The influence of food access on spermatophore production in the pelagic copepod, *Temora longicornis*

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Master thesis

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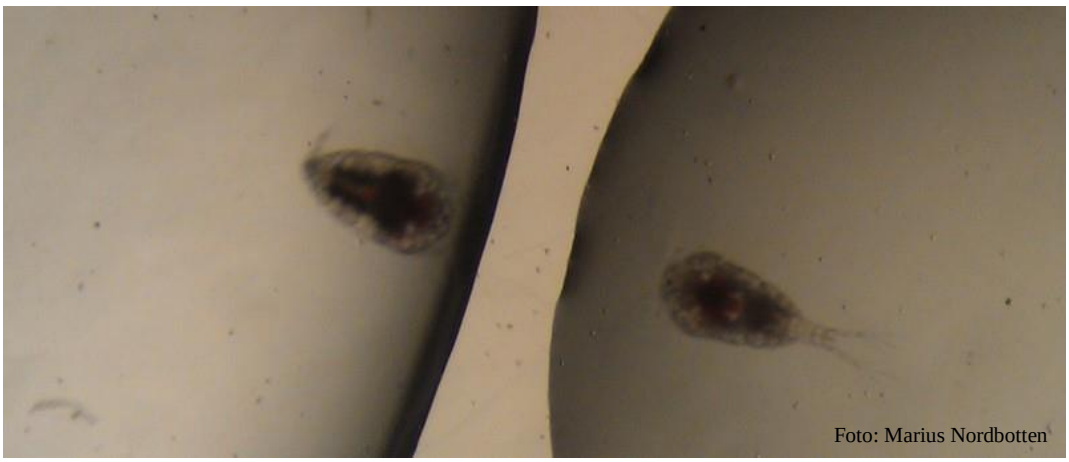

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”Det er ingen ende på naturens tøylesløse påfunn i planktonverden, her kunne en surrealistisk kunstner føle seg overmannet. Om vi ikke hadde hatt annet tidsfordriv på flåten, så hadde det vært underholdning nok å ligge med nesen ned i planktonnettet. Fordi det aldri var noen ende på fantasifulle former og farger om vi spredte planktonet på en fjel og kikket på de enkelte smådyrene hver for seg med blotte øyet. De fleste var ørsmå rekeliknende krepsdyr, Copepoder”.

Thor Heyerdahl, Kon Tiki 1948

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Mamma, pappa og Anders! Tusen takk for at dere alltid er der og hjelper meg med alt som trengs! Og for at dere er så stolte av meg! Dere er verdens beste familie!

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ABSTRACT

In studies on reproductive ecology of pelagic copepods, the role of males is often neglected and male reproductive investment is not well understood. Lately there has been an increasing interest in the topic and it has been indicated that male investment does in fact play an important role in the reproductive dynamics of pelagic copepods. In this experiment, male reproductive investment as a function of food availability has been investigated for the calanoid copepod, *Temora longicornis*. During mating experiments, male spermatophore production has been quantified after three treatments of decreasing food availability. The results have clearly shown that the production of spermatophores is closely linked to food access. Surprisingly, the production is drastically reduced when food access is limited indicating a large investment into spermatophores. However, the direct costs of the spermatophores seem to be minor when their volume is compared to the male body volume. Traditionally, evolutionary theory assumes sperm to be an unlimited resource, but here it is shown that in environments deprived of food, the spermatophore production is limited.

INTRODUCTION

BACKGROUND AND HYPOTHESIS

The planktonic ecosystem is vast, dilute and inhabited by thousands of species. The dominating life form is plankton with copepods as the most abundant component of the metazoan biomass (Verity and Smetacek 1996; Castonguay *et al.* 1998). Although copepods are tiny in size, they provide a crucial source of food for fish larvae, seabirds and many fish, like the zooplanktivorous clupeids (e.g. Sandström 1980; Flinkman *et al.* 1998; Möllmann *et al.* 2000; Casini *et al.* 2004)**.** These tiny animals are also the main grazers of phytoplankton and play an important role in transferring energy to vertebrates.

All animals need energy to function and copepods are no exception. The copepods need energy for a variety of activities, such as locomotion, maintenance of cells and tissues, as well as growth and eventually storage of excess substrates. One of the main energetic components is carbon, and when available, organic carbon is ingested at a constant rate (Kuijper *et al.* 2004). According to the Dynamic Energy Budget (DEB) theory of Koojiman (1995), substrates assimilated from food are directed to reserves, and in turn used for maintenance, growth, development and reproduction. Substrates are firstly utilized to meet the maintenance demands of adults, while the remaining resources are used for growth and gamete production. The copepod biomass is divided between permanent structures and stored reserves. All permanent structures need maintenance and these costs are met using reserves. To maintain the permanent structures, biomass must be produced to replace substrates lost in day-to-day activities.

Pelagic copepods move to find food, mates, avoid predators and stay suspended. The cost of movement and feeding for copepod females has previously been shown to be low (Kiørboe *et al.* 1985). This has been suggested to vary with sex, as there is a difference between the swimming behavior of males and females. Males typically perform active mate searching (Kiørboe 2008) with more movements, significantly higher speed and more directional

precise cruising motions including loops and sharp turns (Kiørboe and Bagøien 2005). Whereas females mostly hover in the water and stay more still (Doall *et al.* 1998). All this movement comes at a price both in terms of metabolic expenses and increased risk of predator encounter (Kiørboe 2008). Males may therefore have an elevated cost related to their reproductive investment due to their swimming behavior.

Traditionally, male copepods have been viewed to spend a relatively small amount of their energy on spermatophore production. The spermatophores are flask like tubes, containing spermatozoa (Hopkins 1978; Mauchline 1998). Females on the other hand, invest more energy into reproduction because egg production is time consuming and requires more energy (Mauchline 1998). However, Mauchline (1998) suggested that copepod males might have a comparable investment in spermatophore production. This is based on the observation that the spermatophore size is relatively big compared to the male body size. A spermatophore is typically 0.5% of the body volume, but there is high interspecific variance in this size (Mauchline 1998). Spermatophore size has been found to depend on nutrient availability and in some cases, males can vary their investment dependent on available resources (Sivars-Becker 2004). A review by Dewsbury (1982) indicate that the cost of sperm production is non-trivial for a variety of other organisms (e.g. nematodes and vipers: Van Voorhies 1992; Olsson *et al.* 1997). This may just as well apply to copepods.

Investment in egg production has been thoroughly studied and has traditionally been used as a proxy for food access in field studies. A quick search on "Web of Knowledge" for "copepod" and "egg production" gives more than 1500 hits. However, research concerning energetic costs of reproductive investment for male pelagic copepods is scarce. In comparison, when searching for "copepod" and "spermatophore production" "Web of knowledge" gives only 23 hits. Exploring such energetic costs may provide knowledge much needed for a better understanding of copepod population dynamics and reproductive outcomes. As previously highlighted by Kiørboe (2007) and reviewed by Titelman *et al.* (2007), relatively high costs of spermatophore production give males potential for sexual selection of future suitable mates. High cost of spermatophores may also explain the relative large amount of unfertilized females found in the ocean (Hopkins 1982; Williamson and Butler 1987; Ceballos *et al.* in review). A study by Kiørboe (2006) showed that only about one third of encountered females

were fertilized. This also implies that spermatophore production may be a high cost for the males.

The cost of spermatophore production may appear in several ways, such as change in size, change in produced number and change in chemical content. In this study I tested the hypothesis that spermatophore production is dependent on food access. This was tested in mating experiments with the species *Temora longicornis,* where the number of produced spermatophores was quantified. The number of produced spermatophores was compared between three different food concentrations to see if the total number produced changed with different feeding conditions. In particular, I focused on the following question:

Is spermatophore production dependent on food availability?

THE BIOLOGY OF *TEMORA LONGICORNIS*

The calanoid copepod *Temora longicornis* is abundant in temperate waters of the northern hemisphere and is mainly found in costal upwelling zones. It represents 35-70 % of the total copepod population in the southern bight of the North Sea (Daan 1989) and is of great ecologic significance in most areas. *T. longicornis* is frequently the most numerically dominant copepod in early summer (Castonguay *et al.* 1998) and maintain a very high abundance well into autumn (Roy *et al.* 2000).

T. longicornis has complex life histories with short generation times and overlapping broods and generations. Up to eight generations per breeding season is possible (Dutz *et al.* 2010). *T. longicornis* is known to produce resting eggs that remain dormant on the sediment surface for long periods. During spring they hatch and give rise to new generations. These eggs are stimulated to hatch when environmental conditions are suitable for nauplii survival in the water column (Lindley 1990; Castellani and Lucas 2003). Temperature, food availability and length of upwelling season are the main factors determining breeding season and govern the amount of generations produced each season (Mauchline 1998). *T. longicornis* adults show strong ageing effects where mortality rates increase and fertility rates decrease rapidly with

age. Mature males and females both have an average adult longevity of 30 days (Sichlau and Kiørboe 2011).

To reproduce, copepods require actual copulation in each generation. When a mate is located, the male approaches the female from behind and captures her by grasping the urosome or caudal furcae (tail segment of copepod body) (Van Duren and Videler 1996; Mauchline 1998) (Appendix I). When the female is captured, the male adjusts his position relative to the female before attaching and transferring his spermatophores directly to the female's genital field. The flask-like spermatophores have a thin neck for attachment to the female. They are able to attach to the females genital field by adhesive secretions extruded from the spermatophore itself during the transfer between male and female (Mauchline 1998). If the spermatophore is correctly attached, it discharges its content (spermatozoa) into the female's genitial antrum where fertilization takes place. The male releases the female directly after placement of the spermatophore. Female *T. longicornis* has place for two spermatophores, but are frequently observed with more placed on them. Misplaced spermatophores can in some cases be able to fertilize the females with the help of a fertilization tube that the spermatozoa can be discharged trough (Mauchline 1998).

T. longicornis females are broadcast spawners (Webb and Weaver 1988) lacking seminal receptacles used for sperm storage (Ohtsuka and Huys 2001). Therefore, one mating may only fertilize one batch of eggs and the females are able to mate several times during their fertile period. This means that the value of each mating is lower for this species than in species which only mate once (Kiørboe 2006). Females can produce eggs for up to 18 days, while males can produce spermatophores only up to 8 days after maturation (Sichlau and Kiørboe 2011). The spermatophore production rate of males is low, only approximately 0-4 day^{-1} (Hopkins 1982; Ianora and Poulet 1993; Ianora *et al.* 1999; Kiørboe 2006; Ceballos and Kiørboe 2010). The males mating rate is assumed to be equal to their spermatophore production rate, due to the transfer of only one spermatophore per mating event (Ceballos in review).

Like most calanoid copepods, *T. longicornis* is an omnivorous opportunistic filter-feeder. Its diet is mostly herbivorous consisting of small algae, yet at times they feed on microzooplankton and ciliates (Peterson and Dam 1996; Gentsch *et al.* 2009). They are suspension feeders, where the swimming mode is associated with the creation of a feeding current (Paffenhofer 1998; Gentsch *et al.* 2009) with which it perceives food and draws the food towards the mouth appendages (Tiselius and Jonsson 1990). This feeding mode enables them to efficiently capture food particles (Tiselius and Jonsson 1990). Adult males continue to feed upon maturation, and although they are able to feed and swim at the same time, the efficiency decreases for both activities (Kiørboe 2008).

MATERIALS AND METHODS

This project was part of a collaboration between two MSc students and one PhD student. The goal was to investigate different aspects of reproductive investment in male pelagic copepods of the species *T. longicornis*. Methods for sampling and incubation were the same throughout the project, however each participant had their own focus (food access, predation and sex-ratio).

SAMPLING AND CULTURES

For studies on spermatophore production, copepods were collected daily at two sites in the Skagerrak Sea (Fig. 1) during a period of 30 days in May 2012. Experiments and sampling were conducted outside the Sven Lovén Centre for Marine Sciences in Kristineberg, Sweden. May is at the end of spring bloom for the phytoplankton when copepods are at their most abundant in the water masses (Castonguay *et al.* 1998). The Skagerrak Sea is running along the southeast coast of Norway, the southwest coast of Sweden and the Jutland peninsula of Denmark, subsequently linking the North Sea in the west and the Kattegat Sea in the east. It has a mean depth of 218 m and a maximum depth of 700 m. The water mass has a salinity ranging from 30 to 35 PSU (Nordberg 1991).

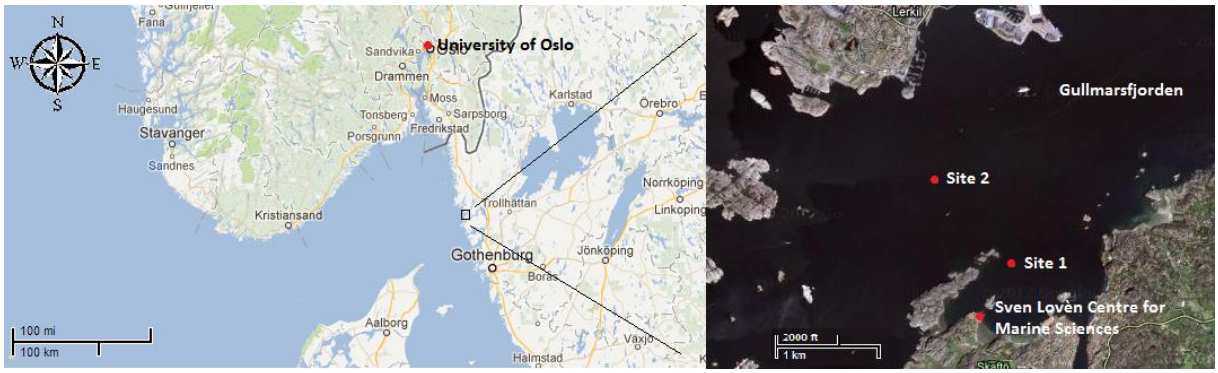

Figure 1: Map of the study site.
Sampling was conducted at two sites in the Skagerrak Sea, outside Sven Lovén Centre for Marine Sciences in Kristineberg, Skaftö island, Sweeden (58°19'30" N, 16°48'0" E). Site 1 has a depth of 20 m and site 2 has a depth of 50 m.

The samples were collected with vertical WP2 net hauls at two different stations at the mouth of the Gullmar fjord. The WP2 net had a diameter of one meter and mesh size of 200 µm with a closed cod end. The net was pulled from 15 m depth to surface at station one and from 40 m depth at station two (Fig. 1). After the haul, samples were transported in thermo containers back to the lab where they were fine-filtered through a 30 µm sieve, sorted and identified live using pipettes and Leica cold-light microscopes.

Collected animals were kept in continuous cultures in aerated 50 L containers in the lab. The animals were reared at 16 ˚C to acclimatize before the incubation. The seawater used for cultures and incubation, was retrieved from the deep sea (32 m) and had a salinity of 32 ± 1 PSU (Sven Lovén Centre for Marine Sciences Station Log 2012). Cultures were fed a single species diet of the cryptophyte *Rhodomonas salina*, which is known to be a good quality food source for cultivated *T. longicornis* (Klein Breteler 1980; Klein Breteler and Gonzalez 1986). *R. salina* was grown in semi-continuous cultures at the lab in a B1 medium added vitamin solution. Cultures were kept in exponential growth by discarding a fraction of the culture stock every 1 or 2 days and replacing it with new B1 medium with vitamins. The discarded fraction was used to feed our copepods.

THE EXPERIMENTS AND INCUBATION

Copepods were incubated in 320 mL screw-cap bottles with three different food concentrations, starvation, limited food and food in excess. The group treated with food in excess was used as control group, in accordance with previous studies of e.g. Kiørboe *et al.* (1985) and Ianora (1998), showing that fecundity is dependent on food quality up to a saturation point where the reproductive rate remains the same. This saturation point range between an algal cell concentrations equal to 400 – 500 µg C L^{-1} (Ianora 1998). The control treatment therefore had a standard of 15 000 cells mL^{-1} *R. salina*, equal to 500 µg C L^{-1} (Kiørboe *et al.* 1985; Klein Breteler and Gonzalez 1986). In the treatment of limited food a concentration of 3 000 cells mL^{-1} equal to 100 µg C L^{-1} (20 % of the excess treatment) was used. While in the starvation treatment, filtered seawater with no algal cells was used. Upon incubation, all individuals were provided food in excess (> 500 µg C L^{-1}). To calculate the

accurate number of algal cells for the incubations, a 1:50 dilution of the algal culture was analyzed with a particle counter (Elzone 180 XY). Cell size was between 5 µm and 7.5 µm as equivalent spherical diameter calculated from Elzone. The formula $C_2V_2 = C_1V_1$ was used to calculate the correct amount of algal cells in the incubation medium. C is the algal concentration and V is the volume of water containing the concentration.

Each incubated bottle contained 3 males and 3 females. Only newly molted virgin males were used. To make sure they were virgins, late copepodite stages, CIV and CV (Appendix II), were incubated individually on 6-microwell plates with food in excess and inspected twice daily. Once molted, they were moved into incubation bottles with mature females. The females used in this experiment were adults of unknown age. To increase males' willingness to mate during the incubation, only females without spermatophores attached were used. After adding the copepods into bottles, the bottles were sealed airtight and mounted randomly on a plankton wheel, slowly rotating at 0.15 rpm. The experiments were conducted in a climate-control room at 15 ˚C on a 12h light: 12h dark cycle. Precautions were taken to minimize the temperature variation to < 2 ˚C. These values are well within the range where the species is reported to thrive optimally (Klein Breteler and Gonzalez 1986; Maps *et al.* 2005).

After two days, the animals were inspected and transferred to a new, identical bottle with fresh food suspension and an equal food concentration. Weak or dead females were replaced with new ones. The water from the used bottles was filtered with a 20 µm filter, flushed onto a gridded petri-dish and inspected under a stereo microscope. Spermatophores, eggs, and nauplii were quantified. Spermatophores can be located either as present on the females or lost (floating free) in the water. Used bottles were rinsed twice to ensure that all specimens were included. After quantifying the spermatophores they were retrieved from the water with glass-pipettes, transferred to cryo-vials and kept frozen in liquid nitrogen on a Dewar flask. A small amount of seawater was added to the vials, to make sure that the spermatophores did not freeze-dry. After four days the incubated bottles were inspected again and the above procedure repeated. After inspection, any females with spermatophores attached, were also retrieved and frozen. All other individuals used in the experiment were preserved by acid Lugol's solution, kept in eppendorf-vials and brought back to a lab at the University of Oslo

for further inspection. The different tasks were distributed randomly between collaborators to ensure unbiased sampling.

COPEPOD SIZE

All preserved individuals from the incubations were photographed and measured at the University of Oslo during August 2012. The individuals were photographed lying in an angle showing their full body length. Photographs were taken with a Canon EOS 7D and a Canon macro photo lens "M8-E 65 mm 1:2.8, mounted on a stand. The camera settings were set on auto, with a magnification of 4 x. For image analysis, the free multi-platform image-analysis software "ImageJ" was used (Rasband 1997-2009; Vogedes *et al.* 2010). The pixel-to-µm ratio was calibrated using the picture of an appropriate calibration slide photographed under the same settings. To allow measurements, the length of the prosome was outlined manually (Fig. 2). The shrinkage effect found when conserving animals in Lugol's solution (Jaspers and Carstensen 2009) was not accounted for. Dry weight was calculated using the formula: $dw = 27.05(prosome\ length)^{2.62}$, where prosome length is in mm and weight comes out in µg (Dam and Peterson 1991). The dry weight of a copepod is about 19 % of the wet weight (Omori 1969) and was converted accordingly to determine their wet weight. To estimate male volume (μm^3) from their wet-weight (µg), they were assumed to have approximately the same density as water and converted accordingly: $1\ cm^3 = 1g\ water \Leftrightarrow 1\mu m^3 = 10^{-6}\mu g\ water$.

Figure 2: To estimate the prosome length of copepods preserved in acid Lugol's solution, a line was drawn manually with "ImageJ" (free multi-platform image-analysis software) from point A to point B.

SPERMATOPHORE AND EGG SIZE

Investigations of the spermatophores (Fig. 3) were done in a lab at the University of Oslo during November 2012. The frozen cryo-vials (Fig. 3) were retrieved from the freezer and the vial opened over a large petri-dish. It is important to make sure no water spills, due to the small size of the spermatophores. Using a micro-pipette, droplets of 100 µL water were placed on a microscope slide and inspected by a Leica DMLS microscope at a magnification of 40 x using an eyepiece micrometer for measurement. When the vial was emptied of seawater, the lid and vial was rinsed well with additional seawater of the same salinity as the water from our experiment. To rinse the vial thoroughly, the vial was slowly rotated with an additional droplet of seawater, to make sure it touched all parts of the inside of the vial walls. After rinsing, the additional water was scanned through in the same manner as previously. For every new sample investigated, a new pipette tip was used. The examination was conducted over a period of several days, and the samples were defrosted a few at a time, to preserve the samples as close to investigation as possible.

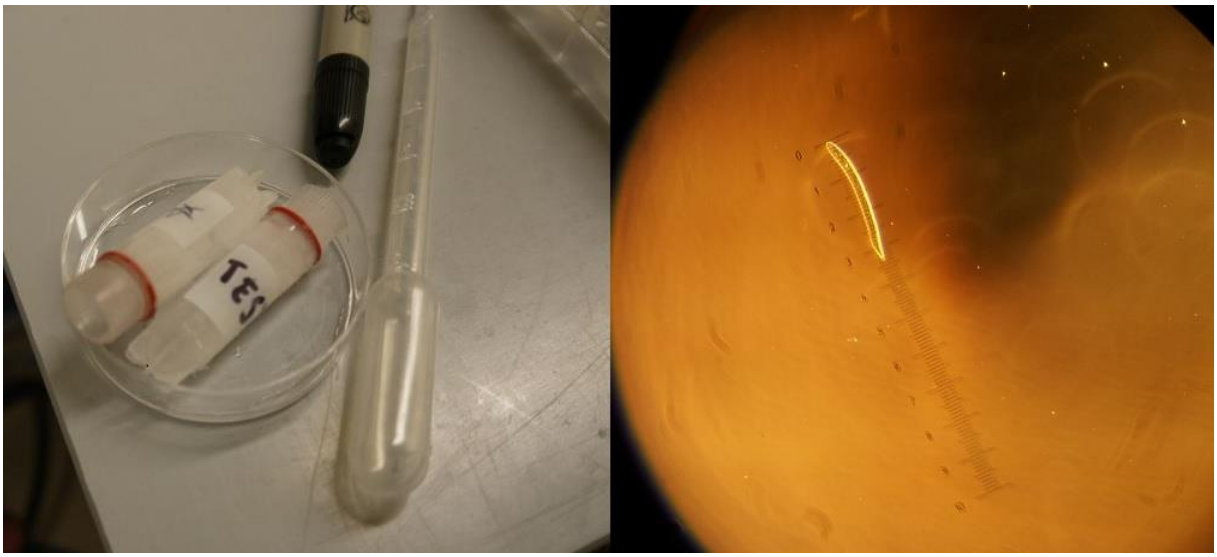

Figure 3: To the left: the cryo-vials retrieved from the freezer. To the right: A spermatophore on under a Leica DMLS microscope at a magnification of 40 x.

The size of the spermatophores were analyzed and compared between the three treatments. There were however problems with locating the spermatophores in the cryo-vials, resulting in many of them being undetected. This was most likely due to transportation loss. The number actually located was too low to conduct any formal statistical analysis. The size was therefore only used to compare size changes between the treatments, and estimate the mean volume of the spermatophores in the three treatments. The volume of the spermatophores was calculated as if they are cylinders, with the formula: $\pi \left(\frac{width}{2}\right)^2 length$. Here mean values for height and width were used.

Similar calculations were done for egg production, where the volume of an egg were calculated as if being spherical, with the formula: $\left(\frac{4}{3}\pi\right) r^3$. The radius was set to be 40 µm, based on previous studies by (Corkett and McLaren 1970; Peterson and Dam 1996; Mauchline 1998) where the diameter of the eggs was measured to be approximately 78 to 80 µm for *T. longicornis*. Hopcroft and Roff (1998) concluded that egg size is less variable than female size within a species. Therefore it is assumed equal egg size and egg volume in all

three treatments. To get the total production volume for a female in the treatments, the mean number of produced eggs in each treatment was multiplied with the estimated egg volume.

To investigate how much males and females invested into spermatophores and eggs the formula $P = \frac{n_s V_s}{V_B}$ was used. Here n_s = production rate (individual^{-1} day^{-1}), V_s = average volume of the products (μm^3 product^{-1}), and V_B = average volume of the individual (μm^3 individual^{-1}).

STATISTICAL ANALYSES

All statistical analysis was done using the open source program R 2.13.1 for Windows.

A Generalized Linear Model (GLM) with a Poisson-distribution was used to test the effect of the food treatments on spermatophore production. This type of model is appropriate because the observations from the experiments are counts. If a stochastic variable Y has a Poisson-distribution with parameter λ, the expectation is equal to the variance, i.e. $E(Y) = var(Y) = \lambda$. In this model, Y is the number of spermatophores produced, such that the GLM with a Poisson distribution may be written as:

$$\log\left(E(Y)\right) = \log(\lambda) = a + b(relative food\ access).$$

Poisson models are very sensitive to any violations to the dispersal assumption. Due to large over-dispersion in the Poisson model it was found better to apply a model with a quasi-Poisson distribution, allowing much more dispersion (McCullagh and Nelder 1989). Log transformation of the variables was not possible due to the occurrence of zeroes in the data set.

The observations were heteroscedastic because the variation increased with mean value, with quite a bit of variation in the control group. Regression analysis using heteroscedastic data still provide an unbiased estimate for the relationship between the predictor variable (here

food access) and the outcome, but standard errors and inferences obtained from the data analysis will be biased.

When using food access a continuous explanatory variable, a linear model will give an estimate of the number of produced spermatophores for any value of food. Such a relationship is only valid if the number of spermatophores changes linearly with food access. A model with food access as a factor variable can only estimate the differences in spermatophore production between the defined factor levels, which do not need to have any natural order. This type of model is more complicated, but also more flexible because it doesn't need to rely on the assumption of linearity. In this experiment two GLMs, one with each type of variables were applied to the data and compared with a likelihood ratio test.

A likelihood ratio test is a statistical test used to compare the fit of two models, where the first model is a special case of the second. I used a likelihood ratio test to test the difference between models treating spermatophore production as continuous variables and factor variables. This ratio is used to compute a p-value that can help decide whether or not to replace the more complex model with a simplified one.

The same type of modeling was done for egg production as a function of food access. Egg production was the total number of eggs and nauplii combined for each of the treatments. Studies by Corkett and McLaren (1970) and Dam and Lopes (2003) have both showed that hatching of *T. longicornis* eggs takes up 48 hours independent of prey type and food concentration for temperatures between 14 and 17 °C. This implies that the nauplii of the two first incubation days might have been produced before the incubation started. However, the correlation between the sum of all eggs and nauplii and the sum of all eggs and nauplii of only the last two incubation days, was very high ($r = 0.9$). Thus, the total number was used.

RESULTS

SPERMATOPHORE PRODUCTION

There was a visible pattern of decreasing spermatophore production in the bottles with decreasing food access (Fig. 4). When deprived from food (0 µg C L^{-1}), males had a much lower spermatophore production, on average 75 % less than when food was provided in excess. There was quite a bit of variance in the data from the excess food (500 µg C L^{-1}) treatment. However, the effect of food depletion was still significant, with both the starved and limited food treatments being different from when food is provided in excess. The treatment of excess food had a mean value of 8.5 spermatophores in the bottles with a maximum of 23 and minimum of 2 produced spermatophores. Males fed limited food (100 µg C L^{-1}) had a mean spermatophore production of 2 with maximum of 11 spermatophores and minimum of 0 spermatophores. In the starved treatment the mean production was 1, with a maximum of 3 and a minimum of 0 spermatophores.

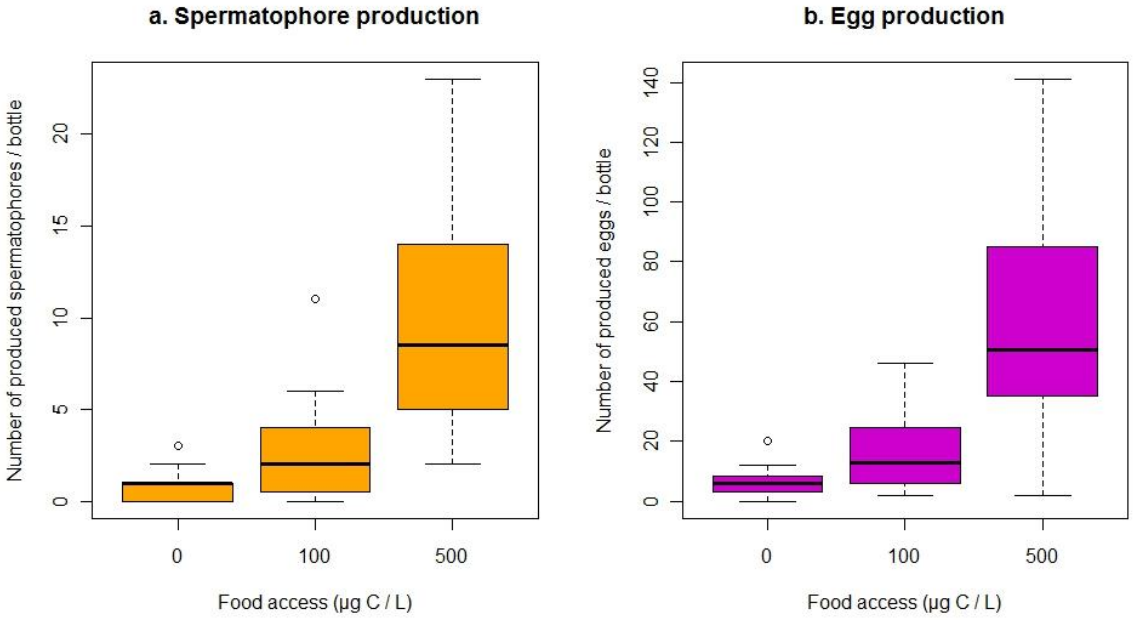

Figure 4: a: Spermatophore production within the bottles for the three different treatment groups (0, 100 and 500 µg C L^{-1}). b: Egg production within the bottles for the three different treatment groups (0, 100 and 500 µg C L^{-1}). In both a and b the bottles contained 3 males and 3 females and was incubated for four days.

Two GLM models were applied with a quasi-Poisson distribution to allow over-dispersion. The first and simplest model treated food access as a continuous variable while the second treated food access as a factor variable with three levels. A comparison of the two models with a likelihood ratio test ($p = 0.13$) showed that the simpler model with food access as a continuous variable gives a parsimonious description of spermatophore production as function of food availability.

In the fitted GLM model for spermatophore production (Table 1.1), log (spermatophore production) is a linear function of food access. The regression slope (b) is 1.97 ± 0.31 (Table 1.1), which means that log (λ) changes by $-1.97 * 0.5$ when food access is reduced by 50%. The mean spermatophore production (λ) is reduced by a factor: $e^{\frac{-1.97}{2}} = 0.37$ when food access is reduced from 1 to 0.5 and spermatophore production decrease exponentially with reduced food access (Appendix III). Spermatophore production thus decreases by 63 % for every 50 % reduction in food access. A 95 % confidence interval for this prediction is 50 %-72 % reduction with 50 % food access. The fit of the model is shown in figure 5.

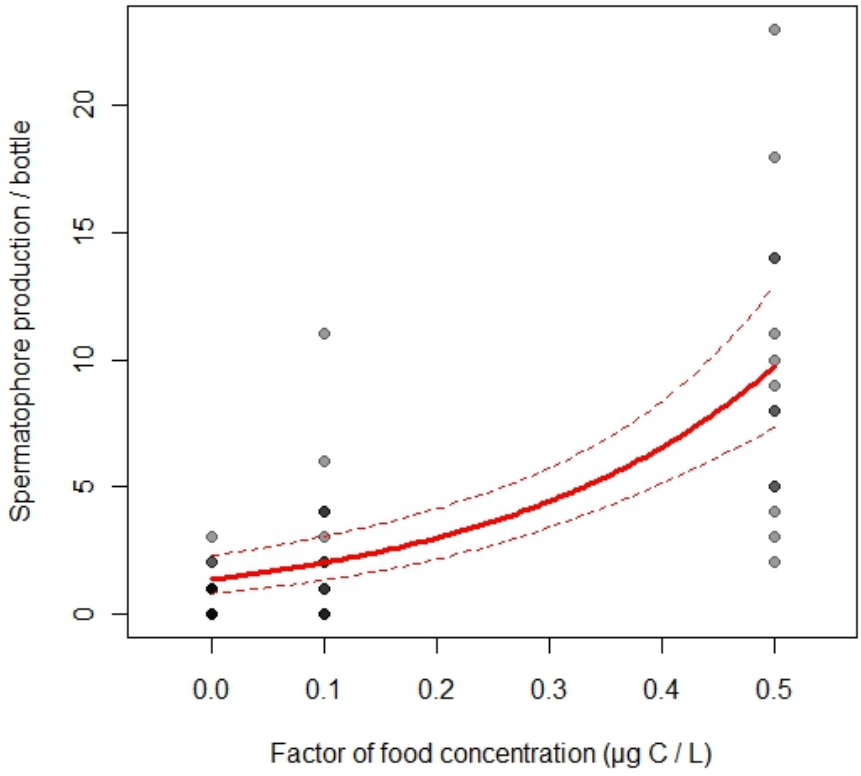

Figure 5: Spermatophore production as a function of food access (µg C L^{-1}). The red curve is predicted from a GLM of the quasi-Poisson family. Dotted lines are 95 % confidence limits for the predicted response. The different shades of gray indicate the number of replicates at each point, black being many and light grey being few.

Table 1: Results from the two GLM models represented by the formula: $\log\left(E(Y)\right) = \log(\lambda) = a + b(relative food\ access)$. 1: estimation of spermatophore production, predicted by food access. 2: estimation of egg production, predicted by food access. The parameter b is the slope of the regression line between log (λ) and relative food access.

1. Model: log(spermatophore production)	
Intercept (a)	0.3
Slope (b) ± SD	1.97±0.31
P-value	$1.31 \cdot 10^{-7}$

2. Model: log(egg production)	
Intercept (a)	2.26
Slope (b) ± SD	1.85±0.27
P-value	$1.4 \cdot 10^{-8}$

EGG PRODUCTION

Egg production decreased with decreasing food access. The treatment with excess food had a mean value of 60 eggs in the bottles with a maximum of 141 and minimum of 2 produces egg. Females fed limited food had a mean egg production of 17 with a maximum of 46 eggs and minimum 2 eggs. In the starved treatment the mean production was 7, with a maximum of 20 and a minimum of 0 eggs.

The egg production in the different food treatments were analyzed in the same manner as spermatophore production. Two GLM models with quasi-Poisson distribution were applied. The first model has food access as a continuous variable and the second model as a factor variable with three levels. A comparison of the two models with a likelihood ratio test ($p = 0.18$) shows that the simpler model with food access as a continuous variable gave an adequate description of egg production.

In the fitted GLM model for egg production the regression slope (Table 1.2) is 1.85 ± 0.27 (Table 1.2), which means that log (λ) changes by -1.85 * 0.5 when food access is reduced by 50%. The mean egg production (λ) is reduced by a factor: $e^{\frac{-1.85}{2}} = 0.4$ when food access is reduced from 1 to 0.5. Egg production decreases by 60 % for every 50% reduction in food access. A 95 % confidence interval for this prediction is 49 % - 69 % reduction with 50 % food access.

SPERMATOPHORE INVESTMENT

The size and volume of the spermatophores varied between the three treatments. The males fed in excess had a mean length of 255 µm and a mean width of 21.1 µm. In the limited food treatment the mean length was 234.5 µm and the width 20 µm. The starved males produced spermatophores with a length of 245 µm and a width of 19 µm.

Table 2: Spermatophore investment as percentage of body volume of the males in the three treatments.

	500 µg C L^{-1}	**100 µg C L^{-1}**	**0 µg C L^{-1}**
Mean male volume (μm^3)	$99 \cdot 10^6$	$97 \cdot 10^6$	$94 \cdot 10^6$
Mean spermatophore volume (μm^3)	$85 \cdot 10^3$	$74\ 10^3$	$69\ 10^3$
Spermatophore production $male^{-1}day^{-1}$	0.8	0.21	0.07
Spermatophore volume $male^{-1}\ day^{-1}$ (μm^3)	$74 \cdot 10^3$	$15 \cdot 10^3$	$4.9 \cdot 10^3$
Spermatophore investment	0.075 %	0.016 %	0.005 %

Male investment in the three treatments declined rapidly (Table 2). Males from the excess food treatment invested 0.075 % of their body volume in spermatophores per day, whereas males fed limited amounts of food invested 0.016 % and starved males invested 0.005 %.

EGG INVESTMENT

The number of eggs produced declined with food access along with female investment (Table 3). Females from the control treatment invested 0.9 % of their body volume in eggs per day, whereas females fed limited amounts of food invested 0.25 % and starved females invested 0.1 %.

Table 3: Egg investment as percentage of body volume of the females in the three treatments.

	500 µg C L^{-1}	**100 µg C L^{-1}**	**0 µg C L^{-1}**
Mean female volume (μg^3)	$130 \cdot 10^6$	$124 \cdot 10^6$	$134 \cdot 10^6$
Mean egg volume (μm^3)	$23 \cdot 10^3$	$23 \cdot 10^3$	$23 \cdot 10^3$
Egg production $female^{-1}\ day^{-1}$	5	1.4	0.6
Egg volume $female^{-1}\ day^{-1}$ (μm^3)	$1149 \cdot 10^3$	$322 \cdot 10^3$	$138 \cdot 10^3$
Egg investment	0.9 %	0.25 %	0.1 %

DISCUSSION

The results from this experiment have clearly shown that spermatophore production is limited by food access for *T. longicornis*. A reduction in spermatophore production found in the starved treatment was not unexpected, considering that any feeding animal would have trouble containing normal activities when starved. However, the limited production in the other treatment, indicate a close connection to food access. The fitted GLM model for spermatophore production implies an exponential dose-response relationship between food availability and spermatophore production, such that when food is reduced by 50 % the males have a 63 % drop in spermatophore production. However, copepods invest a small fraction of their body into spermatophores.

THE COST OF PRODUCTION

It is clear from the results of this study that for *T. longicornis* spermatophore production is limited and directly linked to the amount of food available. This is consistent with previous studies where spermatophore production generally seems to be limited, summarized in table 4. However, males seem to invest a relatively small fraction of their body volume into spermatophores. It is a paradox that the production of something that appears to require such little investment is so drastically reduced when the food access is limited. Especially since evolutionary biology generally assumes that males are able to produce unlimited amounts of gametes and that these gametes can be distributed among females on a "hit-and-run" basis (reviewed by Dewsbury 1982). When comparing gamete to gamete, sperm are considerably smaller and cheaper to produce than eggs. Thus it is reasonable to assume that sperm production is limitless. Reproductive success for males is therefore dependent on number of mates encountered, whereas for females reproductive success is constrained by offspring production (Wedell *et al.* 2002). However, males rarely emit sperm gamete by gamete. They rather discharge the spermatozoa in bundles, ejaculates, or spermatophores containing hundreds of spermatozoa to increase the chances of fertilization, as is the case for *T. longicornis*. The costs of such packages are thus far more expensive than individual

spermatozoa. Therefore sperm production is limited by the number of batches a male is able to produce (Dewsbury 1982).

The energetic costs of spermatophore production have been scarcely investigated, yet a few previous studies have shown results indicating costs connected to spermatophore production for other animals. Wedell *et al.* (2002) found evidence that a restricted diet constrain sperm production in Indian meal moths (P*lodia interpunctella*). Sperm production seem to reduce the life span of *Caenorhabditis elegans* nematodes (Van Voorhies 1992). For the viper snake (*Vipera berus)*, body mass loss during the inactive spermatogenesis stage is as great as during the active mate searching stage (Olsson *et al.* 1997), suggesting that there can be significant energetic demands from spermatogenesis.

The production of eggs and spermatophores responded in much the same way when food access was limited (Fig 4). However, when it comes to investment as a fraction of body volume spermatophores were about ten times cheaper than eggs (Table 2, 3). Females that were fed food in access invested 0.9 % of their body volume into eggs, while males invested only 0.075 %. This is lower than what is expected in comparison to a study by (Mauchline) who found that males invest 0.545 % ± 0.462 % of their body volume into spermatophores. The lower part of this variation-width is however comparable to the size of my results (0.545-0.462 = 0.083). Therefore, even though the direct costs calculated in my experiment are small they are not unrealistic. The seemingly low cost of spermatophores is contradictory to the observed reduced production when food access is limited. This indicates that the spermatophores are of a higher cost than what is derived from their investment.

NUPTIAL GIFT

A possible explanation for the high cost of spermatophore production might be that the seminal fluid contains other substances besides just sperm cells. Little is known about the actual content of the spermatophores. However Defaye *et al.* (2000) found that spermatophores appear to contain two kinds of material, one dense type located close to the

spermatophore neck and one less dense occupying most of the spermatophore. Sichlau and Kiørboe (2011) found that spermatophores contain an average of 1126 ± 92 spermatozoa. Larger spermatophores contain more spermatozoa than smaller ones, however, the sperm content is not proportional to the spermatophore volume (Sichlau and Kiørboe 2011). The seemingly high investment into spermatophores found in my experiment and the indication of variable sizes of the spermatophores, could mean that they contain other substances in addition to sperm cells. Well fed males seem to produce the largest spermatophores. If the spermatophores actually contain other substances, the well fed males are most likely the ones with most additional substances within their spermatophores, due to the spermatophore size and content not being proportional. The additional substances within the spermatophore may include proteins, lipids, carbohydrates, minerals, hormones or other beneficial chemical substances that are supplied to the females. This phenomenon is called nuptial gifts and is common in the animal kingdom, especially among insects (Vahed 1998; Arnqvist and Nilsson 2000).

The receiving females benefit from the nuptial gift with enhanced egg production and elevated longevity (Vahed 1998). For the males however, it is costly to produce nuptial gifts. They may benefit from their investment with enhanced chances of attracting and copulating females. The gift may also contain substances that ensure effective insemination and maximize sperm transfer, resulting in increased number and fitness for offspring produced (Vahed 1998). There is no evidence for copepods actually having nuptial gifts. However, the high investment into spermatophores and the fact that the number of sperm cells in large spermatophores does not increase proportionally with size indicate that the spermatophores may contain nuptial gifts. Insects, like butterflies, beetles and moths all supply nuptial gifts within their spermatophores (Vahed 1998). However, the volume of the spermatophores compared to body volume for these insects are generally higher than for the copepods. Few studies have investigated investment into spermatophores as a fraction of body volume for insects known to have nuptial gifts. Until now, it has been shown that for arctiid moths (*Utetheisa ornatrix*) the spermatophores are of a size 11 % of the body volume (LaMunyon and Eisner 1994), for various bushcrickets spp the investment varies between 2 % and 23 % (Wedell 1993) and for butterflies (*Pieris napi*), the investment is about 12 % (Forsberg and Wiklund 1989). Therefore, to determine whether or not copepod spermatophores contain nuptial gifts, detailed investigations of their content is needed.

MATING LIMITATIONS

Generally, mating has been assumed to be limited by encounter rates rather than spermatophore production. It is assumed that males daily produce sufficient amounts of sperm to inseminate many females (reviewed by Dewsbury 1982). Despite this, only a few females are fertilized even when encounter rates are high (Kiørboe 2007). On average, a male can only mate with 0.9 females per day and only about one third of encountered females are fertilized (Kiørboe 2007). The pelagic environment offer huge challenges for the tiny copepods trying to locate each other. The adaptations of remote detection allow males to search through large volumes of water to such an extent that mating rates are rarely encounter limited (Kiørboe and Bagøien 2005; Kiørboe 2011). Whether a male can utilize these mating opportunities or not depends on if the females are receptive. A female only has room for two spermatophores, and when full, males do not benefit from adding additional spermatophores. Among pelagic copepods, the males typically perform active mate searching. This leads to elevated mortality for the males and can explain why the sex ratio is often female biased (Hirst and Kiørboe 2002; Kiørboe 2006; Hirst *et al.* 2010). The populations may become so female biased that male abundance limits the fertilization rate and consequently population growth. For species like *T. longicornis* that perform multiple mating events, the sexes have a more similar interest in mate encounter and often have a less female-skewed population (Kiørboe 2006). Even at low female concentrations, males typically encounter several females a day and they therefore have more opportunities for mating than they can utilize (Kiørboe and Bagøien 2005; Kiørboe 2007). Therefore there is reason to believe that spermatophore production limits fertilization more than encounter rates.

Males apparently have a limited spermatophore production rate (Table 4). I found that in food deprived environments, the males have an even lower production rate. Therefore it is possible that copepods may alternate their behavior to increase food intake. For suspension feeders such as *T. longicornis*, males may search for females and feed at the same time. Feeding is 50 % more efficient when hovering in the water than when cruising (Kiørboe 2008). If a male is hovering, it has small chances of encountering females in the water due to the large and dilute

environment the copepods inhabit. If there is little food in the water it may lead to a trade off, where males focus on filtering at the expense of cruising. Both Van Duren and Videler (1996) and Kiørboe (2008) found that males offered little or no food had a reduction in swimming activities compared to when they were well fed. This is probably because they switch to hovering to maximize their food intake. When hovering, the males have few opportunities to mate because their ability to encounter females is reduced. It is unknown if males will actually cease their production of spermatophores to focus on feeding when food is scarce. However, if that is the case, it may explain the low production of spermatophores found in the treatments of limited food access in this experiment. It might be that the seemingly high price of production lies in a tradeoff between feeding and mate searching, and when food access is limited, the production is drastically reduced. The non-linear relationship implicit in the fitted GLM model for spermatophore production may reflect a threshold-response. Implying that below a certain food concentration a threshold is met where energy is diverted to focus on feeding rather than production.

IMPLICATIONS FOR POPULATION DYNAMICS

Spermatophore production is closely linked to food access. Whether it is the actual cost of production or food searching behavior that limits it, is difficult to determine. The fact that spermatophore productions is so resource dependent supports assumptions that mating for *T. longicornis* is not random, consistent with recent studies suggesting sexual selection for several species of copepods (Titelman *et al.* 2007; Ceballos and Kiørboe 2010; Sichlau and Kiørboe 2011). If spermatophore production is limited, males may increase their fitness by being selective in their mate choice. Supporting male sexual selection, Ceballos *et al.* (in review) found a high proportion of unfertilized female *T. longicornis* both in field and laboratory cultures. This occurred even with high male availability and with no encounter limitation on mating.

Periods of food deprivation might be the reason for this large proportion of unfertilized females (Ceballos *et al.* in review) and production of sterile eggs (Kiørboe 2007). My results indicate that in periods of food deprivation, the males will have a drastic decrease in their

reproductive output. This is comparable with a study by Ianora *et al.* (1995). Here all copepods were fed different food sources in excess concentrations. When copepods were fed *Skeletonema*, the spermatophore production was very limited. This is probably because S*keletonema* has the ability to reduce its chain length as a response to copepod cues and therefore is a poor food source for copepods (Bergkvist *et al.* 2012). A single species diet of *Skeletonema* can be viewed as similar to no food access. When Ianora *et al.* (1995) fed the copepods *Skeletonema*, they responded with a low spermatophore production of 0.09 a day (Table 4), comparable to my results from the starved treatment, where copepods produced an average of 0.07 spermatophores a day.

T. longicornis has high metabolic requirements with a high biomass turnover and is unable to store lipids as an energy source (Lee *et al.* 2006). They feed mainly on phytoplankton but in periods of limited food supply they need to utilize other food sources like ciliates and other zooplankton to cope with the constant need to feed (Gentsch *et al.* 2009). This ability to change from a strictly vegetarian diet to feeding on microzooplankton increases their food access and will thus enhance production and survival. Since spermatophore production is closely linked to food availability, a change in diet will give them the possibility to increase spermatophore production in periods when phytoplankton access is low. This will also have a positive effect on population growth in less productive periods in the ocean, like during winter when carbon levels sink (Lindahl and Hernroth 1983). *T. longicornis* is at its highest abundance during spring and summer (Castonguay *et al.* 1998; Roy *et al.* 2000) corresponding to periods when the C levels are peaking (Lindahl and Hernroth 1983), inferring spermatophore production should not be limited by food access.

Table 4: Overview of previous studies done on spermatophore production.

Species	Spermatophore production (male^{-1}day^{-1})	Food source (C-concentration)	Paper
Paraeuchaeta norvegica	Up tp 3 during life span	-	Hopkins (1978)
Temora stylifera	0.7 ± 0.6	*Prorocentrum minimum* (30.89 µg C mL^{-1})	Ianora and Poulet (1993)
	0.4 ± 0.4	*Thalassiosira rotula* (15.76 µg C mL^{-1})	
Centropages typicus	< 1 for 12 d	*T. rotula* (15,76 µg C mL^{-1}) *Gonyaulax polyedra* (30.89 µg C mL^{-1})	Miralto *et al.* (1995)
T. stylifera	0.7 ± 0.6	*Prorocentrum* (-)	Ianora *et al.* (1995)
	0.3 ± 0.3	*Isochrystis*(-)	
	0.4 ±0.4	*T. rotula* (-)	
	0.09 ± 0.15	*Skeletonema* (-)	
	0.46 ± 0.21	*Phaeodactylum* (-)	
	0.43 ± 0.35	*Chaetoseros*(-)	
Acartia clausi	< 1 for between 0-55d	*T. rotula* (15.76 µg C mL^{-1}) *P. minimum* (30.89 µg C mL^{-1})	Ianora *et al.* (1996)
T. stylifera	1.4	*P. minimum* (11.6 µg C d^{-1})	Ianora *et al.* (1999)
	0.6	*Gymndinium sanguinium* (6.5 µg C d^{-1})	
	1.2	*G. polyedra* (7.6 µg C d^{-1})	
T. stylifera	0.14	*P. minimum* (-)	Turner *et al.* (2001)
	0.21	*T. rotula* (-)	
	0.61	*Mix of both(-)*	
	0.68	*Alternation* (-)	
A. tonsa	1	*Rhodomonas salina* (1000 µg C L^{-1})	Ceballos and Kiørboe (2010)
Oithona davisa	2.5	*Oxyrrhis marina* (> 500 µg C L^{-1})	Ceballos and Kiørboe (2011)

UNCERTAINTIES AND POSSIBLE BIASES

In my experiment, virgin males were used to maximize reproductive production because it is known that male *T. longicornis* cease to produce spermatophore after only eight days. Females on the other hand are productive up to 13 days after maturation (Sichlau and Kiørboe 2011). However, Heuschele and Kiørboe (2012) found that male *Oithona davisae* (Cyclopoida, Copepoda) can differentiate between virgin and non-virgin females. This new evidence might imply that to maximize spermatophore production in mating experiments with copepods, one should also use virgin females to ensure females are receptive to mating. If the males are able to sense old and non-virgin females, they might lower their production rare, resulting in underestimation of spermatophore production. However. cyclopoid copepods are not broadcast spawners. The females mate once and keep their eggs in an egg-sack. It is therefore of great importance for these males to be able to differentiate between fertilized an unfertilized females. For species like *T. longicornis* where females can and often need to mate more than once, this trait is not as valuable. Therefore the unknown age of females in this experiment probably had little influence on the number of spermatophores produced.

CONCLUDING REMARKS

When food access is limited, male *T. longicornis* drastically reduce their spermatophore production. This comes as a surprise seeing that the apparent cost of spermatophores is low in relation to body volume. Further investigation of the spermatophore content is needed to clarify whiter the reduced production when food is scarce is a result of costly nuptial gifts within the spermatophores, or mainly due to a behavioral trade-off between mate searching and feeding.

Limited spermatophore production will play a major role in regulating the population size of pelagic copepods. It affects the population dynamics by limiting population growth and decreasing reproductive outcomes. Previously it has been assumed that only females are reproductively constrained by food supply. However, here it has been shown that males also have a huge disadvantage in environments with limited food supply because their spermatophore production rate seems dependent on food availability. This thesis therefore present evidence suggesting that spermatophore production is limited and largely controlled by food intake, at least for the species *T. longicornis*. Poor male mating performance will strongly limit female fertilization even with high population abundance and high encounter rates. Therefore it supports recent suggestions that sexual selection might be an underestimated effect on population dynamics in pelagic copepods.

LITERATURE CITED

Arnqvist G. and Nilsson T. (2000). "The evolution of polyandry: multiple mating and female fitness in insects." *Animal behaviour* **60**(2): 145-164.

Bergkvist J. et al. (2012). "Grazer-induced chain length plasticity reduces grazing risk in a marine diatom." *Limnology and Oceanography* **57**(1): 318.

Casini M. et al. (2004). "Feeding preferences of herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) in the southern Baltic Sea." *ICES Journal of Marine Science: Journal du Conseil* **61**(8): 1267-1277.

Castellani C. and Lucas I. A. N. (2003). "Seasonal variation in egg morphology and hatching success in the calanoid copepods *Temora longicornis*, *Acartia clausi* and *Centropages hamatus*." *Journal of Plankton Research* **25**(5): 527-537.

Castonguay M. et al. (1998). "Annual and spatial variabillity in zooplankton biomass and species composition, and in eggs and larvae of mackrel and cod in the southern gulf of St. Lawrence between 1982 and 1991." *Canadian Data Report of Fisheries and Aquatic Sciences*(1035): 1-86.

Ceballos S. and Kiørboe T. (2010). "First evidences of sexual selection by mate choice in marine zooplankton." *Oecologia* **164**(3): 627-635.

Ceballos S. and Kiørboe T. (2011). "Senescence and Sexual Selection in a Pelagic Copepod." *Plos One* **6**(4).

Ceballos S. et al. (in review). "Low fertilization rates in pelagic copepods caused by sexual selection?".

Corkett C. (1967). "The copepodid stages of *Temora longicornis* (OF Müller, 1792)(Copepoda)." *Crustaceana*: 261-273.

Corkett C. and McLaren I. (1970). "Relationships between development rate of eggs and older stages of copepods." *J. mar. biol. Ass. UK* **50**(1): 161-168.

Daan R. (1989). "Factors controlling the summer development of copepod populations in the southern bight of the North-Sea." *Netherlands Journal of Sea Research* **23**(3): 305-322.

Dam H. G. and Lopes R. M. (2003). "Omnivory in the calanoid copepod *Temora longicornis*: feeding, egg production and egg hatching rates." *Journal of experimental marine biology and ecology* **292**(2): 119-137.

Dam H. G. and Peterson W. T. (1991). "In situ feeding behavior of the copepod *Temora longicornis*: Effects of seasonal changes in chlorophyll size fractions and female size." *Marine ecology progress series. Oldendorf* **71**(2): 113-123.

Defaye D. et al. (2000). "Genital structures and spermatophore placement in female *Paradiaptominae* (Copepoda, Calanoida, Diaptomidae)." *Journal of crustacean biology* **20**(2): 245-261.

Dewsbury D. A. (1982). "Ejaculate cost and male choice." *American Naturalist* **119**(5): 601-610.

Doall M. H. et al. (1998). "Locating a mate in 3D: the case of *Temora longicornis*." *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **353**(1369): 681-689.

Dutz J. et al. (2010). "Life cycle and spring phenology of *Temora longicornis* in the Baltic Sea." *Marine Ecology-Progress Series* **406**: 223-238.

Flinkman J. et al. (1998). "Changes in northern Baltic zooplankton and herring nutrition from 1980s to 1990s: top-down and bottom-up processes at work." *Marine Ecology Progress Series* **165**: 127-136.

Forsberg J. and Wiklund C. (1989). "Mating in the afternoon: Time-saving in courtship and remating by females of a polyandrous butterfly *Pieris napi*." *Behavioral Ecology and Sociobiology* **25**(5): 349-356.

Gentsch E. et al. (2009). "Dietary shifts in the copepod *Temora longicornis* during spring: evidence from stable isotope signatures, fatty acid biomarkers and feeding experiments." *Journal of plankton research* **31**(1): 45-60.

Heuschele J. and Kiørboe T. (2012). "The smell of virgins: mating status of females affects male swimming behaviour in *Oithona davisae*." *Journal of Plankton Research* **34**(11): 929-935.

Hirst A. et al. (2010). "Does predation control adult sex ratios and longevities in marine pelagic copepods?" *Limnology and Oceanography* **55**(5): 2193-2206.
Hirst A. and Kiørboe T. (2002). "Mortality of marine planktonic copepods: global rates and patterns." *Marine Ecology-Progress Series* **230**: 195-209.
Hopcroft R. and Roff J. (1998). "Zooplankton growth rates: the influence of female size and resources on egg production of tropical marine copepods." *Marine biology* **132**(1): 79-86.
Hopkins C. (1978). "The male genital system, and spermatophore production and function in *Euchaeta norvegica*, Boeck (Copepoda: Calanoida)." *Journal of experimental marine biology and ecology* **35**(3): 197-231.
Hopkins C. (1982). "The breeding biology of *Euchaeta norvegica* (Boeck) (Copepoda: Calanoida) in Loch Etive, Scotland: Assessment of breeding intensity in terms of seasonal cycles in the sex ratio, spermatophore attachment, and egg-sac production." *Journal of experimental marine Biology and Ecology* **60**(1): 91-102.
Ianora A. (1998). "Copepod life history traits in subtemperate regions." *Journal of Marine Systems* **15**(1-4): 337-349.
Ianora A. et al. (1999). "First evidence of some dinoflagellates reducing male copepod fertilization capacity." *Limnology and Oceanography* **44**(1): 147-153.
Ianora A. et al. (1995). "A comparative study of the inhibitory effect of diatoms on the reproductive biology of the copepod *Temora stylifera*." *Marine Biology* **121**(3): 533-539.
Ianora A. et al. (1996). "The diatom Thalassiosira rotula affects reproductive success in the copepod Acartia clausi." *Marine Biology* **125**(2): 279-286.
Ianora A. and Poulet S. A. (1993). "Egg viability in the copepod *Temora stylifera*." *Limnology and Oceanography* **38**(8): 1615-1626.
Jaspers C. and Carstensen J. (2009). "Effect of acid Lugol solution as preservative on two representative chitineous and gelatinous zooplankton groups." *Limnology and Oceanography-Methods* **7**: 430-435.
Kiørboe T. (2006). "Sex, sex-ratios, and the dynamics of pelagic copepod populations." *Oecologia* **148**(1): 40-50.
Kiørboe T. (2007). "Mate finding, mating, and population dynamics in a planktonic copepod *Oithona davisae*: There are too few males." *Limnology and Oceanography* **52**(4): 1511-1522.
Kiørboe T. (2008). "Optimal swimming strategies in mate-searching pelagic copepods." *Oecologia* **155**(1): 179-192.
Kiørboe T. (2011). "What makes pelagic copepods so successful?" *Journal of Plankton Research* **33**(5): 677-685.
Kiørboe T. and Bagøien E. (2005). "Motility patterns and mate encounter rates in planktonic copepods." *Limnology and Oceanography* **50**(6): 1999-2007.
Kiørboe T. et al. (1985). "Bioenergetics of the planktonic copepod *Acartia tonsa* - relation between feeding, egg-production and respiration, and composition of specific dynamic action." *Marine Ecology Progress Series* **26**(1-2): 85-97.
Klein Breteler W. C. M. (1980). "Continuous breeding of marine pelagic copepods in the presence of heterotrophic dinoflagellates." *Mar. Ecol. Progress Ser* **2**: 229-233.
Klein Breteler W. C. M. and Gonzalez S. R. (1986). "Culture and development of *Temora longicornis* (Copepoda, Calanoida)at different conditions of temperature and food." *Syllogenus* **58**: 71-84.
Koojiman S. (1995). "The stoichiometry of animal energetics." *Journal of Theoretical Biology* **177**(2): 139-149.
Kuijper L. D. J. et al. (2004). "C and N gross growth efficiencies of copepod egg production studied using a Dynamic Energy Budget model." *Journal of Plankton Research* **26**(2): 213-226.
Kwasniewski S. et al. (1991). Atlas of the marine fauna of southern Spitsbergen. Gdansk, Polish Academy of Science, Institute of Oceanology.
LaMunyon C. W. and Eisner T. (1994). "Spermatophore size as determinant of paternity in an arctiid moth (*Utetheisa ornatrix*)." *Proceedings of the National Academy of Sciences* **91**(15): 7081-7084.
Lee R. F. et al. (2006). "Lipid storage in marine zooplankton." *Marine Ecology Progress Series* **307**: 273-306.

Lindahl O. and Hernroth L. (1983). "Phyto-zooplankton community in coastal waters of western Sweden- an ecosystem off balance?" *Marine ecology progress series. Oldendorf* **10**(2): 119-126.
Lindley J. (1990). "Distribution of overwintering calanoid copepod eggs in sea-bed sediments around southern Britain." *Marine Biology* **104**(2): 209-217.
Maps F. et al. (2005). "Egg production and hatching success of *Temora longicornis* (Copepoda, Calanoida) in the southern Gulf of St. Lawrence." *Marine Ecology Progress Series* **285**: 117-128.
Mauchline J. (1998). Advances in marine biology - The biology of calanoid copepods - Introduction. Advances in Marine Biology, Vol 33: The Biology of Calanoid Copepods. London, Academic Press Ltd-Elsevier Science Ltd. **33:** 14-23, 243-294, 279-344, 401-453.
McCullagh P. and Nelder J. (1989). Generalized Linear Models. London, Chapman and Hall.
Miralto A. et al. (1995). "Food type induces different reproductive responses in the copepod *Centropages typicus*." *Journal of plankton research* **17**(7): 1521-1534.
Möllmann C. et al. (2000). "Long-term dynamics of main mesozooplankton species in the central Baltic Sea." *Journal of Plankton Research* **22**(11): 2015-2038.
Nordberg K. (1991). "Oceanography in the Kattegat and Skagerrak over the past 8000 years." *Paleoceanography* **6**(4): 461-484.
Ohtsuka S. and Huys R. (2001). "Sexual dimorphism in calanoid copepods: morphology and function." *Hydrobiologia* **453/454**(1-3): 441-466.
Olsson M. et al. (1997). "Is sperm really so cheap? Costs of reproduction in male adders, *Vipera berus*." *Proceedings of the Royal Society B-Biological Sciences* **264**(1380): 455-459.
Omori M. (1969). "Weight and chemical composition of some important oceanic zooplankton in the North Pacific Ocean." *Marine Biology* **3**(1): 4-10.
Paffenhofer G. A. (1998). "On the relation of structure, perception and activity in marine planktonic copepods." *Journal of Marine Systems* **15**(1-4): 457-473.
Peterson W. T. and Dam H. G. (1996). "Pigment ingestion and egg production rates of the calanoid copepod *Temora longicornis* implications for gut pigment loss and omnivorous feeding." *Journal of plankton research* **18**(5): 855-861.
Rasband W. S. (1997-2009). ImageJ, US National Institutes of Health, Bethesda, Maryland, USA.
Roy S. et al. (2000). "Importance of mesozooplankton feeding for the downward flux of biogenic carbon in the Gulf of St. Lawrence (Canada)." *Deep-Sea Research Part Ii-Topical Studies in Oceanography* **47**(3-4): 519-544.
Sandström O. (1980). "Selective feeding by Baltic herring." *Hydrobiologia* **69**(3): 199-207.
Sichlau M. H. and Kiørboe T. (2011). "Age- and size-dependent mating performance and fertility in a pelagic copepod, *Temora longicornis*." *Marine Ecology Progress Series* **442**: 123-132.
Sivars-Becker L. (2004). "Food and parasites: life hostory decisions in copepods." *PhD thesis. Uppsala University, Uppsala, Sweeden*.
Sven Lovén Centre for Marine Sciences Station Log. (2012). "www.weather.loven.gu.se/en." Retrieved 01.05.2012 - 29.05.2012.
Tiselius P. and Jonsson P. R. (1990). "Foraging behavior of 6 calanoid copepods - observations and hydrodynamic analysis." *Marine Ecology Progress Series* **66**(1-2): 23-33.
Titelman J. et al. (2007). "Copepod mating: chance or choice?" *Journal of Plankton Research* **29**(12): 1023-1030.
Turner J. T. et al. (2001). "Decoupling of copepod grazing rates, fecundity and egg-hatching success on mixed and alternating diatom and dinoflagellate diets." *Marine Ecology Progress Series* **220**: 187-199.
Vahed K. (1998). "The function of nuptial feeding in insects: a review of empirical studies." *Biological Reviews* **73**(1): 43-78.
Van Duren L. A. and Videler J. J. (1996). "The trade-off between feeding, mate seeking and predator avoidance in copepods: behavioural responses to chemical cues." *Journal of plankton research* **18**(5): 805-818.
Van Voorhies W. A. (1992). "Production of sperm reduces nematode lifespan." *Nature*(360): 456-458.
Verity P. G. and Smetacek V. (1996). "Organism life cycles, predation, and the structure of marine pelagic ecosystems." *Marine Ecology-Progress Series* **130**(1-3): 277-293.

Vogedes D. et al. (2010). "Lipid sac area as a proxy for individual lipid content of arctic calanoid copepods." *Journal of plankton research* **32**(10): 1471-1477.
Webb D. G. and Weaver A. J. (1988). "Predation and the evolution of free spawning in marine calanoid copepods." *Oikos*: 189-192.
Wedell N. (1993). "Spermatophore size in bushcrickets: comparative evidence for nuptial gifts as a sperm protection device." *Evolution*: 1203-1212.
Wedell N. et al. (2002). "Sperm competition, male prudence and sperm-limited females." *Trends in Ecology & Evolution* **17**(7): 313-320.
Williamson C. E. and Butler N. M. (1987). "Temperature, food and mate limitation of copepod reproductive rates: separating the effects of multiple hypotheses." *Journal of Plankton Research* **9**(5): 821-836.

APPENDIX I: THE BODY OF *TEMORA LONGICORNIS*

T. longicornis, has a quite large semipellucid (partially transparent) body of faint blue tinge, with cinnamon-colored or reddish patches. The copepod body is divided into several regions; the cephalosome (head region), the metasome and the urosome (hind body). The cepalosome and the metasome together, make the prosome (Fig. 6). The prosome is a clearly defined part of the body and its length from the top of the cephalosome to the edge of the metasome segment 5 is used as a direct measure of body length or size. Because the urosome often is flexed or damaged, this measure is preferred to that of the entire body length (Mauchline 1998).

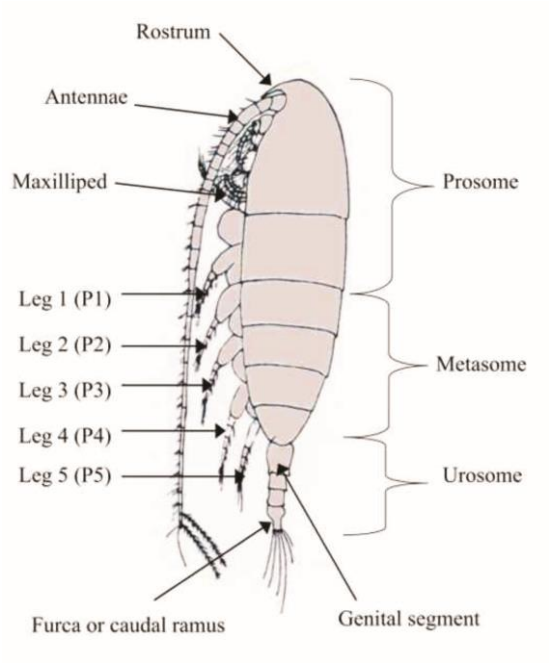

Figure 6: General sketch of copepod morphology (Mauchline 1998)

T. longicornis has an elongated urosome, consisting of three segments in females and four segments in males. The length of males is 1-1.4mm and the females are slightly bigger and can reach 1.5 mm (Kwasniewski *et al.* 1991). The prosome is elongated or pear shaped and the cephalosome is rounded apically. The caudal rami (end segment of urosome, separated in two) are long and symmetric. The metasome comprises of four segments, ending with a terminal metasome segment with rounded corners. The antennules reach beyond the anal

segment (last segment of the urosome). The last pair of legs (P5) are uniramous (has only one branch) and on females they have 3-4 jointed apical spines and males have the distant joint of the right leg bent at 90 degrees, the left leg is chelate.

APPENDIX II: THE DIFFERENT COPEPODITE STAGES OF *T. LONGICORNIS*.

The different copepodite stages are easiest distinguished from each other by counting the number of free thoracic segments, number of urosome segments and number of pairs of swimming legs. Table 5 gives an overview of these features as they relate to *T. longicornis*. The sex of the copepodite stages may only be distinguished after maturation to stage IV, when they form their fifth pair of swimming legs (Corkett 1967).

Tabel 5: Overview of the present segments in different copepodite stages of Temora longicornis (Corkett 1967).

Stage:	**I**	**II**	**III**	**IVF**	**IVM**	**VF**	**VM**	**VIF**	**VIM**
No. of free thoracic segments	4	5	5	4 /5	4 / 5	4	4	4	4
No. of urosome segments	1	1	2	3	3	3	4	3	5
No. of pairs of swimming feet	2	3	4	5	5	5	5	5	5

APPENDIX III MODEL CALCULATIONS

The model formula for spermatophore production is $\log(\lambda) = a - b(food\ inntake)$. When parameters from the model (Table 1.1) are implemented, the calculations are:

$$\log(\lambda_1) = 0.3 - 1.97 * 0.5$$

$$\log(\lambda_1) = 0.3 - 1.97$$

$$\log\left(\frac{\lambda_{0.5}}{\lambda_1}\right) = \log(\lambda_{0.5}) - \log(\lambda_1)$$

$$= 0.3 - 1.97 * 0.5 - (0.3 - 1.97)$$

$$= 1.97 * 0.5$$

$$\frac{\lambda_{0.5}}{\lambda_1} = e^{1.97*0.5}$$

The model formula for egg production is $\log(\lambda) = a - b(food\ inntake)$. When parameters from the model (Table 1.2) are implemented, the calculations are:

$$\log(\lambda_1) = 2.26 - 1.85 * 0.5$$

$$\log(\lambda_1) = 2.26 - 1.85$$

$$\log\left(\frac{\lambda_{0.5}}{\lambda_1}\right) = \log(\lambda_{0.5}) - \log(\lambda_1)$$

$$= 2.26 - 1.85 * 0.5 - (2.26 - 1.85)$$

$$= 1.85 * 0.5$$

$$\frac{\lambda_{0.5}}{\lambda_1} = e^{1.85*0.5}$$