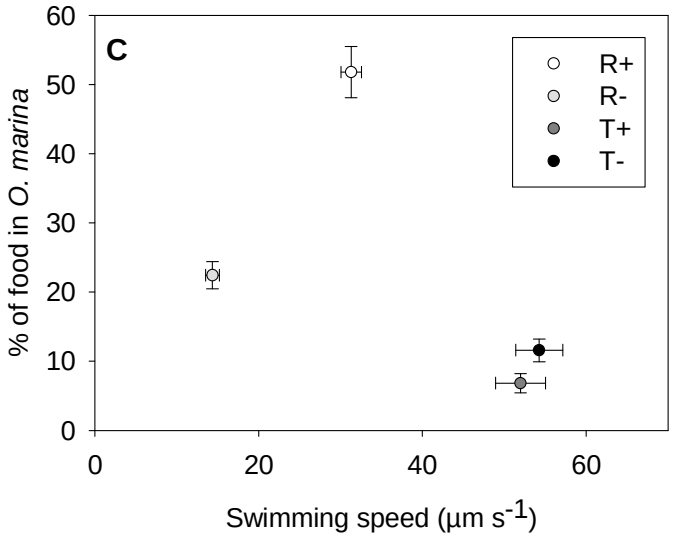


Fig 5.3 Comparison between theoretical assumption of the influence of prey swimming speed on feeding of a motile predator and our results. (A) Prey encounter rate (prey/time) and capture success (prey captured/prey encountered) (B) Predicted ingestion (number of prey ingested/time) for a motile predator. The swimming speed yielding maximum consumption rate is shown by the vertical dashed line (Nislow et al. 1999, modified) (C) Food uptake of *O. marina* grazing on P-replete and P-deplete *R. salina* (R+; R-) and *Teleaulax sp.* (T+; T-) versus the swimming speed of these different algal cells. Values are means and error bars show the standard error of 30 measurements.

Algal escape behaviour

Video recordings were also used to examine the escape behaviour of *Teleaulax sp.* during predator-prey-interactions with respect to the impact of the nutrient treatment. Such escape behaviour was never observed for *R. salina*.

We observed that after an encounter with *O. marina, Teleaulax sp.* increased its swimming speed and changed direction randomly thus actively escaping from *O. marina* (Fig 5.4). This escape behaviour was significantly affected (Student's *t* test, FG=66, $p<0.001$) by the nutrient status of the Teleaulax *sp.* cells (Fig 5.4).

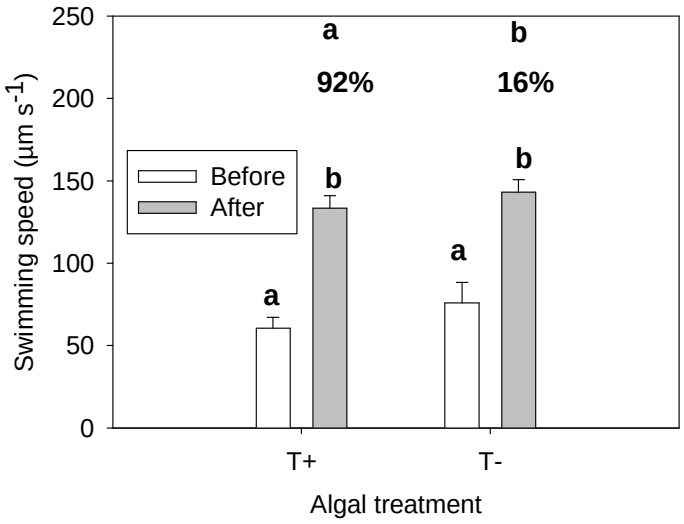


Fig 5.4 Swimming speed of *Teleaulax sp.* grown under P-replete (T+) and P-deplete (T-) conditions before and after encounter with *O. marina*. Bars show the mean and error bars show the standard error of minimum 20 measurements. Different letters (a,b) indicate significant differences (ANOVA, threshold p=0.05). Percentages indicate proportion of cells showing escape behaviour when meeting *O. marina*.

In 92% of the analyzed videos, P-replete *Teleaulax sp.* showed escape behaviour, while P-deplete *Teleaulax sp.* exhibited escape behaviour in only 16% of the interactions. Although this lower percentage was partly due to immobile cells, even among the motile P-deplete *Teleaulax sp.*, only 38% performed this escape behaviour. In those individuals that did show an escape response, the magnitude of the response was not influenced by the nutrient status of the algae (2-way ANOVA, $F_{1.54}=1.6311$, $p=0.21$). Prior to the encounter, *Teleaulax sp.* which escaped showed swimming speeds of 61 µm s^{-1} for P-replete and 76 µm s^{-1} for P-deplete cells. Post-encounter, *Teleaulax sp.* increased its velocity by a factor around 2 (133 µm s^{-1} for P-

replete and 143 μm s^{-1} for P-deplete cells (Fig 5.4) causing successful escape in all observed interactions.

Selective feeding

The grazing experiment's results allowed us to calculate the outcome of selective feeding when *R. salina* and *Teleaulax sp.* are offered together as prey to *O. marina*. We predict substantial selectivity for *R. salina* when both algae are nutrient replete and weak selectivity for *R. salina* when this species is P-deplete (Fig 5.5A). The experiment shows significant (Student's one sample *t* test, FG=7, $p<0.001$) selectivity for *R. salina* in all cases (Fig 5.5B). While no difference could be observed (Student's one sample *t* test, FG=7, $p=0.13$) between theory and observation when both offered algae were nutrient replete, there was a significant difference (Student's one sample *t* test, FG=6, $p<0.01$) between theory and observation when *R. salina* was P-deplete which implies that one of the protagonists modified its behaviour between the grazing and the selectivity experiments.

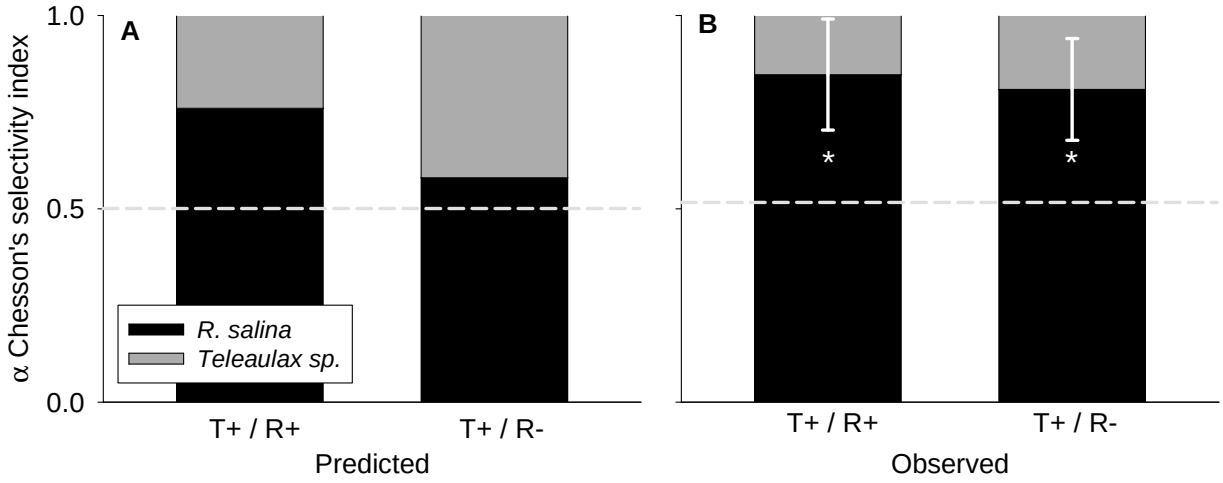


Fig 5.5 Predicted (A) and observed (B) selective feeding of *O. marina* between *R. salina* and *Teleaulax sp.* of different nutrient status (+ for nutrient replete and – for P-deplete), measured using Chesson's selectivity index (Chesson 1978; Chesson 1983). For plot B, values represent mean and standard deviation of 8 replicates. Stars indicate that $\alpha \neq 0.5$ based on Student's one sample *t* test ($p<0.05$).

5.5 Discussion

Nutrient status and motility

Heterotrophic dinoflagellates are important predators in aquatic ecosystems showing a highly variable swimming behaviour (e.g. Sheng et al. 2007). A main advantage of motility is the ability to actively search for food items in the surrounding environment. However, often prey are not passive particles but show motility as well. *O. marina* displayed swimming speeds ranging from 95 to 140 μm s^{-1}. These values are comparable to the data of Tarran (1991). He recorded mean swimming speeds of 90-179 μm s^{-1}, while in other studies higher mean swimming speeds of 307-366 μm s^{-1} were reported (Fenchel 2001; Menden-Deuer and Grünbaum 2006). Although Crawford (1992) suggests an increase of cost for motility in starving heterotrophs, in our study starved *O. marina* showed reduced cell volume and C content but did not decrease their swimming speed, indicating that costs for motility are low, as predicted by Fenchel and Finlay (1983) and Crawford (1992).

Algal swimming speeds differed significantly between species. *Teleaulax sp.* swam twice faster than *R. salina* indicating that prey motility is highly species-specific. We also observed that P limitation strongly affected the motility of both species. Although motile organisms require energy in the form of ATP, it is unlikely that P-limitation reduced ATP availability for motility because the P contained in ATP represents a very small part of the whole organismal P (0.05%) (Elser et al. 1996). Further, it is known that nutrient limitation can result in smaller flagella or deformed cell shape (Donk et al. 1997) what could have influenced motility as well.

Motility and trophic interactions

Since prey behaviour differs among species, the necessity to investigate these differences to analyze feeding activities of organisms is crucial as it influences encounter rates and capture success (Sih and Christensen 2001). Algal motility is important since prey movement enhances encounter rates with predators (Suchar and Chigbu 2006) and is a selection parameter in successful capture (Montagnes et al. 2008). Sih and Christensen (2001) suggested that the encounter rate of a predator with different prey species depends on prey

and predator motility. The encounter model of Gerritsen and Strickler (1977) predicts that slow swimming prey have low encounter rates with a motile predator, while fast prey organisms have high probabilities of encountering a motile predator. Furthermore, Sih and Christensen (2001) assume that prey and predator motility as well as post-encounter defences of prey control the capture success. This assumption was verified by several studies (Buskey 1997; Kiørboe and Titelman 1998; Tillmann and Reckermann 2002). Buskey (1997) offered non-motile diatoms and motile autotrophic dinoflagellates as prey to the pallium feeding heterotrophic dinoflagellate *Protoperidinium pellucidium* and observed that *P. pellucidium* loses contact with the dinoflagellate before the ingestion occurs. In addition, he found that motility of autotrophic dinoflagellates is used for escape and causes the capture filament of *P. pellucidium* to break. Similar results were found by Tillmann and Reckermann (2002) using the heterotrophic pallium feeding dinoflagellate *Oblea rotunda* as predator and the raphiophyte *Fibrocapsa japonica* as prey. In most cases *O. rotunda* failed to attach its tow filament after encounter with *F. japonica* and when attached to the filament, *F. japonica* was able to escape. Thus, capture success is influenced by prey handling ability of the predator and escape behaviour of prey while prey consumption is influenced by prey encounter. Slow swimming preys have low encounter rate with predators, while they are easy to handle for a predator. In contrast, highly motile prey items have higher encounter rate with predators, but they reduce the capture success because of handling difficulties. Thus, slow and fast swimming prey will lead to lower consumption of a predator.

As already mentioned, the swimming speed of algae differed between species and nutrient treatment. P-limited *R. salina* showed low swimming speeds, P-replete *R. salina* swam at moderate speeds and in both nutrient treatments *Teleaulax sp.* showed high swimming speeds. Additional microscopic observations indicated that *O. marina* ingested mainly mobile cells, we therefore used mean swimming speed of motile cells and, as predicted by Sih and Christensen (2001) and Gerritson and Strickler (1977), differences in algal motility influenced grazing activities of *O. marina*. It seems that slow swimming P-limited *R. salina* were easy to handle, but their slow swimming speed reduced the encounter rate with *O. marina* and thus also reduced ingestion. Highest ingestion of *O. marina* was observed when feeding on nutrient-replete *R. salina* indicating that their moderate swimming speed might result in high encounter rate and high handling success. Additionally, we saw in the preconditioning phase as well as in preliminary experiments that *O. marina* is able to graze and grow on

Teleaulax sp. Therefore, although *O. marina* might have had increased encounter rates with *Teleaulax sp.*, lowest ingestion could be due to handling problems with these fast swimming algae.

Algal escape behaviour

Although both P-replete and P-deplete *Teleaulax sp.* swam at the same speed, *O. marina* ingested almost twice as less P-replete algae. Hence, swimming speed differences is not the only parameter explaining variations in ingestion. Since there is a high grazing pressure on phytoplankton exerted by microzooplankton, phytoplankton species developed several defence mechanisms (Verity and Smetacek 1996; Smetacek 2001; Tillmann 2004). These mechanisms can be divided into morphological, chemical and behavioural defences. To deter predators, phytoplankton species have increased in size, formed large chains and colonies or grown spines. Furthermore, there are noxious chemicals which also provide defence. In addition, they can escape by swimming away. Furthermore, it must be noted that there is no single defence mechanism functioning perfectly against the whole range of potential predators (Tillmann 2004) and we observed (unpublished data) that *Teleaulax sp.* escape behaviour and high swimming speed didn't reduce grazing by the copepod *A. tonsa*. Moreover, Yoo et al. (2010) and Jeong et al. (2010) found that the mixotrophic dinoflagellates *Paragymnodinium shiwhaense* and *Gymnodinium aureolum* show positive growth when feeding on *Teleaulax sp*. These dinoflagellates had much higher swimming speeds compared to *O. marina. G. aureolum* presents mean swimming speed of 394 μm s^{-1} and *P. shiwhaense* reaches swimming speeds of 571 μm s^{-1}. Thus, swimming speed is clearly a species specific trait but is also influenced by environmental conditions such as salinity, temperature, and light (Hand et al. 1965; Kamykowski and McCollum 1986; Kamykowski et al. 1988).

We observed that *Teleaulax sp.* P-replete displayed escape behaviour in 92% of the encounter with *O. marina* against 16% by P-deplete algae. These dissimilarities are responsible of the different rates at which both *Teleaulax sp.* nutrient statuses were ingested. Thus, *Teleaulax sp.* showed an escape behaviour which was an effective defence mechanism against grazing of *O. marina*. Furthermore, in the case of *R. salina*, the manipulation of nutrient status caused changes in swimming speeds that significantly affected the grazing of *O. marina*. Thus, the necessity to focus on more than one parameter when studying the impact of prey features on predators' feeding activities appears evident.

Selective feeding

Although we were able to accurately predict the outcome of selectivity when *O. marina* is offered a mix of *R. salina* and *Teleaulax sp.* nutrient replete, the dinoflagellate selected strongly for *R. salina* when this alga was P-deplete while we expected only a weak selectivity. Moreover, Meunier et al. (2012) showed that this dinoflagellate is able to detect food quality differences and actively select for P-rich algal cells. Therefore, neither the swimming speed nor the quality differences of the two algal types are responsible for the divergence between calculated and observed selectivity. Since *Teleaulax sp.* possesses the capacity to escape from a predator by doubling its swimming speed and changing its swimming direction we expect that, although both prey swim at the same speed, it requires more energy for *O. marina* to catch *Teleaulax sp.* nutrient replete than *R. salina* P-deplete. Hence, the selectivity observed might result from a trade-off between energy spent and energy intake and *O. marina* may have grazed preferentially the easiest alga to catch, *R. salina*. This behaviour has been described in the optimal foraging theory developed by MacArthur and Pianka (1966) and successfully applied to various planktonic organisms. As Sih and Christensen (2001) explained, we show that applying successfully optimal foraging theory to motile prey requires knowledge about important parameters such as escape response that are needed to efficiently test the theory, and that with motile prey, differences between prey in vulnerability (encounter rate and capture success) are often more important than variation in predator active choice in determining predator diets.

To conclude, we showed that algal swimming speed is species-specific and strongly influenced by P-limitation. We illustrated that these differences between species and nutrient status had a strong impact on *O. marina*'s food uptake. Finally, we highlighted that *Teleaulax sp.* possesses an escape behaviour which was identified as an effective defence mechanism against grazing.

5.6 Acknowledgments

This study is a part of the PhD study conducted by C.L.M. at the Biologische Anstalt Helgoland, Alfred-Wegener-Institut, Germany, financed by Deutsche Forschungsgemeinschaft (DFG), and complies with current german laws and regulations on animal studies. We thank Martin Löder for useful discussions.

Chapter 6: General discussion

Stoichiometric imbalances

The elemental composition of photoautotrophic organisms is characterized by high plasticity whereas the stoichiometry of herbivores is more constant. Consequently, herbivores are faced with prey not matching their nutrient requirements which has a negative effect on somatic growth and reproduction of the consumer.

Microzooplankters are often exposed to variations of food quality and quantity in their natural habitat (Droop 1973; Hessen 1992; Martel 2010), hence they must have acquired means to handle these fluctuations. Although little is known about protozoan nutrient demands, the growth rate hypothesis allows some predictions. In cells, phosphorus is the main constituent of phospholipids, ATP/ADP, and nucleic acids. Phospholipids being a minor constituent in cells and high energy adenylates contributing less than 1% to dry weight in zooplankters (Båmstedt 1986), nucleic acids contain most of the cellular phosphorus (Elser et al. 1996). When conditions are favourable for growth, genes coding for rRNA production are activated resulting in a large assembly of ribosomes allowing an extensive protein synthesis necessary for high growth rates. Since P is the key element for growth, I hypothesized that fast growing microzooplankton have high P requirements. Hence, microzooplankters must have developed behavioural and physiological adaptations to buffer the impact of limitation by the nutrient they need the most, namely phosphorus.

Stoichiometric regulations

Grover & Chrzanowski (2006) built a mathematical model predicting that the nutrient composition of phagotrophic protists is weakly, but not strictly homeostatic. After a period of nutrient limitation, protists raise the uptake of the previously limiting nutrient once it becomes available again in order to replenish their nutrient stores (1973). But feeding goes beyond this simple replenishment. Luxury consumption results in considerable flexibility in body stoichiometry and allows these organisms to store a particular element in order to be prepared

for future nutrient limitation. Very little was still known on the dynamics of these changes, a gap I wanted to close. I highlighted that stoichiometric response to food quality and quantity change is rapid (see chapter 2) and that, accordingly to what the growth rate hypothesis predicts, the dinoflagellate *Oxyrrhis marina* has a stronger regulation against P-limitation than against N-limitation. This is coherent with other studies showing that the growth of *O. marina* is affected by P- and not by N-limitation (Hantzsche and Boersma 2010). Hantzsche and Boersma showed that whereas N-limited and nutrient replete algae resulted in similar growth rates, P-limited ones had a negative effect on the specific growth rate of *O. marina* thus highlighting that protists are affected by high C:P food in a similar way to crustacean zooplankton.

The prediction of Grover & Chrzanowski (2006) that protists are weakly homeostatic was confirmed by results of Hantzsche & Boersma (2010) and Malzahn et al. (2010). Both used *Rhodomonas salina* as prey and *O. marina* as consumer. Their results highlighted that the stoichiometric imbalances of *R. salina* were transferred to *O. marina*, but in an alleviated form. I observed that even though phosphorus depleted *R. salina* is poor quality food for *O. marina* (Hantzsche and Boersma 2010), the dinoflagellates fed more on this food source when pre-conditioned on nitrogen depleted *R. salina* to compensate for the lack of nitrogen. On the other hand, *O. marina* suffering phosphorus depletion compensated for the lack of elemental phosphorus by increased ingestion rates on nitrogen depleted *R. salina* which are phosphorus rich. This feeding strategy is typical for omnivorous or opportunistic animals (e.g. Pennings et al. 1993; Stachowicz and Hay 1996; DeMott et al. 1998) and allows to compensate the biochemical or elemental deficiencies of the food through compensatory feeding.

Selective feeding

Several studies identified that protists are able to feed selectively. Hansen (1992) and Jakobsen and Hansen (1997) reported that the heterotrophic dinoflagellates *Gyrodinium spirale* and *Gymnodinium sp.* select their prey based on size differences and have an optimum prey size corresponding approximately to their own size. However, Buskey (1997) stated that size is not the only feature influencing the selectivity of dinoflagellates but until now few studies investigated selective feeding with food quality as the only variable parameter. I highlighted that the dinoflagellate *O. marina* (see chapter 3) is selecting for prey items based on food quality differences. As I expected based on the growth rate hypothesis, this

dinoflagellate species, independently on its precondition, always selected for P-rich algal cells.

As I described for microzooplankton, metazoan grazers also experience an unbalanced food supply. I hypothesized that mesozooplankters feed selectively for food quality differences to obtain a balanced diet (Anderson and Pond 2000). In the fourth chapter I showed that, as other species (Villar-Argaiz et al. 2002), the copepod *Acartia tonsa* exhibits strong stoichiometric variations with ontogeny. Consequently, *A. tonsa* developmental stages selected for prey rich in the nutrient they need the most (P for nauplii and N for copepodites). Further, I showed that naupliar density correlates with the rate of change of dissolved N:P ratio. As other studies already suggested (Elser and Urabe 1999; Vanni 2002), this implies that, due to the retention of P as well as the selective removal of this element, nauplii substantially impact dissolved N:P ratios.

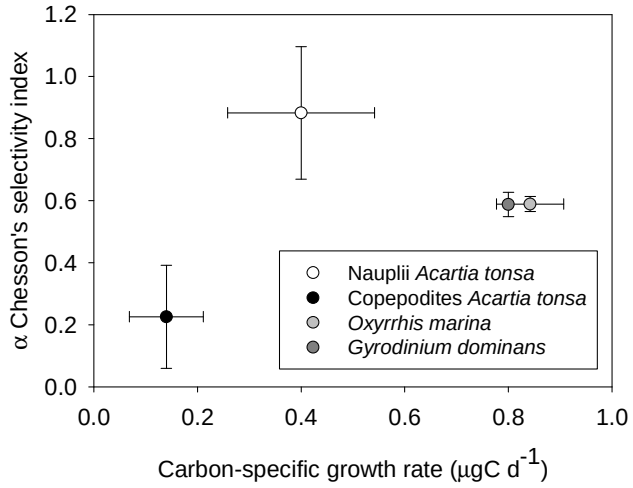


Fig 6.1 Selectivity for P-rich *R. salina* prey of different grazers versus grazers' growth rate. Growth rates of *O. marina* and *G. dominans* are issued of the work by Hantzsche and Boersma (2010) and Nakamura et al. (1995). Data for *O. marina* selectivity and *A. tonsa* growth and selectivity are from chapter 3 and 4.

Based on the growth rate hypothesis prediction that consumers with higher growth rates have higher P requirements, I expect stronger selectivity for P-rich prey, which I observed (Fig 6.1). Since metazoan organisms are more complex than unicellular ones, they may have more developed sensory apparatus for selective feeding explaining stronger selectivity by the copepod *A. tonsa* compared to dinoflagellates.

Stoichiometry, motility and trophic interactions

Planktonic grazers are able to select their prey based on food quality (Cowles et al. 1988; Meunier et al. 2012), taxonomical differences (Flynn et al. 1996; Irigoien et al. 2000; Fileman et al. 2007) and prey size (Paffenhöfer 1988; Hansen et al. 1996) but very little is known on the impact of prey swimming speed and behaviour. As already shown for heterotrophic plankters (e.g. Sheng et al. 2007), I identified that algal swimming speed is species-specific. Since I observed that P-limitation strongly impacts motility, I focused on the indirect effect of P-limitation on food uptake and trophic interactions. My results confirm the predictions of Sih and Christensen (2001) and Gerritsen & Strickler (1977) that the encounter rate of a predator with different prey species depends on prey and predator motility and that prey and predator motility as well as post-encounter defences of prey control the capture success. In order to escape the high grazing pressure exerted by planktonic grazers many phytoplankton species developed escape mechanisms (Verity and Smetacek 1996; Smetacek 2001; Tillmann 2004). I recorded that *Teleaulax sp.* possesses such an escape behaviour which was an effective defence mechanism against grazing of *O. marina*.

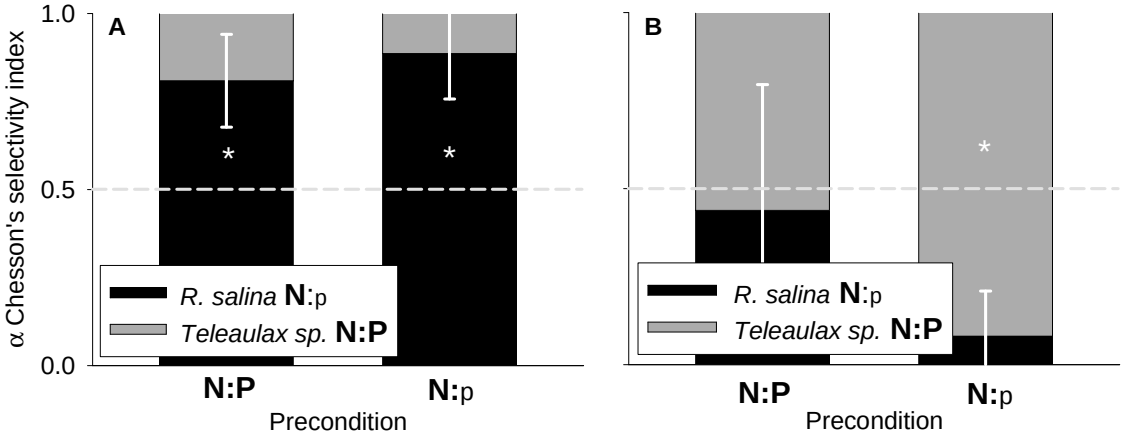


Fig 6.2 Selective feeding between *Rhodomonas salina* P-deplete and *Teleaulax sp.* P-replete of differently preconditioned *O. marina* (panel A) and the copepodite stage of the copepod *A. tonsa* (panel B), measured using Chesson's selectivity index (Chesson 1978; Chesson 1983). Values represent mean and standard deviation of 4 replicates for *O. marina* and 10 replicates for *A. tonsa*. Stars indicate that $\alpha \neq 0.5$ based on Student's paired t-test ($p<0.05$).

High swimming speed and to a second degree escape behaviour had a strong impact on *O. marina* selective feeding (Fig 6.2A, see also chapter 4) while it did not affect grazing of the copepodite stage of the copepod *A. tonsa* (Fig 6.2B, additional results to make the comparison with the data of chapter 4). This confirms that there is no single defence mechanism functioning perfectly against the whole range of potential predators (Tillmann 2004). Although usually selecting for P-rich prey, the dinoflagellate selected, independently on its precondition, for P-deplete *R. salina* instead of P-replete *Teleaulax sp*. On the other hand, when preconditioned on P-limited food, copepodites selected for P-rich *Teleaulax sp.*, while they didn't select when preconditioned on good food quality indicating that grazers are able to recognize their nutrient status and to adapt their feeding behaviour accordingly.

Prey recognition

I showed that consumers, both proto- and metazoans, are able to select for their food based on quality differences. This capacity of grazers to identify the nutritional status of their prey is certainly based on a sophisticated mechanism which, ultimately, enables consumers to buffer nutritional imbalances of their prey via compensatory and selective feeding. Recent studies identified some of the biochemical mechanisms responsible for the ingestion or rejection of food particles in microzooplankters (Nakamura et al. 1995). Roberts et al. (2006) and Wootton et al. (2007) discovered that the cell surface of the heterotrophic dinoflagellate *O. marina* contains lectins, which are highly specific carbohydrate-binding proteins. These proteins might bind with species-specific glycoconjugates of the prey and thus initiate phagocytosis (Martel 2009b). These authors suggested that the nutritional status of phytoplankton cells modifies their structural or species-specific glycoconjugates on the cell surface. Hence, the heterotrophic dinoflagellate may be able to identify the nutritional status of the prey cells during cell-to-cell contact via the detection of these glycoconjugates (Martel 2006; Martel 2009b). Copepods are also able to feed selectively on food particles and, as for microzooplankton, several authors suggested that different cues such as biochemical composition and chemoreception may be involved in prey recognition (Poulet and Marsot 1978; Cowles et al. 1988; Butler et al. 1989). For instance Kiørboe (2011) identified that copepods are able to encounter aggregates such as marine snow by finding and tracking the chemical trail left by the sinking aggregate, similar mechanisms may be involved in prey detection and selection. As a function of their nutritional status or growth stage, algae excrete specific substances (Wetz and Wheeler 2007) composed of different biochemicals such as

carbohydrates and amino acids (Granum et al. 2002) which may serve as attractors or inhibitors for herbivores (Strom et al. 2003; Davidson et al. 2005; Martel 2006; Strom et al. 2007).

Implications for pelagic food webs

As I previously explained, *O. marina* and maybe other protozoans lie, in term of homeostasis ability, between metazoans and autotrophs. Based on theoretical considerations, this would have implications for omnivorous grazers such as copepods. Since they buffer the nutrient imbalances of their food and are trophic upgraders (Malzahn et al. 2010), protozoan grazers are always of better food quality for herbivores compared to autotrophs but are, under normal conditions, very diluted. However, after periods of nutrient limitation (e.g. late spring bloom), protozoan grazers are more abundant and, due to the physiological momentum of their homeostatic regulation, protozoan grazers should represent a substantial part of omnivores' diets. Selective behaviour of mesozooplankters for microzooplankton prey has been reported by several authors (Hansen et al. 1993; Fileman et al. 2007; Löder et al. 2011). Löder et al. (2011) conducted a series of dilution experiments during a mesocosm study which reproduced in the laboratory a phytoplankton bloom. By disentangling the relative impact of micro- and mesozooplankters grazing they highlighted that copepods selected for microzooplankton prey and this selectivity increased at the end of the bloom, certainly due to a decrease in phytoplankton quality.

Implications

The results presented in this thesis allow a better understanding of the importance of nutrient supply rates and ratios and increase our predictive ability on the impact of altered marine biogeochemistry on coastal food webs. Since the industrial revolution, human activity has altered the natural biogeochemical cycle of nitrogen and phosphorus on a global scale, with consistent emissions in waters (Vitousek et al. 1997; Foley et al. 2005; Galloway et al. 2008). As a consequence, eutrophication has lead to e.g. increased micro- and macroalgal biomass, anoxification, especially in deep water layers and changes in species composition and diversity (Carpenter et al. 1998; Cloern 2001; Smith 2003; Smith and Schindler 2009). A

recent analysis of the effects of European environmental policies shows that measures to reduce phosphorus were more successful that those tackling nitrogen (Grizzetti et al. 2012). Moreover, the main source for P fertilizers used in modern agriculture is mined phosphate rock, which is a non-renewable resource and, under a continuously increasing demand, the reserves may be depleted in 50-100 years (Cordell et al. 2009).

Long-term studies evidenced a steady increase of the N:P ratio in coastal waters which could increase eutrophication in N-limited systems, reducing biodiversity and the ecosystem's resilience to future additional anthropogenic stress (Grizzetti et al. 2012). As a result, increased N:P ratios of autotrophic food sources will lower growth rates of P-rich grazers such as microzooplankters and nauplii. Only reduced allocation to ribosomal RNA is possible under P limitation, thus offsetting high growth rates and favouring species with lower growth rates (Peňuelas et al. 2012). Moreover, the raise in dissolved carbon resulting from the amplified CO_2 emissions will increase C availability. This will intensify photosynthesis rates and C uptake, increasing the C:N:P ratios of autotrophs thus reducing further their quality as food. Ultimately these changes in biogeochemical cycles will have a global negative impact on the productivity in the oceans and will not only affect low trophic levels in food webs but potentially also e.g. fish abundances and consequently fisheries and economy, as we know that these food quality effects travel up the food chain (Boersma et al. 2008).

Outlook

My work shows that planktonic grazers possess the capacity to feed selectively based on food quality differences. Nevertheless, we know nothing about the range of food quality variations consumers experience in the nature. To be able to feed selectively, a consumer needs to invest in sensory apparatus which represent a cost. Hence, if herbivores would not face food items of different food quality, it is unlikely that this strategy would have evolved. In order to investigate the magnitude of food quality variations a consumer experience, tools such as the Raman spectroscopy (Colthup et al. 1990) may be useful. This promising technique should allow measuring the elemental content of individual cells, since only the measurement of individual cells will allow us to assess whether selective feeding capability based on within species differences in quality is really necessary.

Moreover, the basic mechanisms behind selectivity remain unknown. For instance, it has not yet been clarified whether grazers' selection is based on pre- or post-contact mechanisms. Observing and filming with high speed video plankters feeding would allow identifying key feeding and motility behaviour. It has been described that prey cells smaller than 5 µm generate too small chemical and hydromechanical signals to allow remote detection; these signals simply dissipate almost instantaneously (Kiørboe 2008). Hence, if selectivity is based on pre-contact signals, grazers may feed selectively only on prey larger than 5 µm. This hypothesis can be tested in microcosms, measuring the ability of herbivores to feed selectively on a mix of nutrient replete and nutrient deplete small phytoplankton on the one hand and larger phytoplankton on the other hand.

I showed that *Teleaulax sp.* possesses the ability to double its swimming speed and change its swimming direction to escape a predator. It might be interesting to observe the behaviour of this species in the presence of an ambush feeder since in this case the best strategy is to stop moving to avoid detection by the predator (Kiørboe and Visser 1999). I observed this behaviour in the dinoflagellate *O. marina* (personal observation) which stopped swimming in the presence of the copepod *A. tonsa* and, once the copepod went away, the dinoflagellate started to swim again. In this case it would be interesting to observe how *O. marina* behaves in the presence of a feeding-current feeder.

Further investigations on the impact of nutrient availabilty on selective feeding as well as on swimming strategies of plankters are crucial to our understanding of aquatic ecosystem functioning and identifying the inherent trade-offs is an issue that needs to be thoroughly addressed when conducting future research in the field of ecological stoichiometry.

Summary

Metazoan grazers such as copepods are homeostatic with respect to their body nutrient composition, which means that they are able to buffer nutrient imbalances between their demand and the supply by their prey, thus keeping their C:N:P stable. Homeostatic regulation can occur post-ingestion, but also several pre-ingestion mechanisms are known, and, when experiencing an unbalanced food supply, grazers may feed selectively for food quality differences to obtain a balanced diet. I showed that the copepod *Acartia tonsa* exhibits strong stoichiometric variations with ontogeny. Consequently, *A. tonsa* developmental stages selected for prey rich in the nutrient they need the most (P for nauplii and N for copepodites). Further, I evidenced that naupliar density substantially impacts dissolved N:P ratios which I attribute to selective removal of P-rich cells as well as selective retention of phosphorus by nauplii.

As mesozooplankters, microzooplankters are often exposed to variations of food quality and quantity in their natural habitat, hence they must have acquired means to handle these fluctuations. I highlighted that the dinoflagellate species *Oxyrrhis marina* selects for prey items based on food quality differences. Independently on its precondition, the dinoflagellate always selected for P-rich algal cells. Even though phosphorus depleted *R. salina* is poor quality food for *O. marina*, it fed more on this food source when pre-conditioned on nitrogen depleted *R. salina* to compensate for the lack of nitrogen. On the other hand, *O. marina* suffering phosphorus depletion compensated for the lack of elemental phosphorus by increased ingestion rates on nitrogen depleted *R. salina* which are phosphorus rich. This feeding strategy allows compensating the biochemical or elemental deficiencies of the food through compensatory feeding. Very little was still known on the dynamics of stoichiometric changes in dinoflagellates, a gap I wanted to close. I highlighted that stoichiometric response to food quality and quantity change is rapid and that *O. marina* has a stronger regulation against P-limitation as against N-limitation. This is coherent with the fact that the growth of *O. marina* is affected by P- and not by N-limitation.

Planktonic grazers are able to select their prey based on food quality, taxonomical differences and prey size but very little is known on the impact of prey swimming speed and behaviour. I

identified that algal swimming speed is species-specific and that P-limitation strongly impacts motility. These differences in motility had a strong impact on *O. marina*'s feeding activity which confirms the predictions that the encounter rate of a predator with different prey species depends on prey and predator motility and that prey and predator motility as well as post-encounter defences of prey control the capture success. In order to escape the high grazing pressure exerted by planktonic grazers, many phytoplankton species developed escape mechanisms. I recorded that *Teleaulax sp.* possesses such an escape behaviour which was an effective defence mechanism against grazing of *O. marina*. High swimming speed and to a second degree escape behaviour had a strong impact on *O. marina* selective feeding while it did not affect grazing of the copepodite stage of the copepod *A. tonsa* showing that there is no single defence mechanism functioning perfectly against the whole range of potential predators.

These results allow a better understanding of the importance of nutrient supply rates and ratios and increase our predictive ability on the impact of altered marine biogeochemistry on coastal food webs. Long-term studies evidenced a steady increase of the N:P ratio in coastal waters resulting in increased N:P ratios of autotrophic food sources which will lower growth rates of P-rich grazers such as microzooplankters and nauplii. Moreover, the increase in carbon availability will increase the C:N:P ratios of autotrophs thus reducing their quality as food. Ultimately these changes in biogeochemical cycles will have a global negative impact on the productivity in the oceans and will not only affect low trophic levels in food webs but also e.g. fish abundances and consequently fisheries and economy.

Zusammenfassung

Herbivore Metazoen wie z.B. Copepoden sind bezüglich ihrer Nährstoffzusammensetzung homöostatisch. Das heißt, sie sind in der Lage Diskrepanzen zwischen dem Nährstoffangebot ihres Futters und ihrem eigenen Nährstoffbedarf abzupuffern. Dadurch halten sie ihr C:N:P Verhältnis stabil. Homöostatische Regulation kann unter Umständen schon vor der Nahrungsaufnahme stattfinden. Im Falle von stark unausgeglichenen Nährstoffverhältnissen zwischen Futterangebot und Bedarf kann es zu selektiver Aufnahme der Nahrungspartikel kommen, die dem Bedarf des Konsumenten entsprechen. Der Copepode *Acartia tonsa* durchläuft während der ontogenetischen Entwicklung starke Veränderungen in seiner Nährstoffstöchiometrie und zeigte ein selektives Fraßverhalten mit Bevorzugung der in der jeweiligen Lebensphase am meisten benötigten Nährstoffe (P bei Nauplien und N bei Copepoditen). Weiterhin konnte gezeigt werden, dass Nauplien durch selektives Fressen von P-reichen Zellen und geringen P-Ausscheidungsraten einen wesentlichen Einfluss auf das Verhältnis von gelöstem Stickstoff zu gelöstem Phosphor haben.

Genau wie das Mesozooplankton ist auch das Mikrozooplankton starken Fluktuationen bezüglich der Nahrungsqualität unterworfen, es kann also davon ausgegangen werden, dass auch diese Gruppe von Planktern Mechanismen entwickelt hat, um hierauf zu reagieren. Es konnte gezeigt werden, dass die beiden Dinoflagellaten *Oxyrrhis marina* und *Gyrodinium dominans* in der Lage sind ihre Nahrung nach Qualitätsmerkmalen zu selektieren. Beide Dinoflagellaten zeigten unabhängig von ihrem eigenen Nährstoffstatus eine positive Selektivität für P-reiches Futter. Zusätzlich konnte gezeigt werden, dass *O. marina* nur bei bereits biochemisch prozessiertem P Selektivität zeigte, während *G. dominans* in der Lage war auch elementares P in der Nahrung wahrzunehmen und positive zu selektieren. Wenn *O. marina* mit N-armer Nahrung vorkonditioniert wurde selektierten sie allerdings positiv zu P-limitierten Algen um ihren Stickstoffmangel auszugleichen. Umgekehrt selektierten *O. marina* die mit P-limitierten Algen vorkonditioniert wurden positiv zu N-limitierten Algen, die reich an Phosphor waren, um ihren P-Mangel auszugleichen. Diese Ernährungsstrategie erlaubt es diesen Dinoflagellaten Nährstoffmängel durch selektive Fressverhalten zu kompensieren. Die Dynamik von stöchiometrischen Veränderungen von Dinoflagellaten war

bisher weitgehend unbekannt. In dieser Arbeit konnte gezeigt werden, dass es zu einer schnellen Veränderung der Nährstoffverhältnisse kommt und dass *O. marina* stärker gegen eine P-Limitation als gegen eine N-Limitation reguliert. Dieses Ergebnis hängt damit zusammen, dass das Wachstum von *O. marina* stärker von P als von N beeinflusst wird.

Die Tatsache, dass planktische Herbivore in der Lage sind nach Nahrungsqualität, taxonomischen Unterschieden und Partikelgröße zu selektieren ist nun hinlänglich bekannt, der Einfluss von Schwimmfähigkeit und Schwimmverhalten von Beuteorganismen ist hingegen wenig erforscht. In dieser Arbeit wurde gezeigt, dass die Schwimmgeschwindigkeit von Algen abhängig von der Art ist und durch P-Limitation stark beeinflusst wird. Diese Unterscheide in der Beweglichkeit von Beuteorganismen hatte einen starken Einfluss auf die Fraßaktivität von *O. marina* und zeigt, dass die Kontaktraten von Räuber und Beute sowohl von der Beweglichkeit von Räuber als auch der Beute abhängig sind. Eine weitere Einflussgröße auf den Fangerfolg ist das Verhalten während der Begegnung zwischen Räuber und Beute. Viele marine Phytoplankter haben Fluchtverhalten entwickelt um erhöhtem Räuberdruck zu entkommen. In dieser Arbeit konnte gezeigt werden, dass *Teleaulax sp*. ein solches Fluchtverhalten an den Tag legt. Eine hohe Schwimmgeschwindigkeit und gekoppelt mit einem gerichteten Fluchtverhalten hatte einen großen Einfluss auf die Nahrungsselektivität von *O. marina*, juvenile Stadien von *A. tonsa* hingegen veränderten ihr Fraßverhalten darauf basierend nicht. Dies Ergebnis zeigt, dass ein einzelner Abwehrmechanismus nicht gegen alle Arten von Fraßfeinden hilft.

Die in dieser Arbeit vorgestellten Ergebnisse führen zu einem besseren Verständnis der Bedeutung von Nährstoffangebot und verhältnis und können dabei helfen bessere Vorhersagen über den Einfluss von Veränderungen der Biogeochemie auf Küstennahrungsnetze zu machen. Langzeitstudien haben gezeigt dass ein ständiger Anstieg des N:P Verhältnis im Küstenwasser zu einer Erhöhung des N:P Verhältnisse in autotrophen Organismen führt. Dieses wiederum führt wahrscheinlich zu einer Abnahme der Wachstumsraten von P-reichen Organismen wie Nauplien und Mikrozooplanktern. Zusätzlich könnte ein erhöhtes Angebot von Kohlenstoff durch eine Erhöhung des CO_2 Gehalts der Atmosphäre zu einer Erhöhung der C:N:P Verhältnisse von Primärproduzenten führen, was zu einer weiteren Verschlechterung der Nahrungsqualität für Herbivore Organismen führen würde. Schlussendlich können diese biogeochemischen Veränderungen dazu führen, dass sich die globale Produktivität der Ozeane verringert, was sich nicht nur auf die niedrigsten

trophischen Ebenen auswirken würde, sondern zum Beispiel auf Fischbestände und damit auch ökonomische Einbußen in der Fischerei nach sich ziehen würde.

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Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit- einschließlich Abbildungen und Tabellen - die ich anderen Werken im Wortlaut oder dem Sinn nach entnommen habe, in jedem Einzellfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie -abgesehen von den auf der folgenden Seite angegebenen Teilpublikationen- noch nicht veröffentlicht worden ist sowie, dass ich solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Maarten Boersma und Dr. Arne M. Malzahn betreut worden.

Helgoland, den 17.03.12 _____

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Curriculum vitae

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Research Interests

- **Trophic interactions** in planktonic food webs
- **Ecological stoichiometry**
- **Behavioural ecology** of plankton

Education

- B.Sc. (Biology), University of South Brittany (France), 2007
  - **Bachelor thesis** within **Roscoff Biological Station** (France, CNRS), supervised by A. Andersen, studying the **beardworms hemoglobin**, 2007
- M.Sc. (**Biological Oceanography**), University of Paris (France), 2009
  - **Master first year thesis** within **Villefranche Zoological Station** (France, CNRS), supervised by A. Sciandra, investigating the **phytoplanktonic lipid metabolism** in day / night cycle, 2008
  - **Master thesis** within **Helgoland Marine Station** (Germany, AWI), supervised by N. Aberle-Malzahn, examining the role of **food quality** and **selective feeding** of **microzooplankters**, 2009
- Ph.D. (Biology), University of Oldenburg (Germany), 2012
  - Thesis within **Helgoland Marine Station** (Germany, AWI), supervised by A. Malzahn, M. Boersma and H. Hillebrand, entitled **you eat what you need: food quality and trophic interactions in planktonic food webs**

Responsibilities

- **PhD representative**, 2010 to 2011
- **Expedition** to the **Lena Delta** (Siberia) during 3 weeks, 2011
- **Responsible** of Helgoland Marine Station's **phyto- and microzooplankton culture bank**, 2009 to 2012

Others

- Diving team chief, 2003 to 2004
- Office job, summer 2004 to 2008
- Activity leader in outdoor centre, summer 2006

Teaching experience

- Teaching in a **secondary school biology class** (6 months), 2005
- Supervision of one **internship student** (6 weeks) on the topic **food quality-induced** prey **selective feeding** by the heterotrophic dinoflagellate *Oxyrrhis marina*, 2010
- Supervision of one **Bachelor student** (8 weeks) on the topic protozoan **food selectivity** linked to **algal motility** and **nutrient status**, 2011
- Supervision of one **Master student** (6 months) on the topic **zooplankton selective feeding** for **intra- and inter-specific food quality differences**, 2011

Other Skills

- Experience with **research technology** (MALLS, Transmission Electronic Microscopy, HPLC, several cell counters, Diffusion Chamber)
- Rescue Diver PADI, 2005

Oral presentations

- **Invited speaker** at Oldenburg University « **Importance of food quality and motility in predator-prey interactions**», 2011
- **ASLO conference** in Puerto Rico « **Selectivity of protozoan and metazoan herbivores for intraspecific food quality differences** », 2011
- **Selected speaker** for the **mid-term evaluation** of the **Alfred Wegener Institute** « **Ecological stoichiometry in aquatic food webs: multitrophic interactions from algae to copepods** », 2011
- **Invited speaker** at the **IFM-GEOMAR** (Kiel, Germany), 2012
- **Invited speaker** at the **Sven Lovén Centre** for Marine Sciences (Kristineberg, Sweden), 2012

Publications

- **Meunier**, C., Andersen, A. C., Bruneaux, M., Guen, D. L., Terrier, P., Leize-Wagner, E. and Zal, F. (2010) Structural characterization of hemoglobins from monilifera and frenulata tubeworms (siboglinids): First discovery of giant hexagonal-bilayer hemoglobin in the former "pogonophora" group. *Comparative Biochemistry and Physiology, Part A,* **155,** 41-48.
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- **Meunier**, C. L., Boersma, M. and Malzahn, A. You are what you eat, or you eat what you are: age specific selective feeding in the copepod *Acartia tonsa*. **in preparation**.

Language skills

- **English : fluent**
- **French : fluent**
- **German : moderate**

Interests

- Fishing
- Rugby
- Diving

Acknowledgments

I thank my advisor Arne Malzahn for making this thesis possible, for always taking time to answer my questions and for sharing his knowledge on fishes and how best to sample them.

I thank Maarten Boersma for his insight, patience and guidance in both professional and personal matters.

I would like to thank my supervisor from Oldenburg University, Helmut Hillebrand, for providing continuous and valuable feedback.

This work was supported by the Deutsche Forschungsgemeinschaft. The financial support is gratefully acknowledged.

This thesis is also the result of the work of a number of students, technicians and collaborators. I owe a lot to Karoline Schulz, Alessandra Dupont, Bettina Oppermann, Julia Haafke, Axel Orban, Christina Krüger, Katharina Funk, Christoph Plum, Florian Hantzsche and Katherina Schoo, with whom it was a real pleasure to work.

A large group of colleagues have inspired and challenged me with their intellect and work ethic. I thank all my colleagues and friends from Helgoland and Oldenburg.

I thank my friends for all the good times we had and for their support in difficult moments: Tommä, Betti, Maddin, Sonni, Judith, Kat, Angel, Matze, Steffi, Nari and the whole OEM group, Rebi for the wonderful last three years, Alice, Elsa and all the ones I may have forgotten.

I thank my parents and my family for their constant support and encouragement.