Effects of predation risk on life history strategies in marine plankton

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Preface

Since the beginning of this PhD project in 2010, several people have been involved at different stages, and they have all been essential for the realization of this thesis.

Firstly, I have been helped and motivated by two outstanding supervisors. Josefin Titelman, thank you for always being eager to answer and discuss my questions, and for sharing clever ideas and solutions, as well as your experiences on life as a scientist. Also, your constant optimism and smiling personality makes everything easier. I have also benefited a lot from Tom Andersen’s seemingly unlimited knowledge in biology and statistics; thank you for sharing R-scripts, motivating comments, and ironic humor! The support from both of you has been invaluable!

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Friends and family; thank you for support, patience and much needed quality time!

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List of papers

Paper I

Paper II

Paper III

Paper IV
Summary

This thesis explores how different aspects of life history strategies in common marine planktonic organisms change when they perceive a chemical cue of predation risk. Predators can have dramatic effects on prey organisms by inducing responses only by their presence. Such induced defenses can actually have stronger effect on a community than the consumption itself, as the defenses influence a larger proportion of the prey and may create cascading effects in the food web. Despite their huge ecological importance much is still unknown about how marine plankton adjust life history traits according to prevailing predation pressure.

Here, I have looked into some life history traits in marine plankton when experimentally exposed to predator chemical cues. Three studies focus on copepods, the dominating group of zooplankton. Importantly, my findings suggest that marine copepods do respond to predator chemical cues by altering several key life history traits. Paper I shows an effect of perceived predation risk on growth and development on naupliar stages of a common coastal species. In paper II non-consumptive effects on egg-carrying were demonstrated. Paper III documents significant costs in male copepod spermatophore production. Perceived risk generally altered swimming behavior and also feeding rates under food limited conditions. All in all, papers I-III demonstrate that vital life history traits in copepods, like growth, development and reproductive investments by both females and males are to some extent regulated by predation risk. Paper IV considers chain length plasticity in a diatom, one of the most ecologically important phytoplankton groups. Based on experiments and an empirical model, we show that chain length plasticity in a common diatom species is consistent with a grazer avoidance strategy.

Altogether, the results presented in my thesis suggest that predation risk is one of the mechanisms that regulate fundamental life history traits in marine plankton.
Introduction

The major objective in the life of any organism is to survive, eat and reproduce. These are the basic components of fitness. Yet, the most critical of these is survival. As the biotic environment is full of life threatening agents, natural selection strongly rewards traits and strategies that protect an organism from deadly interactions. The evolutionary ‘arms race’ between a prey and its predator give rise to ever more sophisticated defenses. However, staying alive is of little importance without being able to acquire resources and eventually reproduce. Exposure to predation risk is very often elevated under feeding activity and reproductive efforts (Magnhagen 1991). Defenses, be they behavioral, morphological or life history traits, may directly conflict with efficient feeding or reproductive rates, and they can be energetically demanding to maintain (Tollrian and Harvell 1999). Thus, the different objectives must be balanced. To optimize such tradeoffs inducible defenses evolved, which in contrast to constitutive traits are expressed only when the risk exceeds a certain level. For inducible defenses to evolve, four requirements must be met: The predation risk must be variable and unpredictable, there must be a reliable cue that informs the prey about the risk, the defense must be effective reducing the risk of predation, and finally there must be a cost to the response. If not, permanent defense mechanisms are more likely to evolve (Tollrian and Harvell 1999).

A typical predation event starts with an encounter, followed by persuasion, attack and consumption (Lima and Dill 1990). Defenses can be behavioral, aiming at avoiding encounter or escaping before the attack. Morphological defenses such as a change in transparency or shape can prevent detection and predator attack (Ohman 1988). And physiological defenses like for example production of toxins can deter the predator from continuing its feeding activity (Kats and Dill 1998). Predation risk may fluctuate in time, depending on the behavior and population dynamics of the predators. The time scale of the risk, relative to the prey might mirror the type of response it induces. Risk can be urgent, inducing escape behaviors. Risk can also be elevated over a time period where the prey may temporarily modify its activities, or even induce morphological traits. In an even longer time
perspective a prey can adjust its life history; the timing of events in a life such as growth, maturation and reproduction. A prey is typically threatened by more than one predator. Sometimes different predators select for contrasting phenotypic responses in a prey (Hoverman and Relyea 2009). Then the relative intensity creates heterogeneity in the risk regime (Gabriel et al. 2005).

Induced defenses can have dramatic ecological consequences. The effects of predators on prey activity, such as reduced feeding and mating, influences energetic and reproductive success of the prey (Boonstra et al. 1998). Furthermore, indirect predator effects can have equally strong impact on community level as the direct effects of predation because they influence a larger part of the population (Peacor and Werner 2001). Also, induced responses influence community structure by promoting coexistence of species (Aránguiz-Acuña et al. 2011).

Papers I – IV explore responses to perceived predation risk in behaviors and investments related to growth and reproduction in two common groups of plankton, copepods and diatoms. In the following I first introduce the background to my studies, and then highlight and discuss the most essential findings from each paper in the context of ecology and life history theory. When possible I refer to review papers rather than original papers. As my papers focus on the role of perceived risk in the form of chemical cues I will focus on this in the following and, with a few exceptions, not elaborate further on other types of signals despite their importance.

**Aquatic chemical communication**

Light is important in aquatic systems, for both bottom-up and top down processes. It is a prerequisite for photosynthesis, and also for visual predation. The vertical gradient and diel cycle of in light intensity to some extent drive distribution and behavior of organisms (Miller 2004). Many pelagic inhabitants have light sensitive organs, yet large groups of animals such as zooplankton have poor vision with no image forming eyes and must rely on other cues to be aware of the more unpredictable events around them.

Chemical signals are an important source of information in any ecosystem, and particularly in aquatic environments (Kats and Dill 1998). In water, diffusion of small molecules is
relatively slow, creating persistent chemical trails and strong concentration gradients (Webster and Weissburg 2009). All organisms emit chemical cues, leaving a complex mixture of chemical information that allows others to track their distance and recent activities. Consequently, aquatic organisms often navigate in the landscape of predation risk, mating opportunities, food patches and competitors using chemosensory assessment. The ability to detect chemical cues is strongly favored among aquatic organisms; hence their chemosensory system is often well developed. Most crustaceans have chemosensory sensillae densely distributed on their feeding appendages and antennae, as well as sensillae on the body surface. These are for remote, as well as contact assessment of chemical cues (Hallberg and Skog 2011).

Chemical information is used in a variety of intra- and interspecific interactions in aquatic environments, e.g. between mates, competitors, parasites and hosts, and predators and prey (Brönmark and Hansson 2012; Roy et al. 2013). There are striking examples of predator-induced responses known from aquatic systems. Rotifers develop long spines (Gilbert 1966), *Daphnia* form ‘helmets’ (Herzog and Laforsch 2013), and carp fish change their body shape (Brönmark and Miner 1992) to become difficult to ingest in the presence of predators. Among life history responses are altered growth rate and onset of reproduction (Macháček 1995; Gutierrez et al. 2010), induction of diapause eggs (Hairston and Walton 1986), and suppression of egg production (Wasserman and Froneman 2013) and mating (Lasley-Rasher and Yen 2012).

The vast majority of studies on aquatic chemical ecology originate from freshwater habitats (Kats and Dill 1998; Ferrari et al. 2010). Perhaps inducible defenses are more common in closed systems where the predator cues predict predation pressure more reliably. On the other hand, the risk regime might be more variable in the sea than in lakes. Nevertheless, evidence of induced defenses in response to predator or grazer chemical cues in the marine environment has accumulated over the last decades, and is still growing rapidly. Of the marine species, benthic and sessile organisms have probably received the most attention (Harvell 1999), but also for plankton the number of reports about chemically induced defenses is growing (Paul and Ritson-Williams 2008; Poulson et al. 2009; Roy et al. 2013; Heuschele and Selander 2014).
Aquatic chemical ecology contributes to better understanding of how predators shape pelagic individuals and communities. Non-consumptive effects of predator presence have shed light on the impact of top-down processes in marine systems, where the bottom-up and harvest-driven perspective has traditionally dominated (Verity and Smetacek 1996).

There are different categories of chemical signals that can be associated with predation risk. One is alarm signals emitted from a conspecific prey that are stressed or injured, another is a digestive signal also originating from the prey as it is being digested by the predator, and a third is kairomones, which are the smell of the predator itself (Hazlett 2011). All of these signals can be important, and sometimes more than one of them are needed for the prey to respond (Schoepfner and Relyea 2005). Yet in other cases, chemical risk signals must be accompanied by other cues, such as light (Effertz and von Elert 2014).

There is still a lot of uncertainty about the chemical structures of the substances involved in predator chemical cues, although the field is in progress (Elert and Pohnert 2000; Poulson et al. 2009; Roy et al. 2013). This is important for understanding the mechanisms for response induction. In this thesis, however, I have focused on responses to chemical cues and their ecological implications, rather than on the chemical properties per se.

The pelagic food web

Planktonic organisms drift with currents in the free water masses, the pelagic habitat. Pelagic food webs are complex, yet size structured (Fig 1). The classical food chain (blue arrows, Fig 1) consists of primary producers, the phytoplankton, which are eaten by zooplankton, such as copepods. The copepods, in turn, are the major trophic link between primary producers and higher trophic levels like vertebrates. Microzooplankton also feed on phytoplankton, and can feed efficiently on the size fraction that is below the optimal size range of copepods (Berggreen et al. 1988). Copepods, are also important grazers on the microzooplankton community (Calbet and Saiz 2005; Calbet 2008). In addition to bacteria and virus, the microbial food web (Azam et al. 1983, orange arrows in Fig 1) also includes the microzooplankton, which eat bacteria and thus bring recycled organic matter back into the classical food chain.
Most aquatic predators eat whole prey organisms, hence prey size set limits to trophic interactions. A predator can be limited by its gape size, but there is also a lower limit on prey size determined e.g. by detection (Aksnes and Utne 1997). Commonly, pelagic grazers and predators are omnivorous to varying degrees, but have an optimal prey size (Berggreen et al. 1988; Hansen et al. 1994). A notable exception to this are some of the dinoflagellates with foraging strategies that allow for ingestion of particles several times their own size (Hansen and Calado 1999). Moreover, trophic interactions are also structured by passive and active selective feeding. Many organisms select prey based on chemical properties of the food or the hydrodynamical signals that they generate (Saito and Kiørboe 2001), in addition to size and shape.

The life cycles and reproductive rates of different functional groups are important for the food web dynamics. Phytoplankton and microzooplankton such as ciliates and heterotrophic dinoflagellates respond rapidly to increased nutrient or food concentrations and may bloom or become abundant in a few days. Others, like copepods, have a longer generation time (see fig. 4) and thus require a longer lag before their abundances peak. This naturally implies that both life history and seasonality is important for the temporal heterogeneity in plankton communities (Kiørboe and Nielsen 1994; Nielsen and Kiørboe 1994).

The pelagic habitat is huge and open, with no obvious physical shelter. Yet, the pelagic is characterized by heterogeneity created by strong vertical gradients in physical and chemical properties. Light, temperature and density, as well as upwelling of nutrients create layers and patches where organisms aggregate according to their preferences. Compared to organisms in terrestrial food webs, plankton have high production and turnover rates, hence grazing and predation pressure is considerable in pelagic environments (e.g. Miller 2004). Certainly, the spatial and temporal heterogeneity of the planktonic community makes predation pressure unpredictable. For instance, for zooplankton predation risk from fish varies between and within seasons, making risk assessment difficult, or even impossible, without a signal.
**Figure 1.** A simplified planktonic food web. Blue arrows represent the classical food chain, and the orange arrows are the microbial food web.

**Aim**

The variability in risk regime and the potential for marine plankton to perceive the level of risk via chemical information is established. Yet, our understanding of responses to risk in fundamental life history traits is scarce, even for plankton organisms as abundant as copepods and diatoms, which I target in my thesis. An organism has a time- and a resource budget that it can adjust in varying degree to optimize feeding efficiency, reproductive output and survival. Hence, predation risk might influence life history traits through time and resource allocation (Pianka 2000). The overall aim of my thesis was to investigate the role of perceived risk for central life history traits in common plankton in different temporal and spatial settings (Box 1). Copepods were target organisms in papers I-III, and their responses to coexisting fish (papers I, III) and mysid (paper II) predators. Paper IV focused on a common diatom in response to a copepod grazer. The specific aims are described below.

**Paper I** targeted the general hypothesis that risk may influence both growth and development in copepods. This was explored using naupliar stages of the copepod *T. longicornis*. Specifically, I tested the hypothesis that *Temora* is sensitive to predator chemical cues and invest less in growth with risk.
In **paper II** the major aim was to determine sex-specific vulnerability to predation risk. Using the copepod *Eurytemora affinis* with the mysid predator *Neomysis integer* I measured both consumptive and non-consumptive effects of predation on sex-ratio and reproduction.

The aim of **paper III** was to quantify energetic investment in copepod male reproduction, and to explore whether the males adjust their feeding, swimming and spermatophore production according to food availability and predation risk. Again, *Temora* was used.

In **Paper IV** I studied the chain length plasticity in the diatom *Skeletonema marinoi*. I tested the hypothesis that *Skeletonema* varies chain length strategically to cope with a grazing regime in the field that selects for contrasting sizes in phytoplankton prey.

**Study organisms**

My studies have mostly been experimental in nature. While, some aspects of the methodology, such as the production of predation risk cues are highlighted in Box 1, the methods are detailed in the respective papers and not elaborated further upon here. The experimental organisms are introduced below.

**Copepods**

Copepods are the most common metazoan on earth (Turner 2004). They occur both in fresh water and in marine habitats, with pelagic, benthic and parasitic lifestyles. Most of the ~13000 species are marine pelagic copepods (Mauchline 1998a). Copepods are small (0.5 – 5 mm) crustaceans feeding on phytoplankton, microzooplankton or even other copepods and detritus, while also being important prey for larger plankton and fish (Mauchline 1998a). They reproduce sexually, but their behavioral strategies and mating patterns vary greatly between species. To find each other, the males search for females and locate them by chemical or hydrodynamic signals emitted by the females (Kiørboe 2011). Copepods have various behavioral and morphological strategies to cope with high predation risk, such as diel vertical migration, rapid escape jumps, small size and transparency (Ohman 1988).

I used the copepods *Temora longicornis* (**papers I, III**) and *Eurytemora affinis* (**paper II**) in my experiments (fig. 2). *Temora* is an abundant coastal marine copepod. It is an omnivore
filter feeder (Tiselius and Jonsson 1990), and is commonly eaten by small fish. *Temora* are broadcast spawners, i.e. they release their eggs freely in the water (Box 2). *Eurytemora* is a euryhaline species, that tolerate fresh-, brackish-, and sea water. *Eurytemora* is also common and sometimes dominating in coastal waters, such as in the Baltic Sea. In difference from *Temora*, *Eurytemora* females carry their eggs in egg sacs until the eggs hatch.

![Figure 2. A: The copepods Temora longicornis that was used in papers I and II (Photo: M. Nordbotten) B: Eurytemora affinis that we used in paper II (Photo: C. Lee).](image)

**Diatoms**

Phytoplankton account for more than 50 % of the global primary production (Field et al. 1998). One of the most diverse and evolutionary successful groups of phytoplankton are the diatoms (Graham and Wilcox 2000). Diatoms are especially important during the spring bloom, where they become very dense, and spike the production in the whole food web. The diatom life cycle alternates between asexual and sexual reproduction (fig. 3B). In asexual reproduction the cells, which consist of two valves divide. The original valves become the outer valves of the daughter cells and new inner valves are formed. In this process the cell size decreases for each cell division (fig. 3). When reaching about 40% of their original cell size the diatoms switch to sexual reproduction in which the original cell size is restored (Graham and Wilcox 2000).
*Skeletonema marinoi* (fig. 3A), which is the study organism in paper IV, dominates the spring bloom in the temperate northern hemisphere. This is a chain forming diatom, where chains are formed by daughter cells remaining attached by a silica frustule after cell division. The chain length is highly variable, and may be up to more than 20 cells, but *Skeletonema* can also appear as single cells. *Skeletonema* cells normally divide once per day (Balzano et al. 2011). The formation of cell chains is ecologically important, influencing the sinking rates, nutrient uptake and interaction with grazers (paper IV).

**Figure 3.** A: The diatom *Skeletonema marinoi*, which we used in paper IV, (Photo: E. Selander). B: The diatom life cycle, showing the formation of cell chains by asexual reproduction (modified from www.bio.vu.nl).
**BOX 1: METHODS**

**Incubations:** Mesocosms were used in paper II. These are large compartments ($\approx 2\text{m}^3$) simulating a natural system. The mesocosms were filled with the natural plankton community from the field, filtered through $50\mu\text{m}$. The large incubation volume restricts the number of possible replicates and requires a high number of organisms.

Bottle incubations on a plankton wheel were used in paper II-IV. The rotating wheel keeps algae in suspension, which is useful in grazing experiments and when growing chain forming algae.

In paper I, I incubated nauplii in $15\text{ml}$ micro wells. This allows for following individuals and performing repeated measurements over time. The small volume entails a risk of stress and infections. Higher bacterial activity may also contribute to more rapid degradation of chemical cues (Loose et al. 1993), which we tried to compensate for by a high cue concentration and renewal of water every day.

**Cues:** We used chemical cues from predators that naturally coexist with the study organisms; the stickleback *Gasterosteus aculeatus* (paper I, III), the mysid *Neomysis Integer* (paper II) and the copepod *Acartia spp* (paper IV). Different approaches to predator chemical cue exposure are possible, each with benefits and drawbacks. Cues can be obtained in different ways; either by filtrates of incubation water where a predator has been kept (paper I), by the predator present (paper II, III), or by having the predator in a cage (paper II). With pre-conditioned water the intensity of the risk signal may be unstable due to degradation. A predator present with the prey organisms gives a natural exposure to the risk cue, however the induced effect can be difficult to distinguish from consumptive effects. A set up where water is shared but organisms kept in different compartments, e.g. a cage, is sometimes more optimal. If water masses do not mix completely, as for example with a small mesh size, the signal will be hampered and not represent the actual density of predators (E. Selander, personal communication).
BOX 2: KEY LIFE HISTORY STRATEGIES IN COPEPODS

Copepods show a wide specter of life history strategies between species, reflecting the variable environments they live in. Some of these strategies are also plastic within species.

- **Seasonal and perennial species.** In high latitudes, copepods are commonly perennial and some species spend the first year maturing. Others, often coastal, species have several generations within a single season (Mauchline 1998a).

- **Diapause.** Some perennial species survive the unproductive season by going into diapause. In autumn the copepods sink down to deeper waters and survive on accumulated fat reserves throughout the winter by decreasing their metabolism (Varpe 2012).

- **Capital and income breeding.** Reproduction can be based on stored energy from previously accumulated resources (capital breeding), or on currently available resources (income breeding) (Varpe 2012). Some species may use both strategies.

- **Egg sac and free spawning.** An important distinction in copepod reproductive strategies is that between species that carry the eggs until they hatch, or those that release the eggs freely in the water. Many fresh water species have egg sacs, while most marine species are free spawners.

- **Diapause eggs** are common in several copepod groups. These eggs are usually produced in autumn. Diapause eggs then remain quiescent during unfavorable conditions, and seed the population the next season (Uye 1985). Many coastal species can alternate between producing subitaneous eggs and diapause eggs.

- **Single and multiple mating.** In some species, females have capacity to store sperm, and can use sperm to fertilize several egg clutches. This is a strategy to enhance reproduction with low mate encounter rates (Kiørboe 2006). Other species lack sperm storing abilities and need to re-mate. Traditional single maters may still have several spermatophores attached suggesting sperm competition and multiple paternity clutches (Titelman et al. 2007).
Life history

Marine plankton has different life history strategies, reflecting the spatial and temporal heterogeneity in the seas. However, life history traits in plankton are shaped by the strong predation pressure in the pelagic environment. This is for example evident in the small size typical for planktonic creatures (Blanckenhorn 2000; Smetacek 2001). There is high variation and plasticity in life history strategies in marine plankton. For instance, the generation time in perennial copepods, like the common *Calanus* spp, can vary with several years, also within species (Falk-Petersen et al. 2009), and life history in cultures often differ from that in the field.

In the following I discuss life history strategies in plankton in the context of predation risk. The most important results from the papers I-IV are highlighted, and the discussion will predominantly focus on the organisms targeted in the papers.

Growth and development

Although growth and development are clearly separate processes they are often treated in synchrony in studies of copepods. However, growth and development scale differently with temperature (Forster et al. 2011) and may also be differently affected by predation risk. Larger size can strengthen the mortality risk by making the prey more easily detectable (Giske et al. 1994). On the other hand, growing and developing as fast as possible can also be an adaptive response, when juvenile stages are more vulnerable (Nylin and Gotthard 1998).

A general hypothesis is that reduced growth and earlier maturation is an adaptive response to higher risk (Nylin and Gotthard 1998). However, there are also examples of prey increasing growth under elevated predation risk (Crowl and Covich 1990, paper I).

Copepods are faced with a multitude of predators with widely different weaponry, suggesting that plasticity in both behavior and life history is necessary. The copepod life cycle usually consist of 6 naupliar stages and 6 copepodite stages, with adult females and males being the final stage (fig. 4). In paper I we studied how potentially perceived risk affects growth and development in the naupliar stages. The results from paper I show that nauplii do respond to
predator chemical cues; risk induced increased growth rate and higher molting probability at some of the stages. This resulted in a larger size throughout naupliar development (fig. 5). Thus paper I suggests that larger size in nauplii can compensate for increased detectability. This is probably not because the nauplii physically grow out the prey size window of their predators, but rather because the morphological and behavioral abilities to avoid predators improve as the nauplii grow and develop. Each molt into the next developmental stage represents increasing number of segments and appendages, and also increasing number of chemical and hydrodynamic sensory cells (Mauchline 1998a; Bruno et al. 2012). The transition from the sixth nauplius to the first copepodite stage also involves metamorphosis into a different body plan, allowing for more efficient escape responses (Titelman 2001; Borg et al. 2012). Development and growth are partially uncoupled processes in copepods (Forster et al. 2011), and paper I indicate that risk may act differently on these processes.

Figure 4. The life cycle of calanoid copepods, consisting of 12 developmental stages from egg to adult. Small coastal copepods may carry out the life cycle in less than a month, while larger species may take several years.
Following individual copepods during development, as in paper I, can reveal effects that are masked by large individual size variation when studying batch cultures, as in most studies of growth rates (Mauchline 1998a). Variation in copepod growth rates is considerable (Hirst and Lampitt 1998; Mauchline 1998a). While responses to food and temperature have received much attention for over a century (Forster et al. 2011), the role of predation risk has largely been ignored in studies of growth and development of marine copepods (but see Gutierrez et al. 2010). Effects of risk is probably more important for growth patterns in marine copepods than previously known (Verity and Smetacek 1996), and is potentially one of the mechanisms that regulate patterns of copepod development in nature.

Reproductive investments

Reproduction can be demanding in terms of time, energy and predation risk (Magnhagen 1991). Reproductive costs are interlinked; for instance, energetic investment requires feeding, which again leaves less time available for reproductive activities and increases exposure to predators additionally (Stearns 1992; Scharf et al. 2012). In sexually reproducing animals, the different costs are unequally shared between males and females. Asymmetric reproductive investments are ecologically and evolutionary fundamental, shaping reproductive strategies and driving sexual selection (Kokko and Jennions 2008). Typically, 

Figure 5. Simulated mean individual mean body size for a copepod population over 14 days from hatching, in ash free dry weight. This is based on responses in stage structure and size at stage, from paper I.
the females are the most burdened with energetic expenses during reproduction. The material contribution to a mating by a male is the sperm, which is traditionally considered to be rather cheap and seemingly unlimited.

The picture may sometimes be different however. In many arthropods, such as fruit flies, butterflies and crickets, sperm is delivered in packages called spermatophores, that is, flasks containing sperm and seminal fluid. Spermatophore production is associated with costs, including energetic costs with effects on longevity (Scharf et al. 2012). Similar to terrestrial arthropods, copepod males produce spermatophores (fig. 6). Although existing estimates (fig. 7, paper III) indicate that spermatophore production capacity in copepods is limited, the energetic costs of reproduction in male copepods are largely overlooked in the literature (but see Burris and Dam, 2015). In contrast, female feeding rates and energetic investments in reproduction is well-quantified in copepods (see e.g. Kiørboe et al 1985), a topic that have received much research attention for over a century (Mauchline 1998b).

Copepod feeding strategies are closely linked to swimming activity and hence also to encounter probability with targets other than food, such as mates and predators (van Duren and Videler 1996; Kiørboe 2008). Males thus need to balance between mate search and feeding and predator avoidance, depending e.g. on the energetic cost of reproduction. Predation risk may alter allocation to reproduction, or modify feeding and mate search in a manner that influence reproductive output in both sexes (papers II and III).
Paper III revealed that the energetic reproductive investment by males is considerable. The production of spermatophores was strongly limited by food availability. Together with scattered spermatophore production rates harvested from different studies in the literature, the pattern was even clearer (fig. 7, paper III). Also, the males continued feeding and even with increased clearance rate with risk in the limited food concentration. The low spermatophore production rate at this concentration however probably prevented a detectable effect of risk on spermatophore production rate per se. The risk also affected motility behavior, with males reducing the time of active swimming when exposed to a risk cue (paper III).

Male Temora have shorter life span than females, and their reproductive potential declines rapidly after 5-7 days (Sichlau and Kiorboe 2011), as is also seen in other copepods (Burris and Dam 2015). As their mating capacity depends on recent ingestion (paper III), the cost of reducing feeding activity in the presence of risk cue might be high, which could explain why we did not find reduced feeding or spermatophore production rate with risk. The strategy to maximize allocation to spermatophore production may be adaptive in nature, where male mating capacity is apparently food limited throughout, or at least for most of the year.

![Figure 7. Spermatophore production rates collected from the literature. Open circles are species which are egg sac spawners, and closed circles are free spawning species. From paper III.](image-url)
Copepod mating behavior involves several components that interfere with predation risk. The mating act itself is a highly conspicuous behavior where the couple spins around rapidly for up to several minutes while the male attaches the spermatophore to the female (Bagøien and Kiorbøe 2005). This attracts attention from predators and also prevents the couple from escaping attacks (Trochine et al. 2005). Accordingly, copepods reduce both mating frequency (Lasley-Rasher and Yen 2012) and mating duration (Maier et al. 2000) upon detection of predation cues.

Van Duren and Videler (1996) documented that both sexes, but males in particular, reduce swimming speed in water with fish cues. We could not find any effect of fish cues on swimming speed but the males reduced the time spent active (paper III). This may also reduce detection by and encounter rates with predators (Tiselius et al. 1997, Titelman and Kiørboe 2003), on the expense of mating opportunities.

While males suffer a higher predation risk by mate search activities, females are put in a vulnerable state during egg production. Spawning strategy has strong effects on predation risk (Box 2). Eggs that are shed directly in the water suffer high mortality, both because they are preyed upon (Bonnet et al. 2004), and also because they can sink out of the productive habitats. Carrying eggs in an egg sac reduces egg mortality considerably (Kiørboe and Sabatini 1994). But egg sacs increase predation pressure on the female, by making the females more visible and their escape responses weaker (Maier et al. 2000).

In the mesocosm experiment (paper II) we found a lower proportion of ovigerous females in treatments with the mysid predator (Neomysis integer) present. Supplementary experiments indicated that this was a non-consumptive effect of the predator presence (paper II). The females either shed their egg sacs prematurely, or they may have delayed egg production in response to the presence of the mysids. Early shedding of eggs in response to predation risk is documented in other species (Saiz et al. 1993; Tiselius et al. 1997). The extent of parental care, like egg carrying, is determined by the trade-off between current and future reproduction (Stearns 1992). Copepods with egg sacs have lower egg production rate than free-spawners (Mauchline 1998b), hence their spawning strategy and amount of parental care reflects their prospects for future reproductions. Yet, there might be a critical level of predator presence, where the parental effort is no longer worth the risk, as indicated in paper.
II. In some species the females shed their egg sacs upon predator attack (Svensson 1997), suggesting that females shed egg sacs to save the offspring, rather than to enhance their own survival. Interestingly, spermatophore production rates are higher in egg carrying species compared to free spawning species (paper III, fig. 7). This could be related to the higher reproductive investment in females with egg sacs, and thus a stronger competition for females in these species.

What paper II also revealed was that in mesocosms with predators, the females were larger than in the mesocosms without predators. This is may suggest that larger size enhance protection against predation, in line with the higher investment in growth during nuapliar development (paper I).

Colony size

Another, although different, aspect of size and predation risk is that of size plasticity exhibited by some colonial organisms. In an environment where the trophic interactions are strongly size structured, size plasticity can be a powerful mechanism to reduce predation risk. The formation of cell chains in the diatom Skeletonema marinoi gives extreme size flexibility (fig. 3). Phytoplankton are subject to grazing by micro- and mesozooplankton; functionally different grazers with contrasting optimal prey size ranges (Hansen et al. 1994, fig 1). Long chains are easily detected and captured by copepods, who are less efficient grazers on single cells (Price et al. 1983). Conversely, a ciliate may ingest single cells but not long chains (see e.g. Hansen et al 1994, paper IV). In a field situation where the relative dominance of these functionally different grazers fluctuates in time, one can imagine that chain length plasticity is an efficient risk avoidance strategy. Since Hessen and Van Donk (1993) first demonstrated grazer induced colony size plasticity, several corresponding findings have been reported. In some systems grazer cues promote colony formation (Lampert et al. 1994; Long et al. 2007) whereas in other systems, they induce splitting of colonies into smaller units (Selander et al. 2011; Bergkvist 2012). In all cases the response protects against the grazer that emit the cue. Clearly, detection of grazer cues and adaptive responses in colony size is prevalent across phytoplankton taxa. Still, it is not clear whether grazer protection is a primary function of chain formation and chain length plasticity in phytoplankton. A reason for this is the lack of evidence of the adaptive value of induced chain length plasticity in field conditions.
In paper IV I found that the grazer induced plasticity measured in laboratory condition does reduce mortality over an annual cycle of natural grazer densities of copepods and ciliates. This was shown by an empirical model using data of grazer densities, grazing rates and the chain length response from experiments and rates in the literature. The complexity of grazer communities and chemical signals in nature is difficult to account for in a model, and also other physical and chemical processes contribute to chain length variation. Yet, paper IV is important, as the first to show the ecological relevance of grazer induced colony size plasticity in phytoplankton. Paper IV, in addition to theoretical and empirical data, suggest that grazer regime may have selected for chain formation in phytoplankton and thus highlights the importance of top-down mechanisms in evolution of functional morphology in phytoplankton.

Closing remarks

Marine plankton holds the most abundant metazoans on the planet and organisms whose biology is fundamental for the major ecosystem function in the ocean. The marine food web is highly dynamic and consumptive effects of trophic interactions are tremendous. Apparently, also non-consumptive effects might be considerable, by influencing vital fitness components in plankton, as shown in this thesis.

Growth, reproduction and survival of organisms determine the community structure and population dynamics. My results suggest that predation risk influence growth pattern and reproductive investments in copepods, with potential effects on the population and community dynamics. Experiments like those in my thesis show the potential for responses in the wild. However, the magnitude of the responses might look different in a field situation with several cues and predators, as well as a multitude of other environmental stressors. Experiments with multiple predators may reveal important trade-offs between responses to different predators.

My findings on the system with Skeletonema and two functionally different predators show that the response from the laboratory is, in fact, ecologically relevant in a field situation with
the same grazers. Yet further studies needs to be done in order to distinguish the effect of predator cues from other environmental variables that may co-vary with predator cues.

In conclusion, predation risk seems to be one of the environmental mechanisms that regulate plankton population dynamics in the pelagic habitat, by influencing life history traits and trade-offs.

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References


