Reproductive biology of the calanoid copepod, *Eudiaptomus graciliodes* (Lilljeborg): Polyandry, Phenology and Life Cycle Strategies.

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A dissertation for the degree of Philosophiae Doctor

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June 2007

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Acknowledgements

First and foremost I would like to thank the Almighty God for making my academic aspiration and accomplishment a reality. Then it is my pleasure to express my heart-felt appreciation and special gratitude to Prof. Ivar Folstad for the opportunity and privilege he gave me to pursue PhD under his supervision. For that and for his unreserved support and guidance during data collection, analysis and the write up of the whole thesis, he is greatly acknowledged. He was always present when I needed his help. I would also like to thank Dr. Raul Primicerio and Dr. Ståle Liljedal for their help during data collection and analysis, for a considerable amount of helpful discussion and comments on earlier drafts of the thesis. I am grateful to Ass. Prof. Jørgen Berge for valuable instruction and assistance on the techniques of morphological measurement and for comments on manuscripts. Thanks to Geir Rudolfsen and Lars Figenschou for helping field sample collection, and to Sissel Kaino for helping in counting copepod sample and taking body size measurements and to the Norwegian State Educational Loan Fund for granting me the scholarship to undertake graduate studies at the University of Tromsø. My special thank goes to dear Kedija Reshid for loving and understanding me, and encouraging me when I was far away from my academic atmosphere. Last but not least, I remain sincere, grateful and indebted to my mother Zubeyda Awole, my father Kelil Ali and all my sisters and brothers, whose words of encouragement, affection and prayer served me as a source of strength and inspiration throughout my study.

Tromsø, June 2007

Abdurhman Kelil Ali

List of Papers

This thesis is based on the following papers, referred to in the text by the Roman numbers given below.

Paper I

Distinct developmental phenology and reproductive strategies in *Eudiaptomus graciloides* populations of two neighbouring subarctic lakes.

Paper II

Morphological correlates of mating frequency and clutch size in wild caught female *Eudiaptomus graciloides* (Copepoda: Calanoida)

Paper III

Influence of female morphology on male mating success in the calanoid copepod, *Eudiaptomus graciloides.*

Paper IV

No apparent fitness benefits of polyandry in female *Eudiaptomus graciloides* (Copepoda: Calanoida)

1. GENERAL INTRODUCTION

1.1 BODY SIZE, DEVELOPMENT TIME AND LIFE CYCLE

Body size is an important trait affecting fitness in many species and several selective mechanisms influencing body size may be identified (Stearns, 1992; Roff, 2002). Yet, fecundity selection and sexual selection are usually the most important mechanisms (Andersson, 1994). In most ectotherms, the number of eggs a female produces increases with body size. Large females are also better equipped to thwart harassing males and reduce direct costs associated with superfluous mating (Crean & Gilburn, 1998). Male body size, on the other hand, can be important in male-male competition for females and for female choice of mates, and body size can also affect the male's mobility and search ability (Andersson, 1994) and the ability to overcome initial resistance of females to mating (Arnqvist & Rowe, 2002; Rowe & Arnqvist, 2002). Additionally, there is often a positive relationship between male size and success in sperm competition in many polyandrous organisms (Nylin & Gotthard, 1998).

While large body size is usually associated with greater mating and reproductive success, several factors also appear to select against it (see review Blanckenhorn, 2000). Adult body size is, for example, a function of growth rate and development time (Abrams *et al.,* 1996), and to achieve a larger size, organisms may have to either grow faster or grow for a longer time (Stearns, 1992; Blanckenhorn, 2000; Roff 2002). However, rapid growth seems to be costly (see review: Munch & Conover, 2003; Gotthard, 2001, 2004), for example, in terms of increased predation risk at higher foraging intensities (Werner & Anholt, 1993; Abrams *et al.*, 1996; Gotthard, 2000; Munch & Conover, 2003) or of physiological costs due to higher metabolic demands (Gotthard *et al.*, 1994; Blanckenhorn, 1998; Fischer *et al.*, 2004). Therefore, growth rates may not always be maximized (Gotthard, 2004). Alternatively, organisms may reach a large size at maturity by growing for a longer time, i.e., by delaying age at maturity (Stearns, 1992; Blanckenhorn, 2000; Roff 2002). However, longer development time may increase the chances of mortality before reproduction (Stearns, 1992; Blanckenhorn, 2000; Gotthard, 2001; Roff, 2002). Given high pre-reproductive mortality, organisms are predicted to decrease their age at maturity (Sibly & Calow, 1986; Abrams & Rowe, 1996), with consequent costs of reduced fecundity associated with a smaller size. Therefore, the advantages of reaching maturity at an early age, and achieving large size may be under conflict. Understanding the optimal balance between these conflicting demands has been the focus of research on life histories (Stearns 1992; Roff 2002). Obviously, the optimal age and size at maturity are expected to depend upon environmental conditions during development (Blanckenhorn, 1998; Nylin & Gotthard, 1998) and the period within which development is constrained (McNamara & Houston, 1996; Abrams *et al,* 1996; Johansson & Rowe, 1999; Gotthard, 2001, 2004)

Environmental variables that are usually invoked to explain the change in development time and final body size include food quality and availability, temperature, and the effect of size-selective predation. For example, studied ectotherms often grow faster and become larger when food is abundant or of high quality (Atkinson & Sibly 1997; Blanckenhorn, 1998). Moreover, the majority of ectotherms grow slower but mature at a larger body size in colder environments (Atkinson 1994; Berrigan & Charnov 1994; Sibly & Atkinson 1994). Such thermal clines in body size are a common phenomenon in species distributed over broad geographic ranges, with the majority of them exhibiting larger adult size in colder environments (Partridge & French, 1996; Ashton, 2004). In addition, numerous experiments have shown that ectotherms grow to large size when reared in cooler condition (see review: Atkinson 1994, Atkinson *et al*., 2003). The inverse relationship between body size and temperature — referred to as *the temperature-size rule* — may be attributed to an evolutionary response resulting from differences among genotypes (Berven 1982; Lonsdale & Levinton 1985; Partridge *et al,* 1994) and/or a developmental response (phenotypic plasticity) to temperature (Atkinson 1994). Moreover, predation, which is often size-dependent, is another important factor that affects development time and body size. For example, small predators select small prey, whereas large predators choose large prey (Nylin & Gotthard, 1998). Thus, when predation from vertebrates that prefer large prey is important, selection may favor a shorter growth period resulting in reduced age at maturity and smaller body size. As body size and clutch size are closely related, selective pressure for smaller size may also result in smaller clutch size. Additionally, in animals that carry their eggs until hatching, for example, in copepods and cladocerans, the risk of predation by visually-oriented planktivorous fishes increase with clutch size as increased clutch size increases the conspicuousness of prey (Svensson, 1995, 1997). The average clutch size of populations exposed to such fish predation has been documented to decrease (Gliwicz, 1981; Dawidowicz & Gliwicz, 1983; Vuorinnen *et al.,* 1983; Gliwicz & Boavida. 1996). Consequently, smaller body size may occur as a correlated response to selection against large clutches. On the other hand, invertebrate predators preferring small prey should favour large body size at first reproduction (Lynch, 1980a, b; Paine 1965; Bertness 1981; Berven & Gill 1983). The different factors favouring or opposing large body size could vary spatially and temporally; under such conditions, discrete populations should evolve different body sizes to suit local selective regimes. Therefore, it is essential to assess environmental conditions during development in order to make reliable predictions about optimal growth trajectories and final body size.

It has also become evident that the seasonal environments in which most organisms live, impose a time constraint on life histories (e.g., Forrest 1987, Nylin *et al*., 1989, Rowe & Ludwig 1991, Wiklund *et al.,* 1991, Rowe *et al.,* 1994, Abrams *et al*.,1996). Especially organisms living in temperate and arctic regions experience only a limited period each year in which conditions for growth and reproduction are optimal. The life cycles of organisms under such condition must be completed within the limits set by seasonality and their fitness is often strongly dependent on successful timing of certain critical life cycle events (phenology) in relation to seasonal changes in biotic and abiotic factors (Gotthard, 2001, 2004). For example, for successful foraging in natural environments, it is important to synchronize the stages that are involved in growth and development with the periods of high abundance and quality of food; otherwise, organisms will experience a strong reduction in fitness in terms of reduced survival or fecundity. Moreover, in order to survive the winter conditions, most temperate organisms have to reach a certain ontogenetic stage, capable of over-wintering. For example, at high latititudes, where the winter conditions are very pronounced, copepods have developed two principal forms of overwintering strategies: the adoption of a resting (or diapause) stage within the developmental sequence together with an ontogenetic, seasonal vertical migration or, alternatively, the production of resting (or diapausing) eggs (Muchline, 1998). Additionally, in sexually reproducing species mating and reproductive activities must start at a time of the year when sexually mature mates are likely to be present (Gotthard, 2001, 2004). Therefore, organisms in seasonal environments are likely to experience selection, not only for utilizing the available time efficiently, but also for synchronization of the life cycle. Under such condition, life history theory suggests that the optimal development time and final body size should vary with time horizons for juvenile growth and development (Werner & Anholt, 1993; Abrams *et al.*, 1996). Empirical studies comparing the timing and duration of critical life cycle events of populations living in different biotopes may therefore lead to a better understanding of the mechanisms linking life history traits and strategies to local environmental conditions.

1.2 SEXUAL SELECTION

Darwin proposed the theory of sexual selection to account for major problems for his theory of evolution by natural selection: why have males of many animals evolved extravagant secondary sexual characters that probably reduce survival? Why are males and females so different in appearance in many species? Sexual selection, Darwin's key explanations, was his shorthand phrase for selection that arises through competition over mates (Andersson, 1994). He posited that sexual selection is different from natural selection. He wrote:

> "*sexual selection ... depends not on a struggle for existence in relation to other organic beings or to external conditions, but on a struggle between individuals of one sex, generally the males, for the possession of the other sex. The result is not death to the unsuccessful competitor, but few or no offspring*" (Darwin, 1859, p88)

Sexual selection could occur either through mate contests, usually male–male competition for mating opportunities, or as a consequence of mate choice, usually female choice for attractive males (Darwin 1871). The fundamental reason for such divergent mating tactic between the two sexes is their difference in gamete size (anisogamy) and associated investment in offspring production. As females generally produce large nutrient-rich eggs and often care for the young, they commonly invest more per offspring than do males. Consequently, males are expected to increase their reproductive success by mating with as many females as possible and, where mating opportunities are limited, to compete for mates. Females, in contrast, are expected to maximize their reproductive success by choosing a high quality male (Bateman 1948; Travers, 1972; Clutton-Brock and Parker 1992).

1.2.1 MECHANISMS OF SEXUAL SELECTION

Darwin's process of sexual selection, mate contest and mate choice, has long been accepted, and the ideas still represent the major concepts of modern sexual selection theory (Andersson and Iwasa, 1996; Kokko *et al.*, 2006). However, the mechanisms by which sexual selection operates are still heavily debated. The controversy over this issue is mainly about what drives female mate choice for male traits. Currently, there are different hypotheses about the mechanisms of sexual selection in general and the evolution of female preference for male traits in particular (Andersson, 1994).

The Fisher Run-away Process

After Darwin, the next major development in sexual selection theory came in 1930 by Ronald Fisher. He filled the gap left by Darwin by proposing a verbal model of female choice. According to this process an initial female preference for arbitrary male character could reach a threshold frequency in the population that selects for more preferred males. Females that mate with males expressing the preferred traits produce more attractive sons who also carry the genes for the mating preference for more preferred male traits. As a result, a genetic coupling (Lande, 1981) will establish between the female preference and the preferred male trait, which in turn will lead to a positive feedback. This creates a self-reinforcing system that continually will exaggerate the female preference and the male trait in the population until the size of male trait compromises male survival to the extent that extra mating advantage cannot balance the reduced survival (Lande, 1981; Kirkpatrick, 1982). However, in spite of being plausible, this process have been criticized for failing to explain how preference could generally be maintained in the face of the costs of choice that might be involved (Andersson, 1994).

Indicator Mechanisms

Fisher (1915) also provided the first outline to another influential idea, variously termed as ''viability-indicator'', ''handicap'' or ''good genes'' model, that sexually selected traits in males could serve as indicators of viability for female choice. This idea received even less attention than his other work on sexual selection, and was largely forgotten (Andersson, 1994). However, the 1960s and 1970s witnessed a revival of interest in Fisher's (1915) indicator idea. The same idea was re-introduced and became more generally known when discussed first by Williams (1966) and later by others (Zahavi, 1975, 1977; Hamilton & Zuk, 1982). An idea much debated under this model was the handicap principle proposed by Zahavi (1975). He presented a verbal idea as a strict alternative to Fisherian runaway selection. Zahavi (1975) emphasized that indicator traits must reduce viability, that is, handicap the survival of the male and the ability to express the traits provides a test of male's genetic quality. Such costly traits should enforce honest signalling by males and thus serve as reliable mate-choice cues for females. Consequently, females enhance the overall quality of genes that they pass on to offspring by choosing the right male (Zahavi, 1975). Co-evolution between the female preference and the preferred male trait would then occur, resulting in exaggeration of display traits with sizable survival costs to its bearer. Several theoretical studies have later provided support for the importance of indicators processes and for the evolution of costly male traits and, also, costly female preference (e.g. Iwasa, *et al.*, 1991; Iwasa & Pomiankowski, 1994; 1999; Pomiankowski & Møller, 1995; Rowe & Houle, 1996; Houle & Kondrashov, 2002)

Direct Phenotype benefits

The above two models of sexual selection both predict indirect benefits to females that exert mate choice. Direct selection for female mate choice could instead arise whenever preferences directly affect female fitness. For example, males may vary in the amount of food they provide, the quality of the nest site they hold, or some other resources that directly affect female fitness. Selection favours females that can recognize males that are superior providers by some feature that is correlated with their ability to provide (see review: Kirkpatrick and Ryan, 1991; Andersson, 1994). Using genetic models, Heywood (1989) and Hoelzer (1989) suggested that non-heritable variation in parental ability may lead to the evolution of male traits that advertise high parental ability. Unlike indirect genetic benefit models, such direct benefit model does not require mechanisms that maintain genetic variance (Andersson, 1994). Additionally, it is easy to see how male traits and female preference for honest signals of a male's ability to increase her fitness can evolve (Andersson, 1994). There is much empirical evidence supporting the direct benefit model and more than for the indirect genetic benefit models. Yet, the latter models may be harder to evaluate, demanding a more sophisticated experimental approach.

Sensory exploitation hypothesis

The sensory exploitation hypothesis (WestEberhard, 1984; Ryan, 1990) is a rather different explanation for the evolution of female preference and male traits. According to this model, preferences may be maintained as pleiotropic effects of natural selection on female sensory systems in the contexts of other selection regimes than mate choice, such as foraging or predator evasion. The male trait is favored simply by virtue of its manipulative effect on a pre-existing bias in the sensory system of females. Contrary to the Fisherian run-away process and indicator mechanisms, the male trait and female preference for the trait are not expected to have a tightly correlated evolution.

Sexually Antagonistic Selection

Most recently it has been suggested that sexual conflict between the two sexes over various aspects of reproduction (e.g., mating rate, female proximate fecundity) could result in the evolution of female mate choice (Arnqvist, 1992; Rowe *et al.,* 1994; Hollan & Race, 1998; Gavrilets *et al* 2001; Chapman*, et al.,* 2003). At the centre of this conflict is the hugely different investment in the gametes by males and females (anisogamy; Bateman, 1948). Since females generally invest considerably more than males, males benefit more from increasing the number of mates and the number of matings than do females (Bateman, 1948; Parker 1979). Thus, males are expected to attempt to seduce or coerce females to mate at a rate beyond their optimum. As mating sub-optimally is costly to females, this generates counter-selection on females to evolve resistance, rather than preference, to the male trait, followed by selection favouring a more extreme male trait to overcome female resistance. Consequently, this will result in the evolution of sexual selected characters along antagonistic, rather than mutualistic, trajectories, which may ultimately lead to viability selection checking further elaboration of the male trait in question (Hollan & Race, 1998; Gavrilets *et al.,* 2001; Chapman *et al.,* 2003; Arnqvist & Rowe, 2005).

1.2.2 POLYANDRY

During the last decade, the increasing use of molecular techniques has revealed that females of many species, spanning a broad array of taxa, mate with multiple males during one reproductive event (polyandry) (Birkhead & Møller, 1998; Arnqvist and Nilsson 2000; Jennions and Petrie 2000). This challenges the long held view of females as the choosy, essentially monogamous sex (see above). Additionally, given that mating is a costly activity in terms of energy or foraging time (Wilcox, 1984; Bailey *et al.*, 1993; Clutton-Brock & Langley, 1997; Watson *et al.*, 1998), predation risk (Arnqvist, 1989; Fairbairn, 1993; Magnhagen, 1991; Rowe, 1994; Ward, 1986) and disease transmission (Daly, 1978; Hurst *et al.*, 1995), polyandry raises the question of why females commonly mate with multiple males (Andersson & Simmons, 2006).

A number of hypotheses have been proposed to explain the evolution and maintenance of polyandry in animal mating system that in some ways parallel the mechanisms of mate choice evolution (see above) (Andersson & Simmons, 2006). These hypotheses can broadly be categorized in to those that based on direct (material) benefits, or indirect (genetic) benefits to female following multiple mating (Hosken & Stockley, 2003). In the former case, polyandrous females may derive benefits such as nutrient received from spermatophores or ceminal fluid, an adequate sperm supply/fertility insurance or additional parental investment that cause female reproductive rate to increase with the number of mating (Thornhill & Alcock, 1983; Arnqvist & Nilsson, 2000; Wiklund *et al.*, 2001; Hosken & Stockley, 2003). Additionally, females may gain indirect genetic benefits that increase offspring fitness (Jennions & Petrie, 2000). Such indirect benefits might arise from improving on earlier mating (good genes models; Thornhill & Alcock, 1983; Simmons, 1987; Olsson, *et al.,* 1996), reducing the risk of genetic incompatibility (Zeh & Zeh, 1996, 1997), inbreeding avoidance (Tregenza & Wedell, 2002), and increased genetic diversity (Baer & Schmid-Hemel, 1999). The different benefits hypotheses have been empirically documented for a variety of organisms (see review: Arnqvist & Nilsson, 2000; Jennions & Petrie, 2000; Hosken & Stockley, 2003). However, the extent to which these hypotheses might apply across taxa is challenged because there are increasing number of cases in which benefits to females could not be found (Kolodziejczyk & Radwan, 2003; Brown, *et al.,* 2004; Maklakov & Lubin, 2004; Patricia & Graeme, 2004)

An alternative hypothesis based on sexual conflict has been proposed to addresses the occurrences of polyandry. This hypothesis invokes that polyandry may evolve as a result of selection on males to copulate as often and with as many females as possible (e.g., Parker, 1979; Rowe *et al.,* 1994; Holland & Rice, 1998; Arnqvist & Nilsson 2000). As males benefit more from higher mating rate than do females (Bateman, 1948), they may seduce or coerce females to mate at a rate beyond their optimum, while females are expected to resist mating attempt to reduce direct costs associated with superfluous mating (Parker, 1979; Arnqvist & Nilsson 2000; Gavrilets *et al.,* 2001). However, pre-mating struggle/resistance is also a costly behaviour to females in terms of energy (Watson *et al*., 1998), foraging time (Wilcox, 1984) and predation risk (Rowe, 1994) and in the face of repeated male harassment, these costs may be even more than the cost of mating itself (see above). Under such condition, female might be better off by accepting superfluous mating, in a strategy of 'making the best of the bad job'. This phenomenon - termed as 'convenience polyandry' - has been proposed as an explanation for the occurrence of multiple mating in many organisms (Thornhill & Alcock 1983; Arnqvist, 1989, 1992; Rowe, 1992; Rowe *et al.,* 1994; Watson *et al*., 1998; Weigensberg & Fairbairn, 1996

Aim of the study

The main objective of this project was to address the question of multiple mating in females of the model organism *Eudiaptmus graciloides* (Lilljeborg). As background knowledge, I also investigated phenology and reproductive strategies of populations living in two neighboring lakes, Takvatn and Fjellfrøsvatn. The specific objectives were:

1. to assess and compare timing of certain critical life cycle events of the two populations in relation to seasonal changes (paper I).
2. to assess and compare development time, body size, clutch size of the populations in relation to environmental conditions during development (paper I).
3. to determine the natural female mating frequency (paper I).
4. to investigate the influence of female morphological traits on the opportunity for multiple matings in wild caught females (paper II, paper IV).
5. to assess the relationships of female's morphology with egg number and egg size (paper II).
6. to investigate the influence of female morphological traits on the outcome of mating encounter under laboratory condition (paper III).
7. to examine direct fitness benefits of multiple mating to female (paper IV).

2.0 Study species and lakes

The lakes sampled

Copepods are small aquatic crustaceans. They are among the most numerous multicellular organisms on earth (Mauchline, 1998) constituting the major components of most marine and fresh water zooplankton communities. I have studied the freshwater calanoid copepod species *Eudiaptomus graciloides* (Lilljeborg) throughout this thesis. Studies on sexual selection included in this thesis were carried out on the population from Lake Takvatn (paper II, III & IV), which showed a greater variation in life history traits and mating frequencies. Comparative work between lakes on life history traits and reproductive strategies of *E. graciloides* also included material from lake Fjellfrøsvatn (paper I). The two sub-arctic lakes (see map: Fig. 1) are situated in northern Norway (69°07□N, 19°05E□). They are characterized by short productive season and cool, oligotrophic waters with similar physico-chemistry and similar ice and snow conditions (ice thickness = 20 – 100 cm & snow depth 5 – 100 cm) (Dahl-Hansen *et al.,* 1994; Primicerio, 2000; Klemetsen *et al.*, 2003). Their surface areas are 15 and 6.5 Km^2 respectively, and both have a maximum depth of 80 m. The fish community is dominated by Arctic char (*Salvelinus alpinus*), which has been introduced into Takvatn from Fjellfrøsvatn (Svenning & Grotnes, 1991). Unlike Takvatn, Fjellfrøsvatn have two morphs of Arctic char, differing in size and timing of reproduction (Klemetsen *et al.*, 2003). Together with the small morph, Planktivorous char is more abundant in Fjellfrøsvatn, implying a greater predation risk for *Eudiaptomus* in this lake (Dahl-Hansen *et al.*, 1994; Klemetsen *et al.*, 2003; Knudsen *et al.*, 2007). For example, Klemetsen and colleague (2003) found that food consumption of Arctic char in Fjellfrøsvatn, without including the small morph, were consistently about twice as high as in Takvatn during winter. Arctic charr are known to feed under the ice (Klemetsen & Grotnes, 1975), at temperatures close to zero (Brännäs & Wiklund, 1992). It has a retinal flexibility (Ali *et al.,* 1984) that allows feeding at low light intensities despite the weak light of the polar night.

Developmental stages

Diaptomid copepods have complete metamorphosis. The eggs hatch into larva called Nauplii. There are six naupliar stages, usually abbreviated N1 to N6. N1 has no oral apparatus, lives on its vitelline reserves and barely move. This suggests that the amount of reserve, which is a function of N1 size, can have an important implication on the survival of the non-feeding stage (Cooney & Gehrs, 1980; Wyngaard, 1986; Guisande *et al.*, 1996). After moulting in to N2, it starts to feed. N6 moults to the first of the five copepodid stages, abbreviated to C1- C5 and finally C5 moults into adults (Dussart & Defaye, 1995; Muchline, 1998).

Figure 1. Map showing the situation of the two examined lakes in Balsfjord, Troms, Northern Norway. Takvatn (214 m.a.s.l.) and Fjellfrøsvatn (125 m.a.s.l.) have a respective surface area of 15 Km^2 and 6.5 Km^2. Water in rivers from the two lakes merges before passing Skjold. Yellow and white lines are roads.

The body and secondary sexual characters

The body of an adult copepod is divided in to three main regions: the cephalosome, metasome and urosome (paper II, Fig. 1). The first two regions are clearly defined parts of the body and they are together known as the prosome, or anterior part of the body. The length of the prosome is usually used as a direct measure of body length or size (Dussart & Defaye, 1995; Muchline, 1998). The urosome, or posterior part of the body, consists of the genital somite and several segments posterior to it, including a pair of furca. Sexually dimorphic characters that develop during the later copepod stages distinguish females and males. Males are usually smaller in size than females (mean prosome length in mm ± SD: male = 0.773 ± 0.018 and female = 0.857 ± 0.028; n=39) and have additional segments in the urosome (appendix 1A, 1C, 1D & 1I). The right antennule of males in calanoid (and both antennules in cyclopoids and harpacticoids) copepods is structurally modified (appendix 1A, 1B) for grasping the female preceding mating (Blades & Youngbluth, 1980; Gilbert & Williamson, 1983; Dussart & Defaye, 1995; Muchline, 1998). Its mid-part is enlarged consisting of a hinge that enables the antennule to fold back upon itself (appendix 1B). Moreover, the fifth pair of swimming legs of male calanoid (and harpacticoids but not cyclopoids) is sexually dimorphic, asymmetrical and adapted for mating activities (appendix 1E, 1F). Its right exopod is modified into a large chela that is used to grasp the female urosome during copulation (appendix 1F). The short and stubby external spine of the left exopod is modified to hold the spermatophore and attach it on the female gential area (appendix 1E). In females, the fifth pair of swimming legs is only slightly modified (appendix 1G).

Spermatophore and its placement

Copepods reproduce sexually requiring copulation. During copulation, the male transfer spermatozoa and associated seminal secretions contained in a package called spermatophore to the female (Fig. 3 & 4). Male *E. graciloides* can attach only one spermatophore in one mating event and the average time between the production of two spermatophores is 43 hours (SD ± 27.1, n = 18; 3 ± 1°C; 18 : 6 h light : dark cycle). The spermatozoa of calanoid copepods are aflagellated and immobile. The development of spermatophores provides an efficient mechanism to transfer gametes to the females (Blades-Eckelbarger, 1991) and it might represent an adaptation to such limitations. Spermatophores of *E. graciloides* (and from the majority of other calanoid copepods) are simple, tube-shaped flasks with mean length of 326 µm (SD: ± 14, n=100) that narrows into a spermatophore neck (Fig (4A) towards its open end (Blades-Eckelbarger, 1991; Hosfeld, 1994). Such simple spermatophores usually adheres to the female by means of cement like substance present on the outside of the spermatophore neck or by secretion extruded from the spermatophore itself (Blades-Eckelbarger, 1991; Hosfeld, 1994; Defaye *et al.,* 2000). However, in some calanoids, the spermatophores possess complex chitin-like plates, termed coupling plates by which they are attached by the male on the female urosome (Blades & Youngbluth, 1979, 1980; Blades-Eckelbarger, 1991). Full spermatophores (Fig. 4A) appear in light microscope to contain two distinct kinds of materials, one less dense, occupying most of the spermatophore, the other, much denser, located close to the neck (Defaye *et al.,* 2000). A completely discharged spermatophore (Figs. 5 & appendix 2C) contains nothing but some remaining secretions, which looks foamy and blistered on higher magnification (Hosfeld, 1994). Placement position of spermatophores on the female urosome differs between species (see: Muchline, 1998; Defaye *et al.,* 2000). In many species of calanoid copepods, males attach the spermatophore over the genital pore within the genital field of the genital double somite, referred to as the direct/correct placement. In other species, however, spermatophores can be indirectly/alternately placed on parts of the genital somite remote from the genital field. Some of these indirectly/alternately placed spermatophores develop a fertilization tube, a prolongation of the original, short spermatophore neck (Hosfeld, 1994). The fertilization tube establishes connection to the genital opening. In yet other species both direct and indirect placements of spermatophores have been observed. (Muchline, 1998; Defaye *et al.,* 2000). In *E. graciloides*, the main spermatophore placement position might be direct. I never observed spermatophores attached far from the genital field. (Figs 3, 5, 6, 7 & appendix 2A). Even females with multiple spermatophores had spermatophores located closely concentrated around the genital field (Appendix 2B). In some cases, single and multiple spermatophores with fertilization tubes were observed in the direct placement position (Fig. 5, 6 & 7 Appendix 2B & 2C).

The phenomenon of multiple spermatophores has been considered a waste of reproductive effort by males (e.g. Katona, 1975; Blades, 1977; Hopkins & Machin, 1977; Hopkins, 1982; Swenson, 1997). Furthermore, the first attached spermatophore on the genital opening of female has been suggested to act as a mechanical barrier that effectively prevents insemination (discharge of seminal products) and subsequent fertilization by other spermatophores (Cuoc *et al*., 1989a, b; Barthélémy *et al.,* 1998; Defaye *et al.,* 2000). I, on the other hand, found multiple spermatophores with fertilization tubes connecting directly to the genital opening of female (Fig. 6). Additionally, electron microscopy studies revealed that multiple spermatophores that was attached on the female genital area reaching the female genital atrium, where fertilization of ova occurs (see appendix 3). Moreover, micro-satellite analysis has recently confirmed the occurrence of multiple paternities within clutches produced by females that had several attached spermatophores (Todd *et al.,* 2005). In sum, these latter observations clearly suggest that attachment of multiple spermatophores on females may not be a waste of reproductive investment.

Female genital area

The genital area of females, where males attach their spermatophores, are located on the ventral face of the genital double somite (Fig. 2). In external view, it is covered with a plate-like flap known as operculum, which is visible in non-ovigerous females (appendix 1). This taxonomically important structure (Defaye *et al.,* 2000) is free distally and articulates with the body wall along a more or less marked anterior hinge. The operculum delimits a small underlying cavity called genital atrium. The atrium opens to the exterior via the atrial slit. The genital atrium is the site of laying and fertilization of the oocytes and of the formation and attachment of the ovisac (Cuoc *et al*., 1989a, b; Barthélémy *et al.,* 1998; Defaye *et al.,* 2000). The spermatophores are deposited on the genital area, where they form seminal pseudo-receptacle, and act as an external chamber, storing sperm until fertilization (Cuoc *et al*., 1989a, b; Barthélémy *et al.,* 1998; Defaye *et al.,* 2000). Females usually detach spent spermatophores with their modified fifth legs before extrusion of egg sacs. The intervals between the two events usually differ within and between species. For example*,* at an average temperature close to that *in situ* (3±1°C; 18:6 h light: dark cycle), females of *E. graciloides* detach their spermatophores in about 28 hours after mating (SD ± 21, n=25) and produce egg sac approximately 47 hours (SD ± 32, n=25) after mating. These intervals might be shorter for other species (Berger and Maier, 2001). The extruded eggs are contained into a sac and carried for some days until the nauplii hatch (Chow-Fraser & Maly, 1988; Berger and Maier, 2001). Diaptomid females lack seminal receptacles to store sperm (Cuoc *et al*., 1989a, b; Defaye *et al.,* 2000) and hence re-mating is necessary to produce a second clutch of fertile eggs (Watras & Haney, 1980; Watras, 1983; Chow-Fraser & Maly, 1988; Berger and Maier, 2001).

Mating behaviour

A high proportion of diaptomid females mate multiply, as indicated by the number of externally attached spermatophores (e.g. Berger and Gerhard, 2001; Paper I, II, and

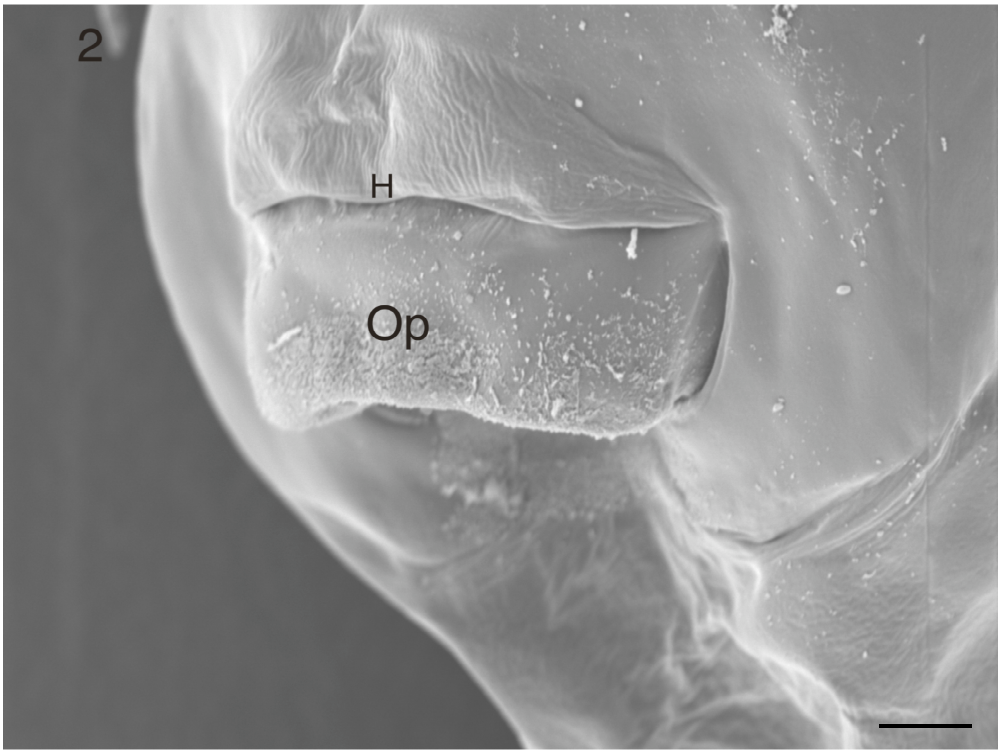

Figure 2. Scanning electron micrograph of genital area of female *Eudiaptomus graciloides*. Note the genital operculum (Op) and its hing (H). Scale bar: 10 µm

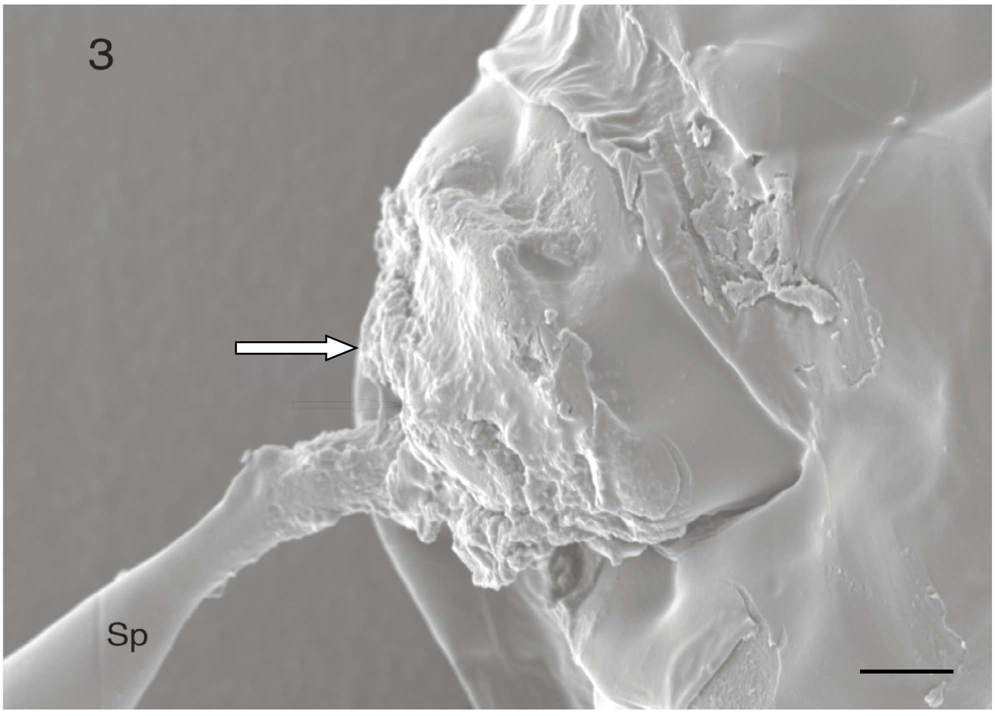

Figure 3. Scanning electron micrograph of spermatophore (sp) placement in *Eudiaptomus graciloides*. Note the genital area and placement site of the spermatophore and cement like material (right arrow). Scale bar: 10 µm

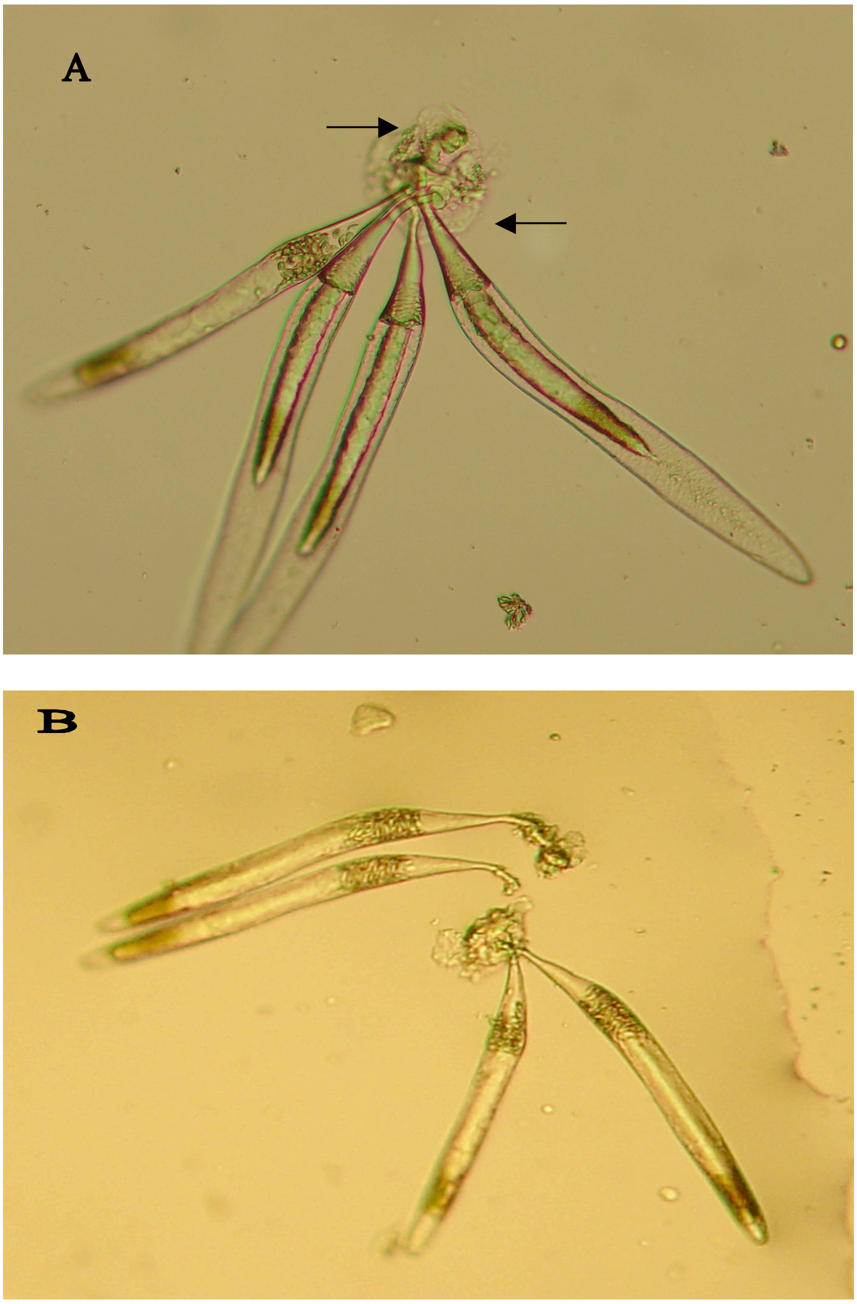

Figure 4. Photographs of spermatophores detached with fine needles from the genital area of female *Eudiaptomus graciloides*. A. Note the adhesive substance (right arrow) attaching all spermatophores together. Left arrow indicates spermatophore neck. B. The two top spermatophores detached with fine needles from the adhesive substance. Note the difference in length.

IV). Moreover, females' rate of mating varies between species, between populations within species, and between individuals within populations. However, a single mating before each clutch production is also sufficient and does not limit female reproductive success (Watras & Haney, 1980; Watras, 1983; Chow-Fraser & Maly, 1988; Berger and Maier, 2001; Paper IV). The question of adaptive significance of polyandry in diaptomid copepod, however, has been given much less attention despite their being an ideal organism for the topic.

Copulation involves a sequence of behavioural as well as morphological interactions between the sexes. Males actively search and chase receptive females and attempt to grasp them. Females respond to this with a vigorous escape reaction and try to dislodge the males (Watras, 1983). During this premating struggle, the male attempts to secure first a furcal and then genital double somite grasp of the female, with its right antennule and right fifth leg, respectively (Berger & Maier, 2001). These two points of attachment are critical for controlling the escaping females and for attachment of the spermatophore externally on the genital double-somite of the females. For females, body size and antennules are probably important in the behavioural response to male mating attempt as these traits are known to be directly associated with mobility and escape ability (Mauchline, 1998). Moreover, morphological segments or contact areas on females, such as the furca and genital double somite, where males make initial contact and position themselves during mating, may also be important in mediating female response to male manipulation.

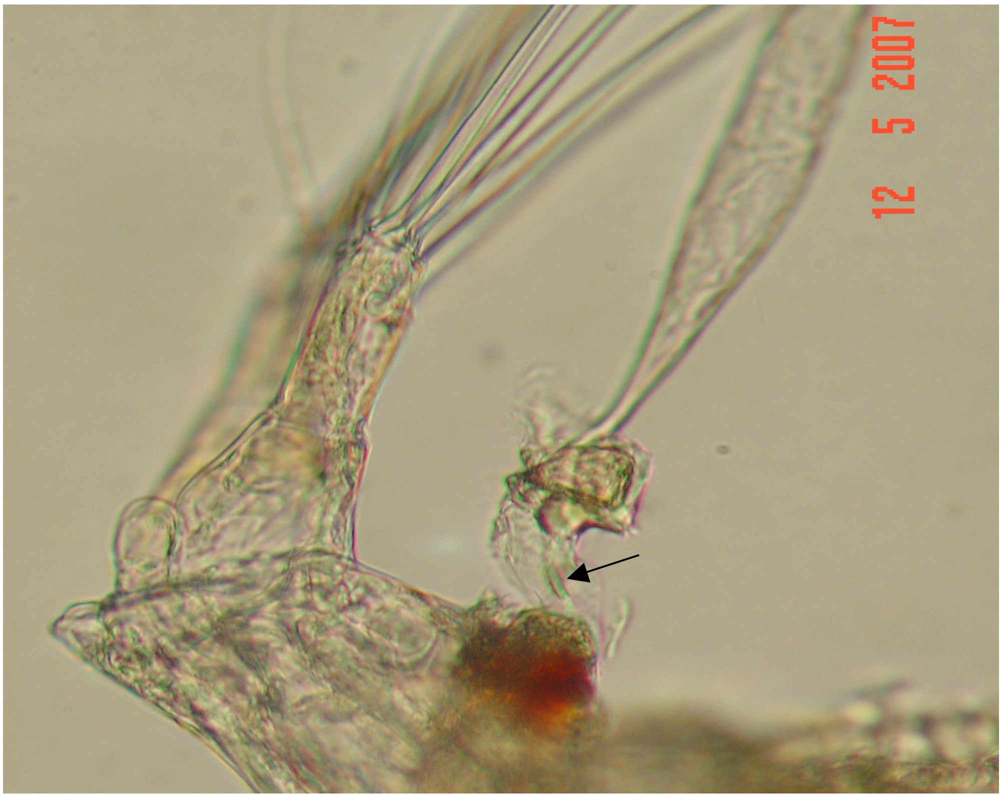

Figure 5. Photograph of posterior part of female *Eudiaptomus graciloides*. Empty spermatophore with fertilization tube (arrow) attached to the genital opening.

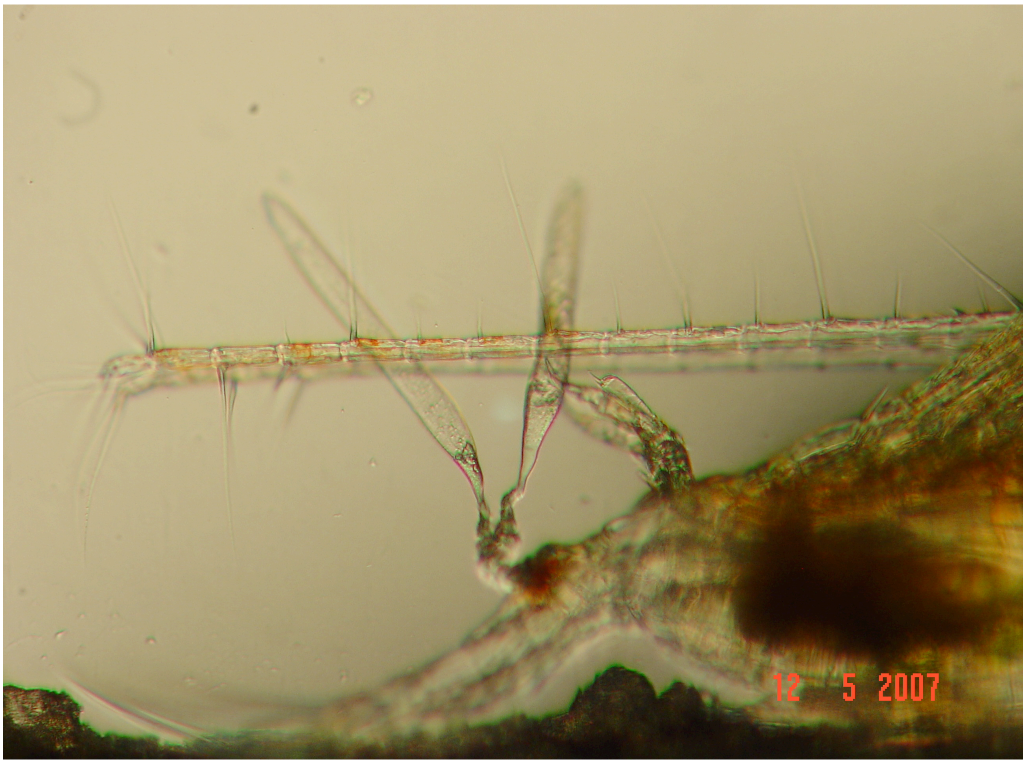

Figure 6. Photograph of *Eudiaptomus graciloides*. Empty spermatophores with fertilization tube attached to the genital opening.

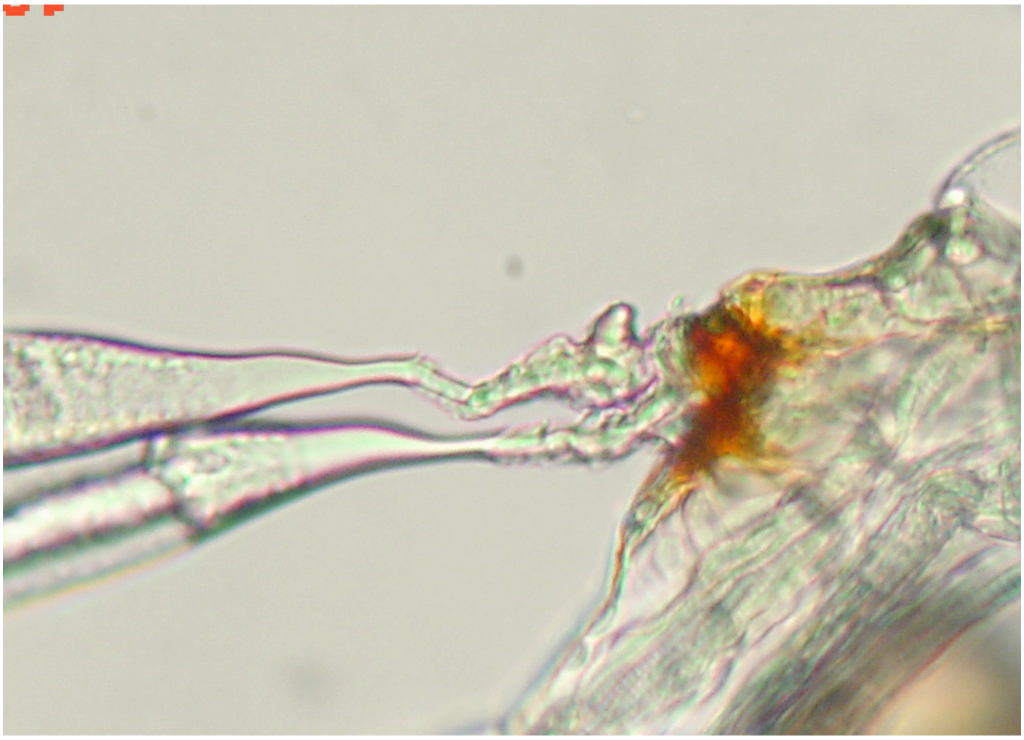

Figure 7. Photograph of posterior part of female *Eudiaptomus graciloides*. Spermatophores with fertilization tube attached to the genital opening.

3. GENERAL MATERIAL AND METHODS

The studies focusing on polyandry, comprising the main part of the thesis (Paper II, III, & IV), were carried out on copepods from lake Takvatn. In Paper I, copepods from Fjellfrøsvatn were also included to provide comparative material on phenology of life cycle and reproduction, and on life history traits. The first two studies (I and II) were comparative and observational, based on fixed material collected in the field, whereas the last two studies (III & IV) were experimental studies performed on live animals under laboratory conditions with controlled light-temperature room (3±1°C; 18: 6 h light: dark cycle, provided by 20 watt incandescent lamps) and provision of lab cultured alga *Scenedesmus gracilis.*

The design of a sampling programme depends on the biological question, the type of information required (qualitative vs. quantitative) and the characteristics of the environment in which the studied organism lives, such as temperature, depth or predation gradients (Mauchline, 1998). In the high latitude lakes studied water temperature is low for most part of the season and copepods exhibit slowed growth rate, extended development time and seasonally restricted breeding periods (Dahl-Hansen *et al.,* 1994; Primicerio, 2000; Klemetsen *et al.*, 2003). Also, in the pelagic, deep area of the lakes, inhabited by *E. graciloides*, horizontal heterogeneity in the relevant demographic and environmental variables is low relative to temporal and between lakes heterogeneity. Therefore, given these conditions and taking into consideration the experience of previous studies (Primicerio & Klemetsen, 1999; Primicerio, 2000), all sampling activities were carried out at fixed stations in the pelagic area, with higher sampling frequencies during the warm, open water period. To check for spatial variation in relevant variables within lakes, additional samples were collected in 2007. Copepod samples were collected with plankton net (mesh size, 50 µm) hauled vertically at a constant speed (0.5 m s^{-1}) from 30 m depth to the surface.

For study I, triplicate samples of copepods and water temperature were collected monthly from January 2003 to January 2004 (except in June, July and October when samples were taken twice a month) from Takvatn Lake and Fjellfrøsvatn Lake. Additionally, triplicate samples from three different stations (>100m apart) were collected once a month from February to April in 2007. The samples were first narcotized (5% ethanol) and then, fixed in (4%, final concentration) formalin solution for later examination. Different developmental stages and sexes were identified based on morphological characteristics (Dussart & Defaye, 1995) and counted from sub-samples under a dissection microscope. Females carrying spermatophores or eggs were also registered, and the number of spermatophores or eggs carried was counted. Adult female body size (prosome length) from selected sampling date (that had ovigarous females) was measured with an ocular micrometer under a dissecting microscope.

For study II, I used five replicate samples collected and fixed (see above) from Takvatn Lake in February and March 2003. From each sampling date, I randomly selected females carrying from one to five spermatophores (10 females in each group) and female carrying from three to eight eggs in egg sac (10 females in each group). Using compound microscope fitted with a drawing mirror, the image of the different body parts, spermatophores and eggs were taken onto a piece of paper and measurements of the drawings were later made to the nearest 0.01 mm. Natural female mating rate and clutch size of females was assessed, by counting the number of externally attached spermatophores and the number of eggs carried in the sac, respectively. Selection of morphological traits for investigation was made based on their importance during mating. Measured traits were analysed to examine their relationship with mating frequency and clutch size.

Mate choice experiment (III) was carried out using live copepods collected from Takvatn in January 2004. The samples were diluted in lake water in 30 l plastic tanks and transported to controlled light-temperature room. Adult males and adult unmated females were sorted and kept separately, to prevent them from mating before the experiment started. They were fed with the alga *S. gracilis* at the final concentration of about $5x10^4$ cells ml^{-1}. After four days of separation, 3 randomly selected receptive females were combined with an adult male in a small vessel containing filtered lake water. A total of 150 experimental trials were performed. After an introduction period of 24 hours, copepods were preserved for subsequent morphometric analyses (see above). The presence of a spermatophore or a fertilized egg on the female genital double somite was considered as evidence of mating. In 58 of my experimental units, I found one mated female and two unmated females. However, as some animals were lost during handling for size measurement, I ended up with 39 experimental units for statistical analysis, each having one male, one mated and two unmated females.

Another experimental study (IV) was performed under laboratory conditions using wild-mated females that had been collected and brought to the laboratory in March 2007. In controlled light-temperature room, I randomly selected 150-mated female, 75 of which were single mated and the rest (75) double-mated, and kept the separately in groups according to their spermatophore (s) number. After 3 days of separation, egg-producing females were transferred to individual chambers containing 20 ml of filtered lake water. As diptomid females need re-mating to produce a new set of fertile eggs (e.g., Berger & Maier, 2001), I introduced a male to each chamber on the same day. The male remained present until the death of the female; if the male died earlier, it was replaced by a new male. During the 3 days period after separation, a total of 62 females (26 single mated and 36 double mated) produced egg sacs, 10 female died (4 single mated and 6 double mated) and the rest (78) failed to produce eggs.

Animals in the individual chambers were fed with the alga *S. gracilis* at the final concentration of about $5x10^4$ cells ml^{-1} three times per week. Females were examined daily for survival and other fitness parameters. Their clutch size and the number of fertilized and unfertilized eggs were noted. Moreover, the date of hatching, the number of egg hatched and the number of alive and dead offspring were recorded. Additionally, females were checked for any change in reproductive phases, mating and additional clutch production. The picture of live adult females and hatching were taken using a light microscope mounted with a digital camera. Measures of morphological data from the digital images of animals were later obtained using CorelDraw 11 and Canvas 8 digital image analysis software. All trait measurements were made to the nearest 0.001 mm. The experiment lasted for two months and provided data about several components of female fitness that was compared between single mated females and double-mated females.

Preliminary studies were also carried out to gather information that were relevant for the experimental studies (III & IV), i.e., time to mating and the length of time between: (*i*) mating (spermatophore attachment) and detachment of spermatophores, (*ii*) mating and egg sac production and (*iii*) egg production and hatching of offspring, (*iv*) the first and the second spermatophore produced by males. A total of 60 experimental trials were performed, each involving the combination of 3 randomly selected receptive females and an adult male in a small glass vessel containing filtered lake water. The experiments were carried out in the abovementioned controlled light-temperature rooms, and animals were fed as mentioned above.

Samples for the different morphological figures presented in the thesis were collected from Takvatn. The techniques employed to gather such figures include, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and light microscopy.

Scanning electron microscopy (SEM). Image of the external morphology of females genital area and the images for spermatophore attachment on the genital opening were made with the SEM technique. In preparation for SEM (Dussart & Defaye, 1995), specimens were cleaned, by rinsing in an aqueous solution of potassium hydroxide. Thereafter, the specimens were fixed, using formalin, and then dehydrated in graded ethanol baths: 50%, 70%, 90%, 95%, and 100%. After that, the specimens were transferred to acetone and critical point dried. Dried samples were mounted with double sided adhesive tape on stubs in the desired orientation under dissecting microscope and coated with gold. Observation and photographing were carried out under scanning electron microscope (model: Jeol JSM 6300).

Transmission electron microscopy (TEM). This technique was used to follow whether or not multiple spermatophores attached on the female genital field reach the genital atrium, where fertilization of ova occurs. Females with multiple spermatophores were first anaesthetized and then, fixed with 3% glutaraldehyde, buffered (pH 3, 0.1 M) mixture of sodium cacodylate solution for 40 minute at 20ºC. Thereafter, specimens were post-fixed in a solution of phosphate buffer (0.1 M) and 1% osmium tetroxide at room temperature for 30 minutes. Then, they were dehydrated in an ethanol, followed by immersion in propylene oxide and finally embedded in araldite. First, several semithine (1 µm thick) section cut were made with glass knives on an ultramicrotome and stained with toluidine blue and examined under light microscopy. The sections were made starting from external ventral face of genital area where males attach their spermatophores. Then, ultrathin sections were cut with a diamond knife and collected on 200-mesh copper grids coated with pioloform support films. They were stained with uranyl acetate followed by lead citrate and examined and photographed with electron microscope (model: Jeol JEM 1010). Staring from the point of attachment of spermatophores on the genital field of females, several ultrathin sections were cut in sequences and examined and photographed until reaching the genital atrium of the females.

4 RESULTS AND DISCUSSION

4.1 Life cycle and natural mating frequency

As background knowledge for subsequent studies (paper II, III & IV), I investigated timing of certain critical life cycle events and reproductive strategies of populations of *E. graciloides* living in two neighboring lakes, Takvatn and Fjellfrøsvatn (paper I). Analyses of the results from one year of regular monthly sampling provided valuable information on seasonal changes in proportion of different developmental stages, incidence of ovigerous and mated females, natural mating frequency of female (measured as number of spermatophores per mated female) body size and clutch size. This information, together with the record of seasonal temperature changes, helped to define the breeding season, the developmental period, the natural mating frequency of female and the annual number of generations of the two populations.

Some aspects of the life cycle followed similar seasonal trends in the two lakes. For example, both populations have only one generation per year. Moreover, they started egg production in mid-winter under the ice cover, before any phytoplankton growth has started, but synchronized the appearance of their later copepodid stages with the seasonal availability of food. The first adults appeared in early autumn, forming the over-wintering population. These findings are in line with those of other studies reporting egg production by *E. graciloides* in late winter, when food availability is low (Ekman, 1964; Menne & Seitz, 1992). Copepods are able to reproduce in periods of low food availability by channeling all the resources available from lipids stored during the productive season into reproduction and maintenance of vital functions (Menne & Seitz, 1992). *Eudiaptomus graciloides* is also able to use low concentrations of food efficiently (Menne & Seitz, 1992). In contrast to adult copepods, which are adapted to fast for long periods, the copepodid stages of *E.*

graciloides need sufficient food resources to develop as well as to store energy in the form of lipids to be used for overwintering and reproduction (Menne & Seitz, 1992). Therefore, *E. graciloides* from the two lakes seem to have adapted to a seasonally variable environment by careful timing of their vulnerable developmental stages to the productive season. This is one of the predicted adaptations assumed for the success of an organism in theoretical studies (Gotthard, 2001, 2004).

Moreover, the field study (I) revealed the occurrence of a moderate level of multiple mating by female *E. graciloides*, that varies between populations as well as between individuals within a population. On average females from Takvatn showed higher mating rate (mean ± SE: 2.4 ± 0.42) relative to females from Fjellfrøsvatn (mean ± SE: 1.7 ± 0.21). The higher predation density in Fjellfrøsvatn compared to Takvatn (Klemetsen *et al.*, 2003) might cause a reduction in the activity of both females and males. This results in a decreased encounter rate between males and females, which tends to reduce mating frequencies (Rowe *et al.,* 1994). The seasonal trends in multiple mating and the trends in egg-sac carrying frequency showed close correspondence and reached the seasonal peak at the same time in both lakes (i.e., March in Takvatn and April and early June in Fjellfrøsvatn). However, both mating rate and egg-sac carrying frequency showed no relationship to adult sex ratio, which, with a few exceptions, remained unchanged in favour of the females. This suggests that a skewed sex ratio does not explain the multiple mating, and that males have the potential to produce more than one spermatophore; a phenomenon confirmed under laboratory conditions. Moreover, According to Maly (1970), if predators are responsible for skewed adult sex ratios, there should be a correlation between predator density and the adult sex ratio of the two populations living in similar habitats. Therefore, the lack of difference in sex ratio between the two lakes differing in predation density (Klemetsen *et al.*, 2003), suggest that predation is unlikely to be responsible for the skewed sex ratio observed within each population.

4.2 Development time, body size and clutch size

Despite the similar seasonal trends, the populations also showed noteworthy differences in other important life history characteristics (paper I). For example, adult female body size and mean clutch size were always larger in Takvatn than in Fjellfrøsvatn. Moreover, in Takvatn, major egg production and development of most early stages occurred in the cooler months, and development from egg to adult took longer time than in Fjellfrøsvatn. These findings are related to one another. For example, in many organisms clutch size is a direct function of body size. Positive relationships between female size and clutch size have been shown in many cases (e.g. Smyly, 1968; Maly, 1973,1983; Checkley, 1980; paper II & IV). Moreover, development time and body size at maturity are the two most important life history traits that are usually inversely related in most ectotherms (Stearns, 1992; Roff, 2002) including copepod (Mauchline, 1998). Additionally, temperature experienced during development is one of the environmental variables that affect the relationship between development time and final body size. For example, the majority of ectotherms grow slower but mature at a larger body size in colder environments (Atkinson 1994; Berrigan & Charnov 1994; Sibly & Atkinson 1994). Therefore, the observed difference in clutch size between the lakes may be explained by the difference in body size between populations. The difference in body size, in turn, may be explained by a negative relationship between size at maturity and temperature experienced during development.

Yet, other important factors that might cause the observed difference in development time and final body size, and hence clutch size, are food quality and availability, and the effect of size-selective predation. The effect of food does not seem important to bring about the observed pronounced difference in the life history traits. This is because the lakes are nearly similar in morphometric, trophic, physico-chemical characteristics; in annual temperature fluctuation and in ice and snow conditions (Dahl-Hansen *et al.,* 1994; Primicerio, 2000; Klemetsen *et al*., 2003; see above). The effect of selective predation may, on the other hand, deserve attention. It is well known that planktivorous Arctic char is more abundant in Fjellfrøsvatn than in Takvatn, suggesting a greater predation risk for *Eudiaptomus* in the former lake (Dahl-Hansen *et al.,* 1994; Klemetsen *et al*., 2003; Knudsen *et al*., 2007). For example, when predation from Planktivorous fish that prefer large prey is important, selection may favor a shorter growth period resulting in reduced age at maturity and smaller body size. As body size and clutch size are closely related, selective pressure for smaller size may also result in smaller clutch size (Nylin & Gotthard, 1998). Additionally, in animals that carry their eggs until hatching, for example, in copepods and cladocerans, the risk of predation by visually-oriented planktivorous fishes increase with clutch size as increased clutch size increases the conspicuousness of prey (Svensson, 1995, 1997). Consequently, smaller body size may occur as a correlated response to selection against large clutches. Even if no conclusive answer can be reached, the effect of selective predation might best explain the observed difference in life history traits between the two lakes.

Moreover, Arctic char displays seasonality in abundance and activity (Dahl-Hansen *et al.,* 1994; Klemetsen *et al*., 2003). For example, in Takvatn and Fjellfrøsvatn, earlier studies showed that feeding rate of char increased markedly in late spring, peaking in summer, and then decreasing again in autumn and remaining low during most of the winter, (with a minimum in March), followed by an increase in May (Dahl-Hansen *et al.,* 1994; Klemetsen *et al*., 2003). Thus, females in Fjellfrøsvatn, which showed peak egg production in late spring and early summer, should be more exposed to predation than females in Takvatn, which showed high egg production in winter, with a peak in March. Greater exposure to visual predators may also help explain the lower mating frequencies observed in Fjellfrøsvatn relative to Takvatn.

4. 3 Benefits of polyandry

Understanding the adaptive significance of polyandry is the focus of much current research work in sexual selection and several hypotheses have been proposed to explain the phenomenon (see section 1.2.2). Under laboratory conditions, I examined the benefits of polyandry by following and comparing egg production, clutch size, fertility, hatching rate of eggs, hatchling body size and early hatchling survival from females that had been mated once or twice. I found no evidence for direct fitness benefits of polyandry in female *E. graciloides.* Double-mated females did not differ from single mated females in several components of female fitness measures. Yet, body size of females explained much of the variation in clutch size among females regardless of whether females had been mated once or twice.

Among the hypotheses proposed to explain the benefits of polyandry, direct benefits are thought to be the origin of polyandry. Fertilization insurance is one such female benefit promoting multiple mating. This may occur when females fail to store sufficient sperm or when male ejaculates are insufficient to fertilize the whole clutch. Under such conditions, multiple mating might therefore enhance female fitness simply by making ample amount of sperm available to fertilize the eggs she produce (Arnqvist & Nilsson, 2000; Hosken & Stockley, 2003). I found, however, no evidences for fertility benefits promoting polyandry in female *E. graciloides*. That is, the fertility and hatching rate of single mated females did not differ from that of double-mated females, suggesting that single mated females were not sperm limited. Moreover, it has also been argued that, by mating with more than one male, females may enhance their fitness via reception of ejaculatory nutrients as shown in mating systems where males transfer spermatophores that contains both sperm and nutritious accessory gland product (Thornhill & Alcock, 1983; Pitnick *et al.,* 1997; Arnqvist & Nilsson, 2000; Stjernholm & Karlsson, 2000; Hosken & Stockley, 2003). During mating, male *E. graciloides* transfer spermatophores that contain both spermatozoa and associated seminal secretions (Blades &Youngbluth. 1980; Dussart & Defaye. 1995). However, the lack of association between spermatophore numbers and clutch size suggests that the accessory seminal secretions of spermatophores are unlikely to have a nutritional function. Therefore, reproduction in female *E. graciloides* does not seem to be constrained by substances derived from spermatophores increaseing the number of eggs produced. Moreover, the accessory substances of ejaculates have also been suggested to have other numerous complex effects on female reproductive performance. One of these is stimulation of egg production. Additionally, the act of mating itself have been suggested to have positive effects on female fitness as it may directly trigger female egg production (Thornhill & Alcock, 1983; Arnqvist & Nilsson, 2000; Hosken & Stockley, 2003). According to these hypotheses, double-mated females should have higher probabilities of egg production than single-mated females. I found, however, no significant difference between the two groups of females in egg production. Therefore, a single mating before production of each clutch seems sufficient and having only one spermatophore seem not to limit female's probability of producing egg sacs (Watras & Haney, 1980; Watras, 1983; Chow-Fraser & Maly, 1988; Berger and Maier, 2001). Additionally, there were no differences in hatchling body size and early survival of offspring from single and double-mated females. Empirical evidence from other copepods show that offspring size is proportional to the probability of survival to adulthood (e.g., Cooney & Gehrs, 1980; Wyngaard, 1986; Guisande *et al.*, 1996) and my findings suggest no indirect benefits in terms of increased offspring viability for double-mated females. Therefore, although every possible benefit of polyandry to female *E. graciloides* from Takvan is impossible to rule out, the obvious direct and indirect benefits were not evident in this study

4. 4 Morphological correlates of mating status explaining multiple mating

Given that mating is a costly activity (Wilcox, 1984; Bailey *et al.*, 1993; Cordts & Parttridge, 1996; Clutton-Brock & Langley, 1997; Watson *et al.*, 1998; Arnqvist, 1989; Fairbairn, 1993; Magnhagen, 1991; Rowe, 1994; Ward, 198; Daly, 1978; Hurst *et al.*, 1995) from which females receive no apparent benefit (paper IV), why then do females *E. graciloides* mate multiply? I examined the relationship between female morphology and mating frequency in wild caught female (paper II & IV). Additionally, I investigated the relationship between female morphology and male mating success using mate choice experiments (paper III). I found that the body size (paper III) and antennules (paper II, III & IV) of females were inversely associated with female mating status (paper II) and female mating frequency (paper II & IV). Moreover, the length of the female furca (paper and II, III & IV) and the size of genital double somite (paper II & III) were directly correlated with female mating frequency and male mating success. In both studies, the selection of morphological traits for investigations was based on their importance during mating. So, consideration of behavioural components and inter-digitations of morphological traits of the sexes before copulation (pre-contact) and during copulation (post-contact) helped to explain the pattern of nonrandom mating observed (Gauld, 1957; Watras, 1983; Roff, 1972; Blades, 1977; Maier; 1995). The patterns were consistent with the predictions and assumptions of the male manipulation hypotheses (Arnqvist, 1989b; Rowe *et al.*, 1994; Allen & Simmons, 1996; Arnqvist, 1997; Holland & Rice, 1998; Blanckenhorn *et al.*, 2000; Gavrilets *et al.*, 2001). These results will be discussed below after considering the possible costs of mating and costs of male harassment to female copepods.

Most of the general costs of mating mentioned in the introduction are likely to apply in diaptomid. For example, pairs in copulation are, compared to single individuals, more conspicuous to visual predators (Hairston *et al.,* 1983; Winfield & Townsend, 1983) and have reduced vigilance and escape abilities (Maier *et al.*, 2000). During copulation, the male is attached to the female with his fifth legs. In some cases, such copulatory position can be maintained for long periods, especially at low temperatures

(Burghard & Maier, 2000). Thus, a receptive female encountering a number of males will experience increased predation risk from multiple mating. Moreover, mating females most likely have higher metabolic expenditures when transporting attached males and probably also suffers a reduced foraging efficiency compared with single females. Additionally, bodily contact with multiple partners can increase the chance of disease transmission (Hurst *et al.,* 1995). *Eudiaptomus graciloides* often suffers from fungus infections, specially on the egg sac (personal observations). Fungus might be transmitted to the females during mating, and infections might affect female body condition and reproduction. Given these costs of mating, from which females receive no appearent benefit, females are expected to evolve resistance to male harassment so as to reduce the cost of superfluous mating. (Parker, 1979; Arnqvist & Nilsson 2000; Gavrilets *et al.,* 2001). Consistent with these predictions, diaptomid females employ resistance to male harassment in the form of escape reactions (Gauld, 1957; Watras, 1983; Roff, 1972; Blades, 1977; Maier; 1995). However, resistance or escape reactions are also costly behaviours to females in terms of increased energy consumption (Watson *et al.*, 1998), reduced foraging time (Wilcox, 1984) and increased predation risk (Rowe, 1994). For example, antennules are traits that power the escape reaction of females. They help generate 20 times faster speed than normal speed, but using them demands a very high energy output (Mauchline, 1998). Moreover, in copepods, swimming and feeding are inseparable (Dussart & Defaye. 1995). Therefore, male harassment, which may cause a switch from maintenance swimming in females to costly displacement swimming, disrupts female feeding (Dussart & Defaye. 1995; Mauchline, 1998). Thus, both multiple mating and escape from mating, in the face of male harassment, are costly for females. Under such condition, females simply may make the "best of a bad job" by going for the less costly option, variously termed “convenience polyandry” (Thornhill & Alcock 1983; Arnqvist, 1989, 1992; Rowe, 1992; Rowe *et al.,* 1994; Watson *et al.*, 1998; Weigensberg & Fairbairn, 1996), “female reluctance” (Rowe *et al.,* 1994; Blankenhorn *et al.,* 2000) or “male manipulation” (Holland & Rice, 1998; Watson *et al.*, 1998) models.

Models of male manipulation predict that the perceived harassment rate may vary among females due to their variability in behavioural and morphological traits. Therefore, under any given average harassment rate, such variation will generate variation among females in mating propensity and mating rate (Holland & Rice, 1998: Watson *et al.*, 1998; Gavrilets *et al.*, 2001). Therefore, as body size and antennule length are directly related to the strength of mobility and escape ability (Dusenbery & Snell, 1995; Hedge & Krishna, 1997; Mauchline, 1998; Maly & Maly, 1999; Biedermann, 2002), large females and females with longer antennules might reject males more easily or with least cost during the premating struggle. Consequently, large females with long antennules mate less frequently than do small females or females with short antennules.

The outcome of premating conflicts is also determined by morphological traits in males and females that function as arms during mating encounter (e.g., Arnqvist 1989; Thornhill & Sauer, 1991; Arnqvist & Rowe, 1995; Sakaluk *et al.*, 1995; Rowe & Arnqvist, 2002). Based on the behavioural sequence and the mechanics of the premating struggle, the two regions of importance for male precopulatory clasping behaviours are female furca and genital double somite. During mating, the male attempts to secure first a furcal grip with his right antennule's and then a genital double somite grasp, with his right fifth leg. He then attaches his spermatophore (Berger & Maier, 2001). These two points of attachment are critical to subdue reluctant (escaping or jumping) females. Thus, a relatively short furca and a short genital double somite might provide mechanical advantage to resisting females. Having a short furca and a short genital double somite make it more difficult for males to grasp them firmly, and/or enable females to be more effective in the degree of downward curling/tilting, thereby making it more difficult for males to secure their mating position and impede access to the genital field where males attach their spermatophores. On the other hand, a female with relatively long furca might have less mechanical advantage to dissociate the male grip than a female with a shorter furca, and an adaptive female behavior in this case could be to accept multiple mating. This could explain the observed association between female traits and spermatophore numbers.

5 CONCLUSIONS

I began this study with the questions: why do some females of *E. graciloides* have many externally attached spermatophores while others have few or one? How much is the extent of polyandry? I have mainly focused on the variation among females in: (*i*) multiple mating and its fitness benefits, and (*ii*) behavioural and morphological traits that influence the opportunity for multiple mating. I have approached this with observational studies in wild caught females relating morphology and mating frequency, measured as number of spermatophores per mated female, combined with experimental studies relating, respectively, female mating rate (status) to female fitness and morphology. As background knowledge, I also investigated the life cycle, the natural female mating rate and some other life history traits of populations from my main sampling lake, Takvatn and from another nearby lake, Fjellfrøsvatn, which were included to provide replicate information for comparison.

The study has revealed the occurrence of moderate level of polyandry in *E. graciloides* that varies between populations as well as between individuals within population. I documented the similarities as well as the difference between the two sub-arctic populations in timing of certain critical life cycle events and in some life history traits. I showed that both populations were univoltine, starting egg production in the winter under the ice cover before any phytoplankton growth has started, but synchronizing the appearance of their later copepodid stages with the availability of adequate supply of food. The first adults appeared in early autumn, forming the over-wintering population. However, I also showed their remarkable differences in: (i) temperature experienced during development; (ii) the length of development time,; (iii) adult body size; and (iv) clutch size. These interrelated findings have been explained in relation to environmental factors such as temperature, food availability and intensity of predation, which fluctuate seasonally in a predictable pattern.

The hypotheses proposed to explain the evolution and maintenance of polyandry are several. I found, however, no evidences for fertility benefit to be the primary mechanism promoting polyandry in female *E. graciloides*. The fertility and hatching rate of single mated females did not differ from that of polyandrous females and single mated females seemed not sperm limited. That is, I found no difference between the two groups of females in egg production, suggesting a single mating before each clutch production is sufficient and does not limit female's probability of producing eggs. Additionally, as the clutch size were not different between monandrous and polyandrous females, reproduction in female *E. graciloides* does not seem to be constrained by substances derived from spermatophores increasing the number of eggs produced. Yet, body size of females explained much of the variation in clutch size among females regardless of the level of female mating rate. Moreover, there were no difference in hatchling body size and early survival of offspring from single mated and double-mated females, suggesting no indirect benefits in terms of increased offspring viability. Although every possible benefit of polyandry to female *E. graciloides* from Takvan is impossible to rule out, the obvious and immediate benefits were not evident in this study.

On the other hand, I found important relationship between the female mating rate and the size of traits that are important before copulation (pre-contact) and during copulation (post-contact). These include, body size and antennule length of females, which were inversely associated, and furca length, which were directly related to female mating frequency. Diaptomid females are known to employ resistance to actively pursuing and harassing males in the form of escape reaction and their mobility and escape abilities are positively related with body size and antennules length. Thus, large females and females with longer antennules should be better able to reject male suitors with least cost during mating encounters. Consequently, these females mate less frequently than do small females or females with short antennules. Additionally, as the furca is the region of importance for male pre-copulatory clasping behaviours, a female with relatively longer furca might have less mechanical advantage to dissociate the male grip than a female with a shorter furca, and an adaptive female behavior in this case could be to accept multiple mating. In sum, the results of both observational and experimental studies are more consistent with predictions and assumptions of the female reluctance hypothesis, which holds that the efficacy of females resistance, and hence their mating activity, is related to the morphological characters that effect such behaviour. Therefore, the most likely explanation for the occurrence of polyandry in *E. graciloides* is that females are manipulated by males into mating at a rate beyond their optimum. This results from selection on males to copulate as often and with as many females as possible. However, genetic benefits of multiple mating to females cannot be ruled out, as I did not directly attempt to quantify indirect payoffs in terms of survival rate, growth rate and reproductive success of offspring. Future studies in this direction will improve our evolutionary understanding of *E. graciloides* mating system.

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Paper I

Distinct developmental phenology and reproductive strategies in *Eudiaptomus graciloides* populations of two neighbouring subarctic lakes.

Abstract

Populations of the diaptomid copepod *Eudiaptomus graciloides* (Lilljeborg) were studied in two subarctic Norwegian lakes (Takvatn and Fjellfrøsvatn) throughout one year. Seasonal changes in proportion of different developmental stages, incidence of ovigerous and mated females, body size and clutch size were considered together with thermal conditions. Some aspect of the life cycle followed similar seasonal trends in the two lakes. Both populations were univoltine, starting egg production in the winter under the ice cover before the phytoplankton growth season, but synchronizing the appearance of their later copepodid stages with the availability of adequate supply of food. The first adults appeared in early autumn, forming the over-wintering population. However, the populations exhibited remarkable differences in female mating rate, clutch size, body size, seasonal timing of peak egg production, maxima reached by the different developmental stages and hence, timing and duration of major growth periods. On average females from Takvatn showed higher mating rate relative to females from Fjellfrøsvatn. Adult female body size and mean clutch size were always larger in Takvatn than in Fjellfrøsvatn. Moreover, in Takvatn, major egg production and development of most early stages occurred in the cooler months, and development from egg to adult took longer time than in Fjellfrøsvatn. Phenology and duration of the life cycle are considered to explain the marked between-lake differences in body size and clutch size in relation to environmental variables such as temperature, food availability and intensity of predation, which fluctuate seasonally in a predictable pattern.

INTRODUCTION

The calanoid *Eudiapomus graciloides* is one of the most common copepod species in continental European lakes (Kiefer, 1978; Einsle, 1993), where it is often the dominant herbivore. *E. graciloides* shows considerable variation with regard to reproductive strategies and life cycle across its geographical range. This variation is likely to reflect variation in temperature, food availability and predation pressure. For example, both univoltine populations (Alxelson, 1961; Ekman, 1964; Menne & Seitz, 1992) and populations with more than one generation per year are found (Nauwerck, 1963; Bosselmann, 1975; Hofmann, 1979). Moreover, some populations reproduce in winter without feeding, presumably using the energy stored during the previous phytoplankton bloom **(**Ekman, 1964; Menne & Seitz, 1992**).** Other populations reproduce in winter, under poor food conditions, as well as during a short period in the summer months, when food is available (Bosselmann, 1975). Still others reproduce throughout the year (Nauwerk, 1963). *E. graciloides* is known to undergo diapause as eggs, old copepodid stages and adult stage, to overcome unfavorable time periods (Pasternak & Arashkevich, 1999; Santer *et al*, 2000; Zeller *et al.*, 2004), but the timing and importance of diapause also vary considerably among lakes (Pasternak & Arashkevich, 1999; Santer *et al.*, 2000).

Populations of *E. graciloides* exhibit considerable inter-population variation in clutch size. In diaptomid copepods, clutch size is generally positively related with female body length (e.g. Smyly, 1968; Maly, 1973,1983; Checkley, 1980). In turn, size at maturity is a result of intermoult duration and growth increment at moulting, which are inversely related to temperature (Runge & Myers, 1986; Mauchline, 1998 and reference therein), and may be modified by the quantity and quality of available food (Mauchline, 1998). For a given female body size, food availability and water temperature can further influence clutch size. In many diaptomid species, the mean clutch size is a negative function of temperature (Elmore, 1983; Chow-Fraser & Maly, 1991) and a positive function of food concentration (Williamson & Butler, 1987; Chow-Fraser & Maly, 1991). In environments with low food availability, such as high latitude lakes, copepods tend to produce few large, energy-rich eggs (Dunbar, 1968; Clarke, 1979; Mauchline, 1998). Additionally, experimental and field studies suggest that females are adapted to produce a few large eggs when food is scarce and many small eggs when food is abundant (Hutchinson, 1951; Guisande, 1996), exhibiting strong plasticity in life history traits. Producing large, energy-rich eggs, is an adaptive response to a resource-limited environment, as it enables the early juvenile stages to rely on internal energy reserves for development, making them relatively independent of the adverse feeding conditions (Auel, 2004).

Size selective predation may also represent an important source of selection on clutch size through its effect on size at maturity. In the presence of planktivorous fish that selectively feed on large copepods, small body size at maturity should be favored (Gliwicz & Pijanowska, 1989; Svensson, 1995, 1997). Visual predators may further influence clutch size by selectively feeding on those females that, due to their larger clutches, are more conspicuous (Gliwicz & Pijanowska, 1989; Svensson, 1995, 1997). Predators may further affect size at maturity and clutch size by influencing the phenology of reproduction. Timing the vulnerable, reproductive stage to periods of low predatory activity, typically winter, helps to reduce predation risk (Hairston, 1988), but will also affect growth rates and clutch size.

The present work is a comparative study of the life cycle and reproduction of *E. graciloides* living in neighbouring lakes (Takvatn and Fjellfrøsvatn, Northern Norway). The two lakes have similar morphometric and physico-chemical characteristics, but differ in terms of abundance of planktivorous fish. The seasonal changes in abundance of various developmental stages, mating frequency, egg production, and clutch size was monitored in the two lakes together with temperature changes. This information would help define the breeding season, the developmental period and the annual number of generations. Seasonality of phytoplankton production and predator abundance and activity in the lakes is known from previous studies (Dahl-Hansen et al., 1994; Primicerio, 2000; Klemetsen *et al.*, 2003). This predictable, seasonal fluctuation in relevant environmental variables is used to interpret the timing of the breeding season and the development period in the two populations. Differences in female body size, mating frequency and clutch size between lakes are related to discrepancies in developmental and breeding phenology, and to environmental differences between lakes.

MATERIAL AND METHODS

Study sites

The sub-arctic lakes Takvatn and Fjellfrøsvatn are situated in northern Norway (69°07′N, 19°05E′. At these high latitudes, there is a strong seasonality with respect to temperature and photoperiod, pelagic primary production and predator abundance and activity (Dahl-Hansen et al., 1994; Primicerio, 2000; Klemetsen *et al.*, 2003). The area has two months of midnight sun, from end of May to end of July. The sun stays below the horizon from end of November to end of January. The maximum change in photoperiod is more than half an hour per day in the weeks before and after these events. The ice-free season usually lasts from end of May, early June, to middle of November, early December. Both lakes have similar ice and snow conditions (ice thickness = 20 – 100 cm & snow depth 5 – 100 cm) (Klemetsen *et al.*, 2003).

Lake Takvatn is an oligotrophic lake with a surface area of 15 Km^2 and a maximum depth of 80 m. The lake is surrounded by a birch wood landscape and located at 214 m a.s.l. The water of the lake is cold (Fig. 1) reaching a maximum epilimnetic temperature of about 14 °C during summer and a minimum temperature of 0.1 – 0.3 °C (down to 40 m) during winter (Dahl-Hansen, 1995). A detailed description of the lake and its biota is given in Klemetsen *et al.* (1989). The fish community in the lake is dominated by Arctic char, which was introduced into Takvatn from Fjellfrøsvatn (Svenning & Grotnes, 1991). Lake Fjellfrøsvatn has morphometric (surf. area, 6.5 Km^2; max depth, 80 m), trophic and physico-chemical characteristics, including yearly temperature fluctuations (Fig. 1), that are similar to Takvatn. Planktivorous char is more abundant in Fjellfrøsvatn than in Takvatn, implying a greater predation risk for *Eudiaptomus* in this lake (Klemetsen *et al.*, 2003; Knudsen *et al.*, 2007; Persson *et al.* 2007). Food consumption of Arctic char in Fjellfrøsvatn was about twice as high as in Takvatn during winter (Klemetsen *et al.* 2003). Arctic charr are known to feed under the ice (Klemetsen & Grotnes, 1975), at temperatures close to 0 °C (Brännäs & Wiklund, 1992), having a retinal flexibility (Ali *et al.,* 1984) that allows visual feeding at the low light intensities experienced in winter. Arctic char displays a strong seasonality in abundance and feeding activity, both increasing markedly in late spring, peaking in the summer, and decreasing again in autumn and winter (Dahl-Hansen *et al.,* 1994; Klemetsen *et al.*, 2003).

Sampling and analysis

In both lakes, water temperature measurements and triplicate samples of copepods were taken monthly from January 2003 to January 2004, except in June, July and October when samples were taken twice a month. Additionally, triplicate samples were collected once a month from February to April in 2007, during the reproductive season. The samples were collected from stations situated in the deep part of the lakes. The sampling activities and the choices of sampling site location were made based on the experience of previous studies (Primicerio & Klemetsen, 1999; Primicerio, 2000). For sampling, plankton net (mesh size = 50 μm, mouth diameter =30 cm) was hauled vertically from a depth of 30 m at a constant speed (ca 0.5 m s^{-1}). The samples were fixed, after narcotizing with 5% ethanol, in 4% formalin solution.

Adults and copepodid stages were taxonomically identified, based on morphological characteristics (Dussart & Defaye, 1995), and counted, and the stage and sex were registered, in triplicate whole or sub-samples under a dissection microscope. Females carrying spermatophores or eggs were also registered, and the number of spermatophores or eggs carried was counted. As diaptomid females normally carry their eggs until the nauplii hatch, detached egg sacs were assumed to have been knocked off from egg-carrying females after sampling. Accordingly, the number of detached egg sacs was recorded and added to the total number of egg carrying females. Adult female body size (prosome length) from selected sampling dates (with ovigerous females) was measured with an ocular micrometer under a dissecting microscope. Differences in mean female body size between lakes, years and months were analyzed using three ways ANOVA.

RESULTS

Developmental phenology

The first appearance of copepodid stages and adult males and females followed similar seasonal trends in the lakes, the main distinction being in the timing of seasonal maxima (Fig. 2). At the beginning of the sampling season, in January, under ice cover, the populations were exclusively represented by adults in both lakes. Adults were present throughout the entire sampling period. In Takvatn, the proportion of the adult population started to decline in April with the first appearance of early copepodid stages, and reached a seasonal minimum by July. The proportion of adults increased again in autumn, and remained almost constant thereafter, suggesting the appearance of a new over-wintering adult population (Fig. 2A). In Fjellfrøsvatn, the adult population started to decline in June, after the ice-break, and reached a seasonal minimum in August (Fig. 2B).

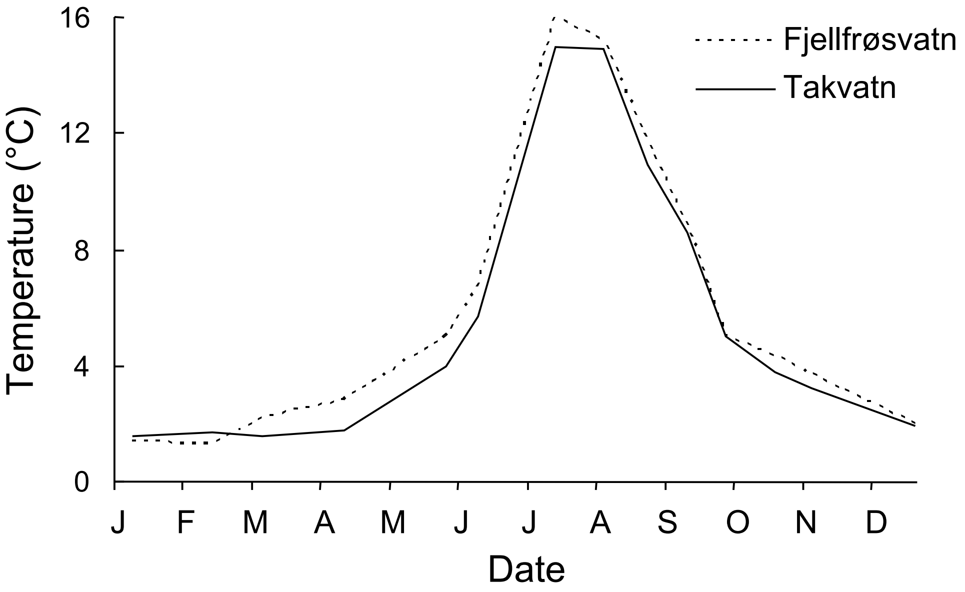

Figure 1. Seasonal changes in water temperature near the surface of Lake Fjellfrøsvatn and Lake Takvatn.

In Takvatn, copepodid stages (CI–CV) were registered from April until the last sampling date, in January 2004, but were noticeably less abundant from September and onwards. In Fjellfrøsvatn, copepodids were found from June to October. The lakes exhibited remarkable differences in the timing of maximum abundance of the copepodid stages. In Takvatn, the early copepodid stages (CI–CIII) showed a maximum in late June, whereas in Fjellfrøsvatn, the maximum took place in late July. Thereafter, their proportion declined rapidly, with a nearby disappearance by September in Fjellfrøsvatn and by October in Takvatn (Fig. 2). Moreover, in Takvatn, late copepodid stages (CIV–CV) peaked in July, but in Fjellfrøsvatn they reached their maximum proportion only in August.

The lakes exhibited also remarkable differences in the timing of peak egg production (Fig 2) and in the duration of development time, from peak egg production to adult

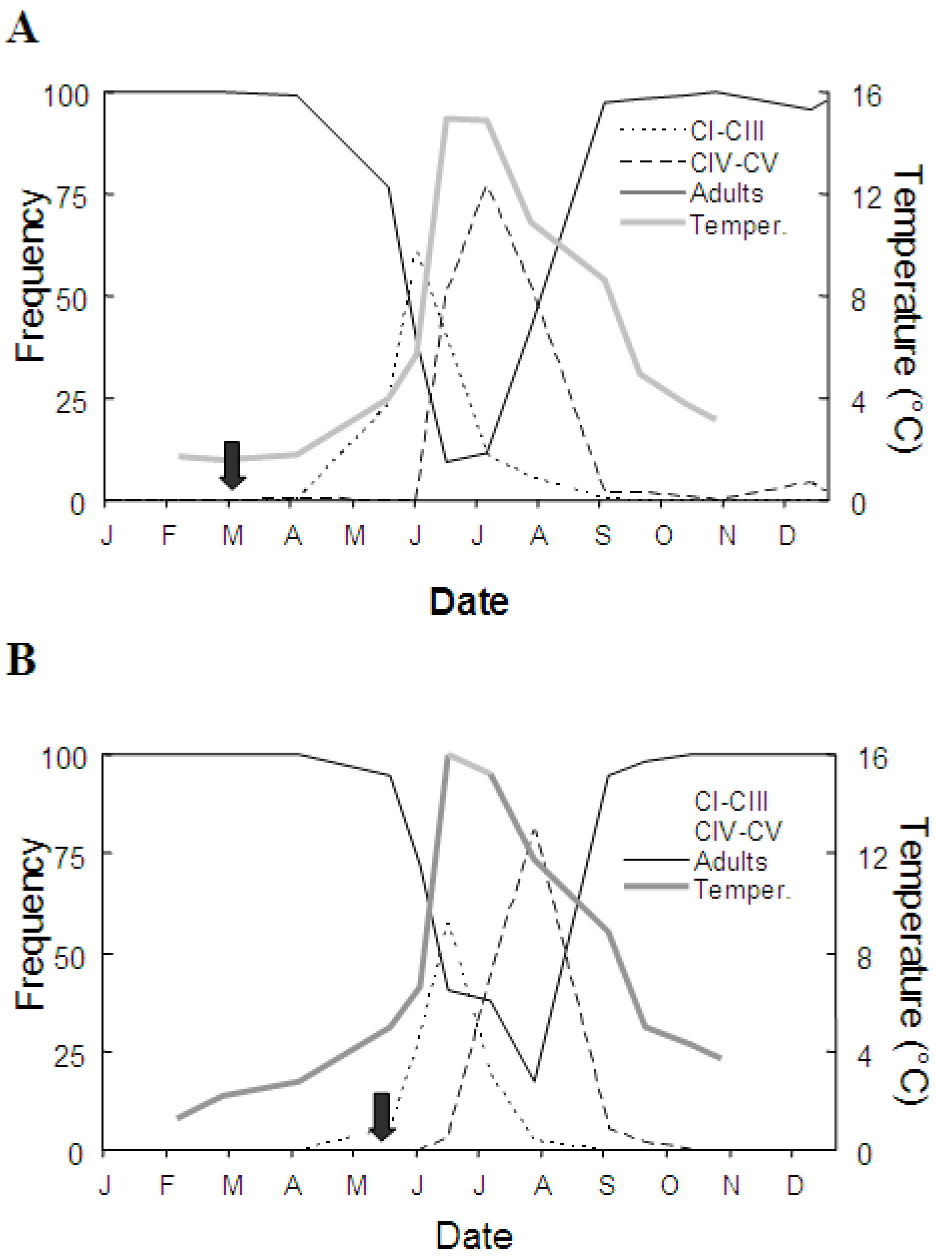

Figure 2. Seasonal changes in water temperature near the surface and proportion (%) of copepodids (CI-III & CIV-V) and adults (females and males pooled) of *E. graciloides* in (A) Lake Takvatn and (B) Lake Fjellfrøsvatn. Arrows indicate time of peak egg production.

stage. In Takvatn, peak egg production occurred in March (Fig. 2A) at a water temperature of 1.6º C, while in Fjellfrøsvatn it took place in early June (Fig. 2B) at a water temperature of 5º C. In Takvatn, the first copepodids became adults in August, nearly five months after the time of peak egg production (Fig. 2A). In Fjellfrøsvatn the first copepodids became adults in September, only three months after peak egg production (Fig. 2B). Thus, unlike in Fjellfrøsvatn, in Takvatn the embryonic, naupliar and most of the early copepodid stages occurred mainly in the cooler months. Additionally, individuals in Takvatn took relatively longer time to develop from egg to adult, than individuals in Fjellfrøsvatn.

Sex ratio

The proportion of adult males had two peak periods in both lakes (Fig 3). The first major peak occurred in early June in Takvatn (60 %), and in April in Fjellfrøsvatn (54.91%), whereas the second peak occurred in early July in Takvatn (54 %), and in November in Fjellfrøsvatn (50%). Apart from these two periods, the adult sex ratio was slightly biased in favor of females for the rest of the sampling period and showed minor seasonal changes in the lakes (Fig 3). Taken over the whole year, the adult sex ratio was slightly skewed toward the females (percent males, expressed as mean ± SD, was 44.40 ± 6.86 in Takvatn and 45.31 ± 3.87 in Fjellfrøsvatn). There was no difference in sex ratio between the two lakes (*t*=0.621, *P*=0.5397).

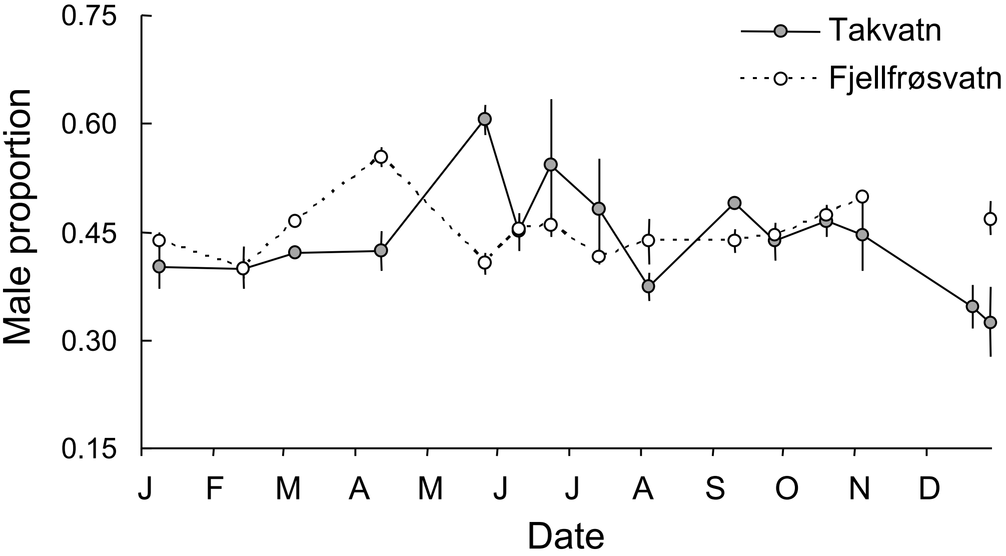

Figure 3. Seasonal variation in sex ratio of adult *E. graciloides* in lakes Takvatn and Fjellfrøsvatn. Vertical bars depict standard errors.

Seasonality of mating

In Takvatn, females with attached spermatophores were found throughout the year (Fig. 4). In January, at the start of sampling activity, females with attached spermatophores were low. They remained low (below 10 %) during late winter and spring. Afterward, mating activity increased to reach a maximum in early July, at a time when there were proportionally a very low frequency of adults (Fig 2A) with a male biased sex ratio (54 %, Fig. 3). Breeding then declined to the lowest level between August and early November. In December, however, mating activity started to increase sharply and continued to do so in January, suggesting the start of new breeding season for the new adult generation. In Fjellfrøsvatn, females carrying spermatophores were not found in January (Fig 4). In February, however, there were high proportions of females with spermatophores, suggesting the start of breeding season. Mating activity remained high in March, and then, declined and remained low until early June. Thereafter, breeding increased to a second peak in late June and early July, followed by a decrease to zero in August (Fig. 4). The second peak in mating activity occurred when there were proportionally very few adults.

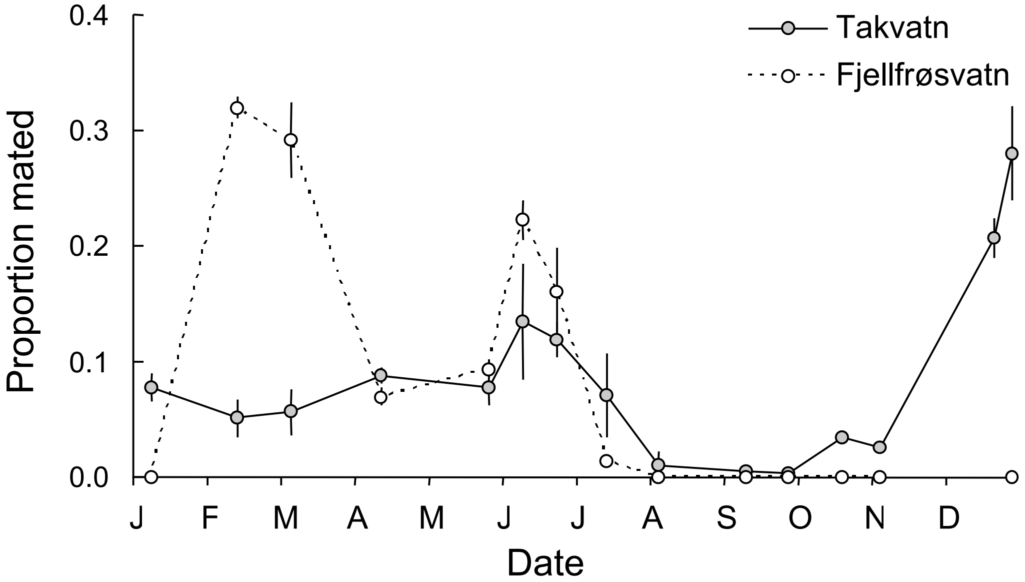

Figure 4. Seasonal variation in the proportion of females of *E. graciloides* with spermatophore in Lake Takvatn and Lake Fjellfrøsvatn. Vertical bars depict standard errors.

Mating frequency

In Takvatn, the mean number of attached spermatophores increased steadily, until the seasonal peak was reached in March (mean ± SE: 2.4 ± 0.42 - Fig. 5). Thereafter, spermatophore numbers declined gradually and reached the lowest level between late July and early October. After October, it increased slowly for the rest of the sampling season. In Fjellfrøsvatn, peaks in spermatophore numbers occurred in April (mean ± SE: 1.7 ± 0.21) and early June (Fig. 5).

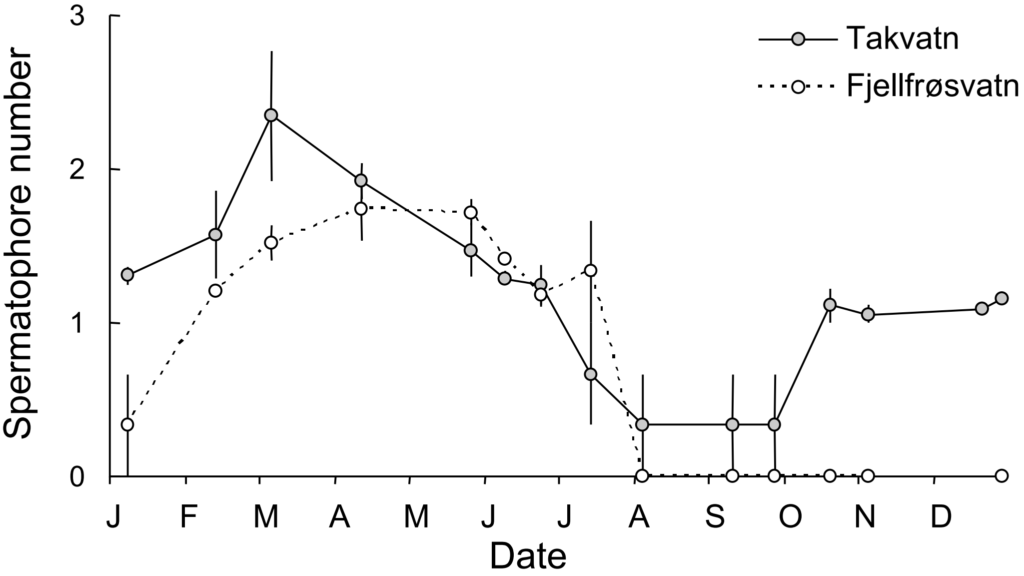

Figure 5. Seasonal variation in the mean number of spermatophores per mated females of *E. graciloides* in lakes Takvatn and Fjellfrøsvatn. Vertical bars depict standard errors.

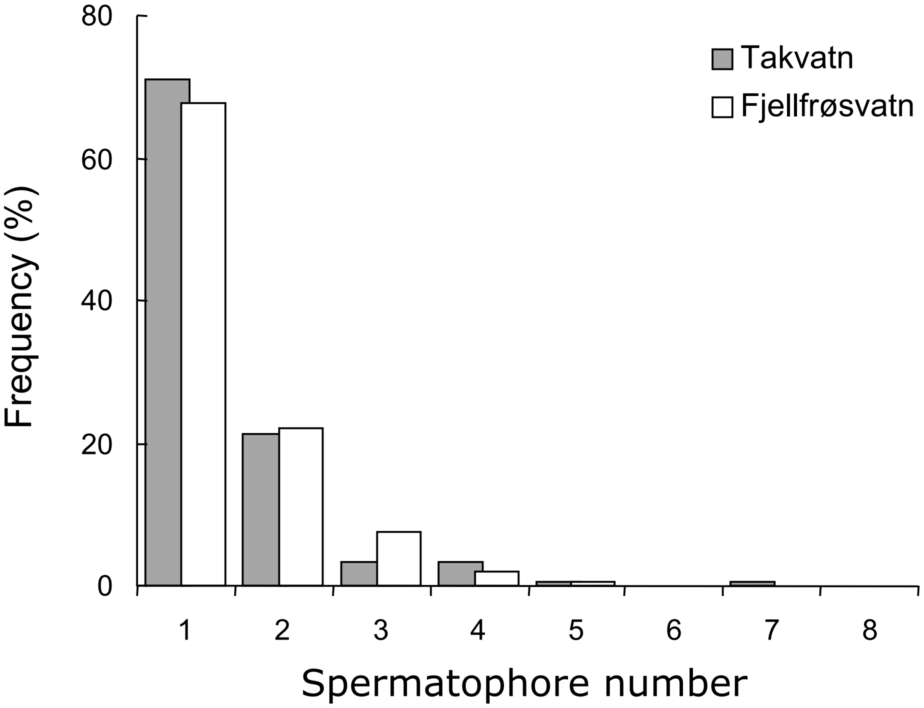

Figure 6. Frequency distribution of number of spermatophores attached on adult females *E. graciloides* in lakes Takvatn and Fjellfrøsvatn.

The frequency distributions of spermatophore numbers per female, based on the entire sampled material for the two lakes, were skewed, females with more than three spermatophores being rare (Fig. 6), and varied seasonally (not shown here). Adult females were found carrying up to 7 (in Takvatn) and 8 (in Fjellfrøsvatn) spermatophores. Females with three or more spermatophores were mainly found from February to early June in Takvatn and from March to late June in Fjellfrøsvatn.

Seasonality of reproduction

Females in both lakes started egg-laying under the ice, but exhibited clear differences in the duration of the breeding period and in the timing of maximum reproductive activities (Fig. 7). In Takvatn, breeding went on for a large part of the year, but egg laying was noticeably less frequent between July and early October, ceasing altogether in late October and November. Thereafter, reproduction resumed and began to increase during December and January. Frequency of ovigerous females was high in January (86.2%), and peaked in March (89%), at a water temperature of 1.6 ºC. In Fjellfrøsvatn, ovigerous females were found from February, albeit with low frequency (5.1 %), to late July (Fig. 7). Thereafter, reproduction increased steadily and reached maximum frequency in early June (83.1%), at a water temperature of 5 ºC, followed by a sudden decline in number of females carrying eggs, with a total disappearance by August.

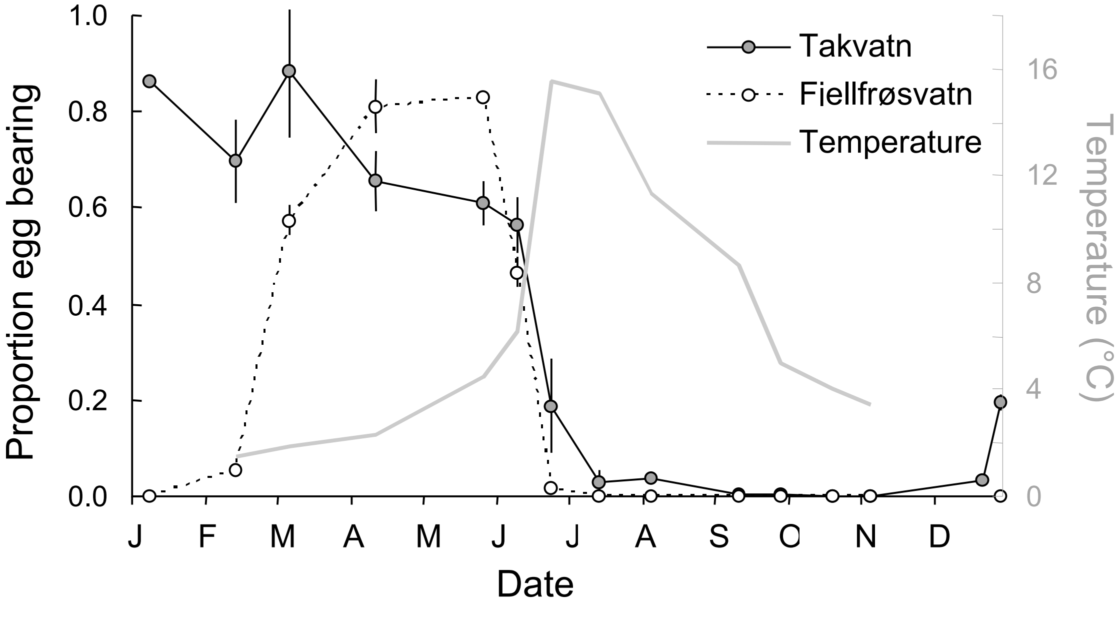

Figure 7. Seasonal variation in egg-carrying frequency of *E. graciloides* in Lake Takvatn and Lake Fjellfrøsvatn. Vertical bars depict standard errors. Mean water temperature near the surface from the two lakes is shown for reference.

Clutch size

Mean clutch size was always greater in Takvatn than Fjellfrøsvatn (Fig. 8). The maximum registered clutch size was 10 eggs in Takvatn and 6 eggs in Fjellfrøsvatn (Fig. 9). At the beginning of the sampling period, the two populations followed a

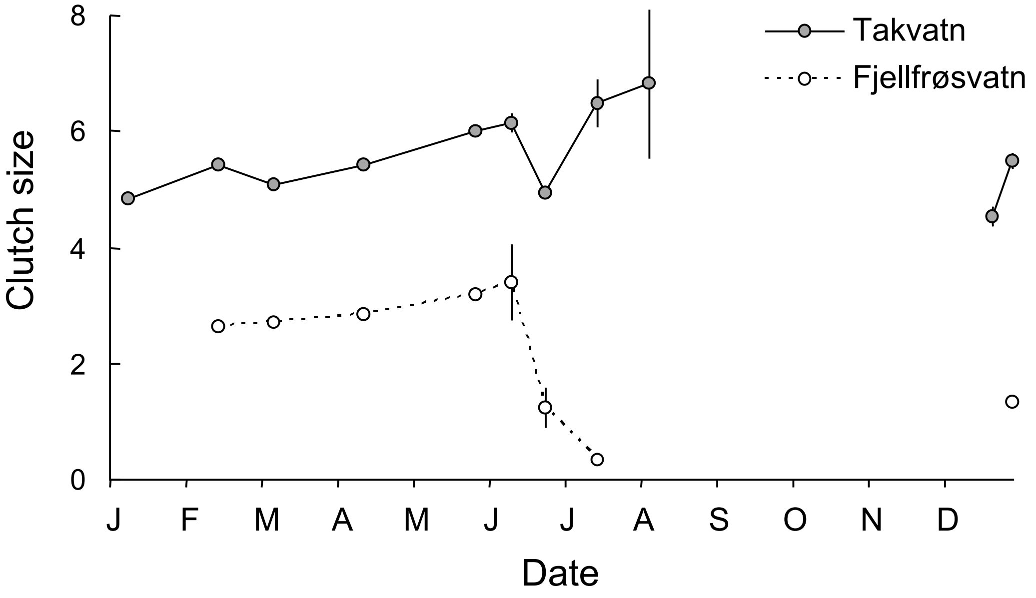

Figure 8. Seasonal variation in number of eggs per egg sac of *E. graciloides* in Lake Takvatn and Lake Fjellfrøsvatn. Vertical bars depict standard errors.

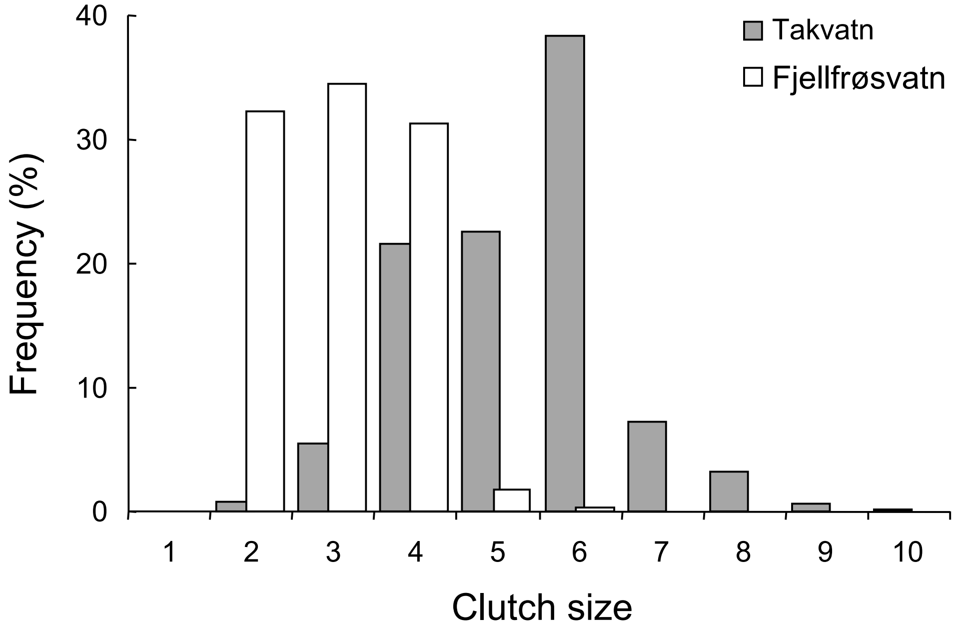

Figure 9. Percentage frequency distribution of adult females *E. graciloides* carrying different number of eggs per egg sac in Lake Takvatn and Lake Fjellfrøsvatn.

similar tendency, with a steady and slight rise in mean clutch size, which peaked in June (Takvatn, mean eggs per egg-sac ± SE: 6.1 ± 0.18; Fjellfrøsvatn, mean eggs per egg-sac ± SE: 3.4 ± 03) thereafter, seasonal tendences differed. From July to mid August, the mean clutch size in Takvatn fluctuated between 4.9 and 6.9 eggs. In Fjellfrøsvatn, on the other hand, it suddenly decreased to 1.86 eggs in July.

Body size

Adult females of *E. graciloides* showed a distinct size difference between lakes, with females from Takvatn being larger than in Fjellfrøsvatn (*P*<0.0001, Fig. 10). Body size did not vary throughout the winter (February to April, *P*=0.704), but did vary between years, with relatively smaller females in 2007 (*P*<0.0001).

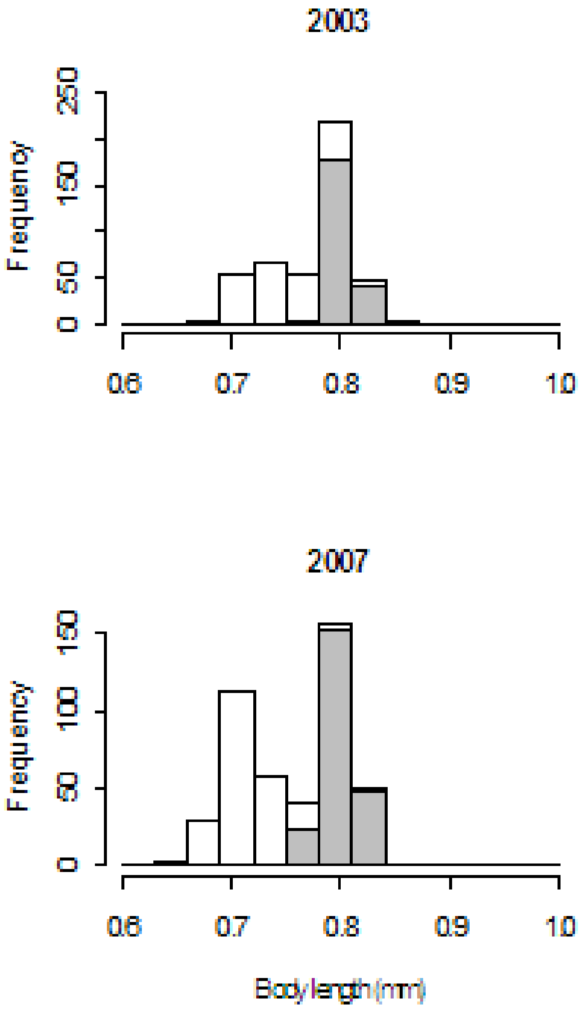

Figure 10. Female body size distribution in the lakes Takvatn and Fjellfrøsvatn in 2003 and 2007 (gray bars, Lake Takvatn).

DISCUSSION

The life cycles of the two populations of *E. graciloides* displayed similar characteristics. The copepods overwintered as adults, reached peak reproduction in late winter, and continued breeding until late July/August, when the last adults disappeared. The copepodids reached maximum abundance in July/August, and started maturation in August/September, forming the new over-wintering population of adults. The data on seasonal development and breeding confirm the univoltine nature of these populations, as expected on the basis of the low temperatures and productivities experienced in the lakes (Alxelson, 1961; Ekman, 1964; Menne & Seitz, 1992).

Analyses of the frequency distribution of spermatophores on adult females have revealed the occurrence of moderate levels of multiple mating by female *E. graciloides*, with substantial seasonally variation. On average females from Takvatn showed higher mating rates relative to females from Fjellfrøsvatn. The higher predation risk experienced in Fjellfrøsvatn (Klemetsen *et al.*, 2003) might cause a reduction in the activity of females. Such a reduced activity would result in decreased encounter rates with males, thereby reducing mating frequencies (Rowe *et al.,* 1994). The seasonal trends in multiple mating, and the trends in egg-sac carrying frequency showed close correspondence and reached the seasonal peak at approximately the same time in both lakes (i.e., March in Takvatn and April and early June in Fjellfrøsvatn). However, both mating rate and egg-sac carrying frequency showed no relationship with adult sex ratio. Rather, adult sex ratio remained relatively stable in favour of females. This suggests that sex ratio does not reflect the observed changes in multiple mating, and that males have the potential to produce more than one spermatophore; a phenomenon confirmed under laboratory conditions (personal observation). Moreover, according to Maly (1970), if predators are responsible for skewed adult sex ratios, there should be a correlation between predator density and the adult sex ratio of two populations living in a similar habitat. Therefore, the lack of difference in sex ratio between the two lakes, even if they differ in predation risk (Klemetsen *et al.*, 2003), suggests that predation is unlikely to be responsible for the skewed sex ratio observed within each population.

Despite the difference in the timing of peak egg production, females in both lakes started egg-laying under the ice cover, before any phytoplankton growth has started (Primicerio 2000). These findings are in line with those of other studies reporting egg production by *E. graciloides* in late winter, when food availability is low (Ekman, 1964; Menne & Seitz, 1992). Copepods are able to reproduce in periods of low food availability by channeling all the energy available from lipids stored during the productive season into reproduction and maintenance of vital functions (Menne & Seitz, 1992). *Eudiaptomus graciloides* is also able to use low concentrations of food efficiently (Menne & Seitz, 1992). Moreover, *E. graciloides* synchronized the appearance of its later copepodid stages with the availability of food. In contrast to adult copepods, which are adapted to fast for a long time, the copepodid stages of *E. graciloides* need sufficient food resources to develop as well as to store energy in the form of lipids to be used for overwintering and reproduction (Menne & Seitz, 1992). *Eudiaptomus graciloides* in the examined lakes seems to have adapted to a seasonally variable environment by careful timing of its vulnerable developmental stages to the productive season.

Populations in the two lakes exhibited remarkable differences in temperature experienced during development, in the length of development time, in adult body size and clutch size. In Takvatn, most embryonic as well as early postembryonic development occurred in a relatively cooler period than in Fjellfrøsvatn. Additionally, individuals in Takvatn took relatively longer time to develop from egg to adult stage than individuals in Fjellfrøsvatn. Moreover, in Takvatn, adult females were larger and produced larger clutch sizes than females in Fjellfrøsvatn. These findings can be interrelated considering that the observed differences in clutch size may be explained by the difference in body size between lakes (e.g. Smyly, 1968; Maly, 1973,1983; Checkley, 1980; Paper II & IV), which in turn can be due to the negative relationship between size at maturity and temperature experienced during development. Indeed, the majority of ectotherms grow slower but mature at a larger body size in colder environments (Atkinson 1994; Berrigan & Charnov 1994; Sibly & Atkinson 1994).

Another factor that deserves attention is the effect of selective predation on size at maturity and clutch size. When predation from planktivorous fish that prefer large prey is important, selection may favor a shorter growth period resulting in reduced age at maturity and smaller body size. As body size and clutch size are closely related, selective pressure for smaller size may also result in smaller clutch size (Nylin & Gotthard, 1998). Additionally, in animals that carry their eggs until hatching, like copepods and cladocerans, the risk of predation by visually hunting planktivorous fishes increases with clutch size due to increased prey conspicuousness (Svensson, 1995, 1997). Consequently, smaller body size may result as a correlated response to selection against large clutches. Currently, planktivorous char is more abundant in Fjellfrøsvatn than in Takvatn, implying a greater predation risk for *Eudiaptomus* in the former lake (Klemetsen *et al.*, 2003). In both Takvatn and Fjellfrøsvatn, the feeding rate of char increases markedly in late spring, peaking in the summer. Feeding rate decreases again in autumn and remains low during most of the winter, shows a minimum in March and is followed by an increase in May (Klemetsen *et al.*, 2003). Thus, *Eudiaptomus* females in Fjellfrøsvatn, which showed peak egg production in late spring and early summer, should be more exposed to predation than females in Takvatn, which showed high egg production in winter, with a peak in March. Greater exposure to visual predators may also help explain the lower mating frequencies, which reduce conspicuousness and vulnerability, observed in Fjellfrøsvatn relative to Takvatn.

To conclude, the seasonality of *E. graciloides* life cycle and reproduction followed similar trends in the two lakes, the main distinction being the timing of peak egg production and of maxima reached by the different developmental stages and hence, of major growth periods. Such differences provide valuable insight for understanding the observed variation in body size and clutch size between populations in relation to environmental variables such as temperature and intensity of predation, which fluctuate seasonally in a predictable pattern. The study also revealed different levels of multiple mating by female *E. graciloides*, suggesting the influence of environmental or internal sources of variation in mating frequencies.

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Paper II

Morphological correlates of mating frequency and clutch size in wild caught female *Eudiaptomus graciloides* (Copepoda: Calanoida)

Abstract

A high proportion of female in diaptomid copepod mate multiply and more often than needed for each clutch production. Our understanding of the adaptive significance of this behavior and the underlying process is limited. Theory suggests that polyandry may arise when whatever benefits females accrue from multiple mating outweigh the costs, or males manipulate females against the females' best interests. I tested these predictions by examining the relationship between female body size, clutch size and mating frequency, as indicted by the number of spermatophores in a wild caught copepod *Eudiaptomus graciloides* (Lilljeborg). Large females carried more eggs than small females. Additionally, the frequency of mating (i.e., the number of spermatophores) was inversely related to the size of female and the length of antennules and directly related to the size of furca and genital double somite. Diaptomid females are known to employ resistance to actively pursuing and harassing males in the form of escape reaction and their mobility and escape abilities are positively related with body size and antennules length. Consequently, large females might reject males more easily and hence mate less frequently than do small-sized females. My results do strongly suggest that multiple mating in *E. graciloides* is, at least in part, maintained by sexual conflict over mating rate, and that females are manipulated by males into additional mating against their best interests.

INTODUCTION

Theory suggests that each mating with an additional female increases male fitness, whereas fecundity rather than the number of mating partners limits female's reproductive success (Bateman 1948). Contrary to these predictions, however, females of many animal species mate multiply (e.g. Arnqvist & Nilsson, 2000; Jennions & Petrie, 2000; Hosken & Stockley, 2003). This apparent discrepancy between theory and empirical observations has generated an extensive body of theoretical and empirical research. The two most often quoted hypotheses for the evolutionary origin and maintenance of polyandry are the female benefit hypothesis and the male manipulation hypothesis. The former explanation invoke benefits to the female from multiple mating, either directly or indirectly. Direct benefits, such as nutrient received during mating (Thornhill & Alcock, 1983), cause female fitness to increase with the number of mating (Wiklund et al., 2001). Additionally, female may derive indirect genetic benefits that increase offspring fitness (Jennion & Petrie, 2000). Such indirect benefits might arise from improving on earlier mating (Thornhill & Alcock, 19883; Simmons, 1987; Olssen, *et al.*, 1996), reducing the risk of genetic incompatibility (Zeh & Zeh, 1996, 1997), inbreeding avoidance (Tregenza & Wedell, 1998, 2002), and increased genetic diversity (Baer & Schmid-Hemel, 1999).

In contrast, the male manipulation hypothesis propose that frequent female mating may result as a by-product of selection on males to increase their fitness (Parker, 1979; Holland & Rice, 1998; Arnqvist & Nilsson, 2000; Crudgington & Siva-Jothy, 2000; Byrne & Roberts, 2000; Stutt & Siva-Jothy, 2001). In general, males benefit more from higher mating rates than females (Bateman 1948; Arnqvist & Nilsson 2000). Moreover, elevated mating rates are costly to females (e.g., in terms of increased predation risk, time and energetic expenditure), which in turn renders conflict over mating rates between the sexes to be prevalent in mating systems (Arnqvist & Nilsson 2000). The exact form of the relationship between mating rate and female fitness depends on the interaction between costs and benefits associated with multiple mating (Watson *et al.*, 1998; Arnqvist & Nilsson 2000; Arnqvist *et al.,* 2004) and may, therefore, differ among females due to variability in behavioural, physiological, and morphological traits that may arise due to genetic differences, developmental trajectories or proximate environmental factors (e.g. Chapman &

Partridge 1996; Jennions & Marion, 1997). Hence, it is essential to characterize females mated at a wide range of rates and relate the effect of mating rates to female fitness in natural populations. Such data help us understand the potential causes of female mating rates and some of their implications.

Daiptomid copepods offer an excellent system in which to investigate these topics. In this system, a high proportion of females mate multiply, as indicted by the number of externally attached spermatophores, despite their inability to store and use sperm over more than one oviposition (Watras & Haney, 1980; Watras, 1983; Chow-Fraser & Maly, 1988; Cuoc *et al.*, 1989a, b; Berger & Maier, 2001). Moreover, females' rate of mating varies between species, between populations within species, and between individuals within populations. For example, some females of *E. gracilis* and *E. graciloides* have been observed carrying up to seven and nine spermatophores, respectively, while others have one or just a few (Berger & Maier, 2001;Paper I). However, our understanding of the adaptive significance of this behavior and the underlying process is limited. Yet, several authors attribute such phenomenon as a waste of reproductive effort by males resulting from overcrowding or high male to female sex ratios (e.g. Katona, 1975; Blades, 1977; Hopkins & Machin, 1977; Hopkins, 1982; Swenson, 1997). However, without considering the mechanism behind such mating rate and its ultimate consequences in terms of sexual selection, it might be unsafe to characterize such male behavior and effort as useless and as waste. Moreover, males should not mate indiscriminately as the costs of producing spermatophores may be high, unless selection favored male behavior that promotes the insemination of many females (i.e., when the reproductive benefits of such behavior outweigh the costs).

The assumptions of the male manipulation hypotheses explicitly generate predictions about the nature of association of mating rate with female traits and fitness. As superfluous carry direct costs to females, they are expected to evolve vigorous rejection behavioral responses to bring mating rates back to their optimum. Consequently, females mating rate would be inversely related: (*i*) to any morphological and/or behavioral trait that affect such female response and (*ii*) to their fitness. Alternatively, the female benefits hypothesis assumes that additional direct or indirect benefits accrued by females from multiple mating and that such benefits thus must be strong enough to outweigh the costs of multiple mating. Therefore, females that are mated with multiple males will have, on average, higher fitness than females that are mated with a few or one mate.

In order to determine the potential causes of multiple mating, I tested this predictions by examining the relationship: (*i*) between females morphology and a wide range of mating frequencies, (*ii*) between the same females morphology and different egg number and egg size in wild caught copepod *Eudiaptomus graciloides* (Lilljeborg). These relationships would possibly allow me to indirectly relate any effect of mating rates on such fitness measures through the common morphological characteristics of both groups of females. I estimated the natural female mating rate per reproductive bout by simply counting the number of externally attached spermatophores that females carry and assessed the fitness measures of females by counting the number and measuring the size of fertile eggs that females carry externally.

Copepods reproduce sexually. Males copulate with females, and transfer spermatophores (simple flask-like container) that contain spermatozoa and seminal secretions to the genital double-somite of the females. There are fairly general behavioural patterns of events followed by almost all calanoid copepod during mating (Gauld, 1957; Katona, 1975; Blades & Youngbluth, 1979; Jacob & Youngbluth, 1983; Berger & Maier, 2001). Initially, the male grasps the female's caudal furcae with his geniculate right antennule. Following this, he move into the copulatory position by releasing his antennule and then grasping her genital double-somite with his right fifth leg. Then, using his left fifth leg, he (extrudes and) attaches a spermatophore externally on the female's genital double-somite by means of cement like substance present on the tapered, open end of the flask. Finally, he releases the female. The duration of this process is short (i.e., 2 min. in *E.gracilis*) but varies within the same species and between different species of the same taxonomic group (Berger & Maier, 2001). Additionally, it may involve behavioural as well as morphological or physiological mechanisms, which ensure proper spermatophore placement (Blades & Youngbluth, 1980). Mechanisms, such as sex attractant pheromones released by females, may also help copepods to locate and identify each other prior to mating (Katona, 1973; Griffiths & Frost, 1976).

Several male diaptomid species selectively engage in mating and spermatophore transfer usually with gravid females containing mature oocytes. Females lack seminal receptacles to store sperm (Cuoc *et al.*, 1989a, b) and hence re-mating is necessary to produce fertile eggs again (Watras & Haney, 1980; Watras, 1983; Chow-Fraser & Maly, 1988; Berger & Maier, 2001). Spermatophores discharge their contents over the genital area of females where they form an attached spermatophoral mass (Cuoc *et al.*, 1989a, b). Females usually detach spent spermatophores with their modified fifth legs before extrusion of egg sacs. The eggs are extruded into a sac and carried for some days until the nauplii hatch (Chow-Fraser & Maly, 1988; Berger & Maier, 2001).

MATERIAL AND METHOD

Eudiaptomus graciloides were collected in February and March 2003 from Takvatn lake, Northern Norway (69°07'N, 19°05'E; for a detail description of the lake, see Dahl-Hansen *et al.*, 1994) in March 2003. Copepod samples were collected from 30 m depth to the surface using a 30 cm diameter conical net fitted with a mesh size of 50 µm. Before fixation samples were narcotized with 5% ethanol to avoid violent reactions such as dropping of egg sacs (Gannon & Gannon, 1975) and then preserved in 4% formalin.

In the laboratory formalin persevered samples were rinsed with tap water and females carrying either spermatophores or eggs were isolated and grouped according to their number of spermatophores (range 1-11) and number of eggs in the sac (range 2-9). From each sampling date, I randomly selected 10 females with spermatophores and 10 with eggs from each group for measurements. However, in both February and March samples, very few females carried more than five spermatophores, I obtained, therefore, five groups with females carrying one to five spermatophores (10 females in each group). There were also very few females that carried fewer than three eggs and more than eight, hence, I obtained six groups with females carrying three to eight eggs (10 females in each group) from each sampling dates. Only undamaged specimens with a relatively straight body were chosen for measurements. Using a dissecting microscope and a fine needle, I gently removed the spermatophore and egg sacs. By the use of a compound microscope fitted with a drawing mirror, I draw the image of the different body parts, spermatophores and eggs onto a piece of paper and linear measurements of the drawings were later made with a ruler to the nearest 0.01 mm. Antennule length was obtained by following the curved drawing traced on the paper with a measuring wheel. While detaching and drawing the spermatophore and the egg sac, the animals were placed separately in a drop of water on excavated slides. Drawings of the genital double-somite, furca and eggs were made at 160× magnifications while for the rest body parts and the spermatophores 100× magnifications were used.

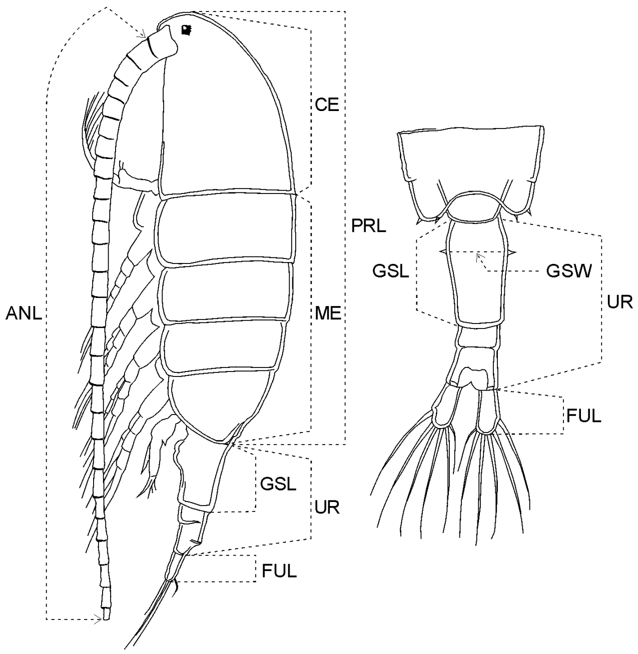

Figure 1. Diagrammatic illustration of the external morphology of female *Eudiaptomus graciloides* indicating measurements taken and other body parts. PRL, prosome length; GSL, genital double-somite length; GSW, genital double-somite width at the spine; FUL, furca length; ANL, antenna length; CE, cephalosome; ME, metasome; UR, urosome. Drawing by Tove Midttun.

Body size (i.e., prosome length and width), antennule length and morphological parts that males usually hold on to (i.e., furca and genital double-somite) during mating were included in the measurements (Figure 1). Additionally, the length of spermatophores and the diameter of eggs were measured. To estimate the repeatability of measurements, all traits were measured twice on 30 randomly selected individuals. The traits included in the measurements, for females with spermatophores and for females with eggs are given in figure 1, and the repeatability of the size measurements where high (R^2 between 0.99 and 0.97, table 1). From prosome length and width measurements, I calculated the volume of prosome using the formula for an ellipsoid (Kuosa & Göran, 1989). For statistical analysis, I used the mean of left and right antennules and of furca.

Table 1. Traits included in the measurement and their repeatability estimated as the proportion of the total variance attributed to between-individual variance.

Traits	R^2
Prosome length	0.99
Prosome width	0.99
Right antennule	0.98
Left antennule	0.98
Genital double-somite length	0.98
Genital double-somite width at the spine	0.97
Right furca	0.99
Left furca	0.98
Spermatophore length	0.99
Egg diameter	0.99

Statistical Analysis

Before analyses, all morphological traits values were standardized $(zi = (x_i - x)/s_x)$. For both mating frequency and clutch size, univariable and multivariable selection coefficients were calculated according to the multiple regression methods of Lande & Arnold (1983). Univariable selection coefficients are estimates of the direct effect of selection on a given trait as well as of the indirect effects of selection on correlated characters, whereas the multivariable selection coefficients measures only the direct effects of a given character on the dependent variable, adjusting for the effects of indirect selection on the statistically correlated characters included in the analysis. Ordinary least squares regression analyses were used for all tests of significance of single partial regression coefficients and for total models. The relationship between egg length and number was estimated using partial correlation between egg length and egg number having controlled for the effect of body size. All statistical analysis reported in this paper were performed with the R software. Sampling date did not affect any of the morphological traits measured (e.g body length t=0.046, P = 9637), so it was not considered in the statistical analysis.

RESULTS

Up to eleven spermatophores were observed at the genital double-somite of a female. However, generally one to two spermatophores were the most common. The position of the spermatophores on females is remarkable. Both on females with single and multiple spermatophores, I did not observe any attached spermatophores far from the genital field. Even the multiple spermatophores were closely concentrated around the genital field.

Female size, components of size, egg number and size

Univariable and multivariable regression coefficients as predictors of female clutch size are presented in Table 2. The analysis is based on data from March sample. Yet, the inclusion of the February sample did not noteworthy change the results. Univariable analyses of the relationship between different morphological traits of females and clutch size, revealed that body size (length and width) and furca length were positively related to number of eggs carried per female. However, when the effect of body size was held constant, furca length was not related to clutch size. The rest of the traits measured were not significantly related to female clutch size in univariable and multivariable models (Table 2). Therefore, large females had many eggs compared to small females.

Table 2. Standardized univariable [±SE] and multivariable [±SE] sexual selection coefficients for female body size measures as predictors of female clutch size. Significance of the parameter estimates and of the model were tested by ordinary least squares regression analyses (n=60). SE= Standard Error.

Traits	Univariable	Multivariable
Prosome Volume	**0.754 (0.201)*** **	**0.902 (0.201)** **
Genital somite width	0.371 (0.221)	0.013 (0.233)
Gentital somite length	-0.051 (0.226)	-0.173 (0.211)
Antennule	-0.281 (0.223)	-0.465 (0.293)
Furca	**0.541 (0.215)***	0.447 (0.220)
		F* = 3.592*
		df = 5, 54

*P<0.05, **P<0.01 ***P<0.001

Moreover, egg size did not differ among females (F = 0.277, P = 0.598) and mean egg size was not significantly related to female body size (b = 0.186, df = 58, P = 0.154, r^2 = 0.035) or to egg number, even after having controlled for the effects of body size (r = 0.186, n = 60, P = 0.154).

Female size, components of size, spermatophore number and size

Female size (body length and width), antennules and furca influenced relative mating frequency (Table 3). In univariable analyses, mating frequency was negatively related to body size and antennules length, and positively related to furca length and genital double somite width. However, when analyzing all measured traits using a multivariable model, the effect of body size became non-significant, while that of antennules, furca and genital double somite revealed the same pattern. Therefore, the detected effect of body size was apparently due to indirect influence of phenotypically correlated traits. This was confirmed by the observation that body size and antennules length were highly correlated (Pearson correlation coefficient = 0.429). These analyses suggest that antennules size, furca size and genital double somite were the most important traits explaining the variation in female mating frequency and that the detected effect of body size was apparently due to indirect influence of antennules that is correlated with body size.

Table 3. Standardized univariable [±SE] and multivariable regression [±SE] coefficients for female body size measures as predictors of female mating frequency. Significances of the parameter estimates and of the model were tested by ordinary least squares regression analyses (n=100). SE = Standard Error.

Trait	Univariable	Multivariable
Prosome Volume	**0.301 (0.140)***	-0.143 (0.133)
Genital somite width	**0.361 (0.139)***	**0.298 (0.128)***
Gentital somite length	-0.245 (141)	-0.202 (0.126)
Antennule	**-0.458(136)*****	**-0.441 (0.136)****
Furca	**0.475 (134)*****	**0.467 (0.129)*****
Spermatophore size	**-0.434 (137)*****	
		F=8.393 ****
☐		*df* = 5, 94

**P*<0.05, ***P*<0.01, ***P<0.001, ****P<0.0001

The mean length of spermatophores per female was negatively related to the number of spermatophores and the size of furca in univariable analysis (regression coefficient [±SE] = - 0.238 [0.095], *P*<0.05 and - 0.488 [0.135], *P*<0.05, respectively). However, in multivariable analysis, only furcal length remained negatively related to the mean length of spermatophores of females (regression coefficient [±SE] = -0.357, [0.140], *P*<0.05).

DISCUSSION

My study shows that large females have higher clutch size than small females. Similar relationships have been found in other calanoid copepods (e.g. Smyly, 1968; Maly, 1973,1983; see Mauchline, 1998 for more examples) and in a variety of animal species (see Andersson, 1994 for review). Such a relationship is often expected simply because larger females have more volume for eggs and more internal space for storing resources (e.g., energy) than do smaller females (Wickman & Karlsson, 1989; Roff, 1992). Many calanoid copepods store lipids in oil sacs (Mauchline, 1998) and this has also been observed in *E. graciloides* (own observations). In some high latitude copepods, reproduction is less dependant on ambient food concentration. These species over winter as copepodid V, and adult stages, and mobilize lipid stores for egg production (Mauchline, 1998). In this regard, *E. graciloides* might not differ from these species as females have peak sexual activity during colder months of the year (Paper two). During this period females might largely depend on the stored energy reserves for reproduction rather than depend on their immediate supply of food. Under such condition the size of the females might reflect their potential capacity for storing resources and hence relate to their clutch size.

On the other hand, egg size did not differ significantly among females and mean egg size was not related to female body size (both length and width). This might suggest that selection act directly to reduce the variance in egg size through stabilizing selection. That is, females may be selected to maximize clutch size and the strength of this selection pressure may override the positive effects of egg size on offspring fitness, reported for other copepods (e.g., Cooney & Gehrs, 1980; Wyngaard, 1986; Guisande *et al*, 1996). Moreover, I found no relationship between the number of eggs carried by a female and the average size of the eggs even after having controlled for the effects of body size. My result indicates that, for *E. graciloides,* estimates of female reproductive fitness based on clutch size measures alone are sufficient and that the inclusion of egg size measures unlikely provide additional information.

The primary motivation for this study was to address the question of multiple mating by female *E. graciloides*. Direct benefits hypotheses for the evolution of multiple mating predict that female reproductive rate should be increased with the number of matings, as these provide females with direct resources (Thornhill & Alcock, 1983; Wiklund *et al.*, 2001). However, the mating system of *E. graciloides* seems non-resource-based, as males do not control resources that seem to be important to the females. Moreover, the findings of a higher mating rate in small females compared to large females, and the lower clutch size in small females than in large females, suggest that it is unlikely that multiple mating in this system is maintained by direct benefits, that is, that females are mating with additional males to increase their fitness. In sum, the lack of evidence for early-life indirect benefits from multiple mating suggest that indirect benefits of multiple mating are likely to be swamped by the direct costs of superfluous mating, supporting the theoretical predictions of Kirkpatrick & Barton (1997). However, extenuating effects of indirect benefits to females, for example, via sexy sons or good genes cannot be ruled out..

In contrast, the male manipulation hypotheses for the evolutionary origin and maintenance of multiple mating states that frequent female mating evolve as a by-product of selection on males to increase their fitness (Holland & Rice, 1998; Arnqvist & Nilsson, 2000; Crudgington & Siva-Jothy, 2000; Byrne & Roberts, 2000; Stutt & Siva-Jothy, 2001). As mating at very high rates is costly to females, they are expected to evolve behavioural responses, such as mate rejection, to minimize the cost of unwanted copulations (Arnqvist, 1989, 1992; Rowe, 1992, 1994; Fairbairn, 1993; Watson *et al.*, 1998). The level of females' resistance to male mating attempts and the perceived male harassment rate may vary among females. Such variation may partly be caused by behavioral and morphological traits that affect harassment and resistance – traits that results from genetic differences, developmental trajectories or proximate environmental factors. (e.g., Chapman & Partridge 1996; Jennions & Marion, 1997). These variations generate variation among females in mating propensity and mating rate under any given average harassment rate (Watson *et al.*, 1998). The observed patterns of mating are compatible with such predictions. That is, I found higher rate of mating on females with longer furca and larger genital double somite. Observations on behavioural components and inter-digitations of morphological traits of the two sexes during copulation from other copepods may help explain this (i.e. in diaptomids; Watras, 1983; in calanoids; Gauld, 1957; Roff, 1972; Blades, 1977; in cyclopoids; Maier; 1995). Mating is usually initiated when the male grasp the female on her caudal furcae with his geniculate (right) antennule. Following this the female swim about and makes vigorously attempts to escape from the male's grip. Females are often successful in escaping before the male holds on to her genital double-somite with his fifth leg and secure his mating position. During this premating struggle, the ability of a male *E. graciloides* to hold effectively onto a female, and of a female to dissociates the males grip is probably related to the size of female's furca and genital double somite. Thus, the longer the furca and/or the larger the genital double somite the less likely a male will be shaken loose by the movement of the female. Consequently, more males would be able to mate with such females. Moreover, the inverse relationship between female size (in terms of prosome volume and antennule length) and frequency of mating observed further suggest that another major determinant of the rate of mating in *E. graciloides* appears to be the strength of a female to repel mates during the premating struggle. Mobility and escape ability are known to be directly associated with body size and/or prolonged organs such as antennules that are involved in detection of stimulus and powering fast jumps (Dusenbery & Snell, 1995; Hedge & Krishna, 1997; Mauchline, 1998; Maly & Maly, 1999; Biedermann, 2002). Consequently, large body size and large antennules appear to be advantageous to the females when rejecting males during the premating struggle. Collectively, these results are in agreement with predictions generated by the male manipulation hypotheses. This hypothesis further predicts that elevated mating rates should have fitness consequences for females (Holland & Rice, 1998; Arnqvist & Nilsson, 2000; Gavrilets *et al.*, 2001). Yet, my separate analysis - characterizing females that are mated at a wide range of rates and that produced different number of eggs - do not allow me to distinguish the effect of elevated mating rate on female reproduction rate. For example, small females that are less vigorous are less able to avoid unwanted mating and must tolerate more mates than more vigorous large females. Consequently, such females might have lower clutch size than large females because they are inferior in quality or more affected by the costs associated with elevated mating rates, or because of a combination of both these two mechanisms.

In conclusion, my results demonstrate an association between the variation in mating rate and the phenotypic value of the traits that are important for mobility and escape ability of females, i.e., the region of importance for male pre-copulatory clasping behaviors on female or for thwarting the other sex. This is consistent with the predictions of female avoidance of male-imposed costs of multiple mating. Although my analysis do not allow me to distinguish the effect of elevated mating rate on female reproduction rate from those stemming from female quality, my results do suggest that multiple mating in this system is maintained by sexual conflict over mating rate. Whatever benefits females may accrue from multiple mating must be large enough to outweigh the costs. Given the lack of indirect evidence for increased clutch size and early-life indirect benefits from multiple matings (paper IV), this is less likely. However, in order to fully understand the effect of multiple mating on overall female fitness, further studies are needed that employ manipulation of mating rate, on successful reproduction, time to oviposition, egg production, and offspring body mass as well as the fitness measures of offspring such as survival rate, growth rate and reproductive success (Arnqvist & Nilsson 2000). To distinguish the confounding effects of elevated mating and female quality on female reproduction rate, the studies need to account for female body size and other traits influencing both female reproduction rate as well as the outcome of premating struggle and hence mating rate. Such studies would enhance our understanding of how sexual conflict over mating rate interacts with life-history traits to affect female fitness.

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Paper III

Influence of female morphology on male mating success in the calanoid copepod, *Eudiaptomus graciloides*.

Abstract

In species exhibiting inter-sexual conflict over mating, the outcome of female-male sexual encounters may depend on the phenotypic value of traits of either sex that are important for pre-copulatory behaviors. To explore this prediction, I investigated the relationship between female morphology and male mating success in mate choice experiments with the diaptomid copepod, *Eudiaptomus graciloides* (Lilljeborg), for which inter-sexual conflict is suggested to occur. Three randomly selected receptive females were introduced to an adult male in a small glass vessel containing filtered lake water. After staying together for a period of 24 hours, copepods were preserved for subsequent morphometric analyses. I examined whether measurements of the different body parts of the males, the mated and the unmated females explained the female mating status/male-mating success. The results suggest that mated females had relatively longer furca than unmated females, confirming the pattern observed in earlier field studies. Moreover, mated females had smaller body size and had longer genital double somite compared with the unmated females. The results of the experiment are consistent with predictions generated by a model of sexual conflict. Based on the behavioural sequence and the mechanics of copulation in this system, I discuss the influence of such traits on the observed outcome of mating interactions.

INTRODUCTION

Due to the basic asymmetry in the prevalent fitness optima between the sexes, males will usually benefit from securing multiple mating, even if females have mated previously (Bateman, 1948; Trivers, 1972). Despite the potential (genetic) benefits of polyandry to females, costs from mating at a high rate with multiple males may outweigh the benefits (Arnqvist & Nilsson, 2000; Jennions & Petrie, 2000). For example, females may increase predation risk (e.g. Fairbairn, 1993; Rowe, 1994; Arnqvist, 1997), reduce foraging time, reduce foraging efficiency (e.g. Wilcox, 1984; Watson *et al*., 1998), or increase energetic expenditure (e.g. Arnqvist, 1989a; Watson *et al*., 1998) from multiple mating. Such a situation poses inter-sexual conflict over the mating rate between the sexes (Parker 1979, 1984). This conflict is predicted to result in the evolution of traits in males that coerce females into mating at a rate beyond their optimum (Clutton-Brock & Parker, 1995) and in traits of females that reduce the number of costly superfluous copulations (Parker, 1979). These traits can be manifested behaviorally and morphologically (Arnqvist, 1989b; Rowe *et al*., 1994; Allen & Simmons, 1996; Arnqvist, 1997; Blanckenhorn *et al*., 2000). Male traits that overcome female reluctance can be under strong selection potentially leading to the evolution of inter-sexual arms races (Arnqvist & Rowe, 1995, 2002a, b; Race, 1996). Substantial empirical evidences for such coevolutionary scenario have been documented in several species. For example, many male insects possess claspers that enhance control of copulation (Thornhill & Alcock, 1983; Thornhill, 1984; Arnqvist, 1989b, 1995). Likewise, females possess morphological adaptations to aid in rejection of unwanted mates (Arnqvist & Rowe, 1995).

The behavioral interactions between the sexes of diaptomid copepods during mating appear to be inter-sexual conflict over the mating rate. Males actively search and chase receptive females and attempt to grasp them. Females respond to this with a vigorous escape reaction and try to dislodge the males (Watras, 1983), indicating female reluctance to mate. Such female responses upon physical encounter with males are also reported for other copepods and cladocerans (Roff, 1972; Blades, 1977; Maier, 1995; Roff, 1972; Tsuda & Miller, 1998; Brewer, 1998; Forro, 1997). During this premating struggle, the male attempts first to secure a furcal and then a genital double somite grasp of the female, with his right antennule and his right fifth leg, respectively (Berger & Maier, 2001). These two points of attachments are critical to subdue reluctant (escaping or jumping) females, and males of diaptomid and several other species of copepods possess geniculated antennule(s) and a modified fifth leg that are adapted to grasp females during mating (see Mauchline, 1998). Additionally, most of the above-mentioned costs of mating are likely to apply in diaptomids. For example, pairs in copulation are, compared to single individuals, more conspicuous to visual predators (Hairston *et al.,* 1983; Winfield & Townsend, 1983) and have reduced vigilance and escape abilities (Maier *et al*., 2000). Thus, a receptive female encountering a number of males will experience increased predation risk from multiple matings. Moreover, diaptomid females lack seminal receptacles (Cuoc *et al*., 1989a, b) and hence cannot store sperm. A single mating before each clutch production is often sufficient and does not limit female reproductive success (Watras & Haney, 1980; Watras, 1983; Chow-Fraser & Maly, 1988; Berger & Maier, 2001; Paper IV). Additionally, males seem to provide nothing but gametes. Yet, a high proportion of diaptomid females mate multiply both in laboratory and in the field (e.g. Berger & Gerhard, 2001; Paper I, II, and IV), suggesting a inter-sexual conflict over the mating rate.

Any trait that would increase the ability of females to resist males during the premating struggle would decrease the overall costs to females imposed by males (Arnqvist & Rowe, 1995). Likewise, a trait that would enhance the ability of males to subdue reluctant females would improve male mating success at a cost to the female (Thornhill & Alcock, 1983; Thornhill, 1984; Arnqvist, 1989b, 1995). Large body size and prolonged organs such as antennules that are involved in the detection of stimulus and powering fast jumps (Mauchline, 1998) are associated with, for instance, higher mobility or higher search and escape ability (Dusenbery & Snell, 1995; Maly & Maly, 1999; Hedge & Krishna, 1997; Biedermann, 2002). I hypothesized that large body size or well-developed locomotive and sensory organs would increase female's ability to thwart harassing males and thereby increase the female's control over the rate of copulations. At the same time, relatively short morphological segments or contact areas on female where males make initial contact and further position themselves during mating would have similar effect as that of large body size and prolonged organs, as this would make it more difficult for males to grasp females firmly. Thus, if the female reluctance hypothesis is the primary mechanism of selection in *Eudiaptomus graciloides* (Lilljeborg), as suggested by previous observations (Paper

II), then male mating success would be (*i*) negatively related to female body size or segment size (e.g., such as size of antennules that are important for mobility and escape ability of females), and (*ii*) males should more easily manage to mate with females with large caudal furca and large genital double somite, all regions of importance for precopulatory clasping behaviors. Large size of these traits should ease a male's ability to hang on to a resisting female. Here, I test these predictions in *E. graciloides* using a mate choice experiment.

MATERIAL AND METHOD

Live *E. graciloides* were collected from lake Takvatn by vertical hauls from 30 m depth to the surface using a conical net (diameter, 30 cm) fitted with a mesh (50 μm). The copepods were kept in lake water in 30 l plastic tanks and transported to the laboratory. Here the animals were transferred to 25 l maintenance aquaria and kept in a controlled light-temperature room (3±1 °C; 18:6 h light: dark cycle, provided by 20 watt incandescent lamps generating about 0.25-0.30 W m^2 to the water surface depending on the position of the aquaria). The next day, adult males and adult unmated females were sorted with the aid of a dissecting microscope and kept separately in 2 l beakers containing filtered lake water (100 animals per bottle), to prevent them from mating before the experiment. The animals were fed *ad lib* with lab cultured *Scenedesmus gracilis* at the concentration of about 5x104 cells ml^{-1}. In diaptomids, mating usually takes place when the female have ripe oocytes (Chow-Fraser & Maly, 1988). After four days of separation, three adult females with ripe oocytes, recognized as dark bands in the prosome (Berger & Maier, 2000), were randomly selected and combined with an adult male in a 30 ml glass vessel containing about 25 ml of filtered lake water. Three females were used in each vial to increase the chance of mating. To obtain a large number of mated animals, covering a broad range of size-ratios, 150 experimental units were used. The vials were kept under the same condition as described above. After a period of 24 hours, copepods were fixed using a 4% formaldehyde solution for later measurement, after narcotizing them with 5% alcohol to avoid violent reactions that may result in dropping of spermatophores or egg sacs (Gannon & Gannon, 1975).

The presence of a spermatophore or fertilized eggs on the female genital double somite was considered as evidence of mating. This method of scoring does not capture attempts that have been made but that have not resulted in extrusion of the spermatophore, nor does it include matings that have taken place but for which no evidence of attached spermatophore or fertilized eggs is left on the females (it is difficult to differentiate such mated females from unmated ones). Yet, differentiation was made between females with fertilized clutches and females with unfertilized clutches; unfertilized eggs disintegrate and appear as a gray mass in the clutch sac, whereas fertilized ones remain distinct and dark (Watras & Hanery, 1980). Females with unfertilized eggs were considered unmated. Since diaptomid produce only one clutch per mating event (Watras & Hanery, 1980; Watras, 1983; Williamson & Butler, 1987), the fertilized clutches were assumed to be produced from the experimental mating. In 58 of my experimental units, I found one mated female and two unmated females. However, as some animals were lost during handling for size measurement, I ended up with 39 experimental units for statistical analysis, each having one male, one mated female and two unmated females.

The relevant body parts of the males and females were measured as follows. With the help of a drawing mirror fitted on a compound microscope, I draw the images of the different body parts of interest onto a piece of paper. The drawing of female and male antennules, the female furca and the male right fifth leg, including the claw, were taken after dissecting or detaching them from the body. Linear measurements of the drawings were later made with a ruler to the nearest 0.01 mm. The length of antennules and the male right fifth leg, including the claw, were obtained by following the curved drawing traced on the paper with a measuring wheel. While dissecting, detaching and drawing, the animals were placed separately in a drop of water on excavated slides. Drawings of the female genital double somite, the female furca and the male right fifth leg, with the claw, were made at 160× magnifications, whereas for the remaining body parts 100× magnifications was used. Body size (i.e., prosome length and width) and antennules length of both sexes, and morphological parts that males use to hold on females (i.e., female furca and genital double somite) and those male traits used to hold the female during mating (i.e., male right antennules and right fifth legs) were included in the measurements (Fig. 1). Measurements of these body parts or morphological traits were highly repeatable, with R^2 ranging from 0.97 to 0.99 (see paper II for details). Consequently, each measurement was only taken once.

Statistical Analysis

All morphometric measures were centered and standardized before the analyses were carried out. All selection coefficients reported are thus standardized selection coefficients (Lande & Arnold, 1983; Endler, 1986). The influence of morphological characters on mating likelihood was estimated by conditional logistic regression (Campbell, 2006). Conditional logistic regression was required due to the complex study design with matched unmated and mated females (1-2 case-control design, see Hosmer & Lemeshow, 2000). To measure the direct effect of a given trait as well as of the indirect effects of any correlated characters, I used conditional logistic regression analyses of female mating status (1 = mated, 0 = unmated) on the different measures of female body size components, employing separate analyses for each trait. To measure the direct effect of a given trait independently of the others, I used multiple, conditional logistic regression analyses of female mating status on all female morphological variables. To assess any potential relationship of male morphology with female morphology affecting male mating success, I used conditional logistic regression analyses of female mating status on the logarithms of the different size ratios between the sexes (log (*female/male*)). Significances of the parameter estimates (for both simple and multiple logistic regressions) and of the model (for the multiple logistic regression) were determined by likelihood ratio chi-square tests.

RESULTS

Summary statistics for female and male size measurements are given in Table 1. Univariable and multivariable regression coefficients as predictors of the mating status of females/male mating success are presented in Table 2. Separate analyses of the impact of the different morphological components of females on mating status revealed that genital length and furca were positively related to male mating success. The rest of the traits measured were weakly related to male mating success in univariate models (Table 2).

When analyzing all measured traits of females using a multiple regression model that adjusts for the effects of selection on correlated characters, furca size of females were found to be positively related to mating status, while body length and antennule length was negatively related to mating status (Table 2). The mated females had longer genital double somite, longer furca, and shorter body length relative to the unmated females.

Table 1. Means and standard deviations for various female and male morphological characteristics measured (in mm).

Trait	Female	Male
Body Length	0.857 (0.028)	0.773 (0.018)
Right antennule	1.174 (0.062)	1.050 (0.039)
Left antennule	1.172 (0.064)	1.062 (0.042)
Genital somite length	0.139 (0.008)	
Furca	0.068 (0.003)	
Fifth leg		0.285 (0.008)

Table 2. Standardized univariable and multivariable coefficients (±SE) for female body size measures as predictors of female mating status/male mating success Significance of the parameter estimates and of the model (for the multiple logistic regression) was tested by logistic regression using likelihood ratio chi-square tests (LR, likelihood ratio test; n=39).

Trait	Univariable	Multivariable
Female traits		
Body length	0.075 (0.197)	-0.355 (0.272)*
Genital double somite length	0.310 (0.202)*	0.237 (0.254)
Antennule	0.399 (0.225)*	-0.315 (0.585)
Furca	0.401(0.234)*	0.489 (0.294)*
		LR*
Log (*female traits / male traits)*		
Body length/body length	2.490 (5.96)	-17.211 (9.79)*
Genital d. somite length/fifth leg	7.120 (4.13)*	0.907 (9.79)
Furca/right antennule	9.74 0(5.59)*	21.506 (9.09)**
Right antennlue/right antennule	8.830 (4.67)*	-16.201(7.47)**
		LR***

P≤0.1, **P<0.05, ***P<0.01,*

Among the different sex size ratios (log (*female/male*)) analyzed in univariable models, the size ratios of female furca to male right antennule, female genital double somite to male fifth leg, and female right antennule to male right antennule produced positive association with male mating success. In the multivariable model, male mating success were negatively associated with the size ratios of female to male body size, and of female right antennule to male right antennule, and positively associated with the size ratios of female furca to male right antennule. Hence, males are more successful in mating with small females, females with longer genital double somite, shorter antennules and longer furca.

DISCUSSION

I predicted that individuals of species exhibiting inter-sexual conflict over the mating rate, such as *E. gracilodes,* should exhibit correlations between mating success and the phenotypic value of traits that are important for mobility and escape ability of females and regions that are of importance for male precopulatory clasping behaviors or for thwarting the other sex. In accordance with the expectations, male mating success is inversely associated with female body size and antennules (weakly). A study of sexual selection relating female morphology and mating frequency, as indicated by the number of externally attached spermatophores, in wild caught *E. gracilodes* has demonstrated that large females and females with prolonged antennules, short furca and small genital double somite are mated less frequently than do small females and females with short antennules, long furca and large genital double somite (Paper II & IV). In this study, the lack of strong negative association between female mating status and antennules might be the effect of the experimental container, which might have been small for female to fully use the potential of their escape reaction. However, these findings are in accordance with my predictions developed from the behavioral sequence and the mechanics of the premating struggle described in the introduction.

In a system involving inter-sexual conflict over the mating rate, the major determinant of mating success appears to be the strength of a female to repel mates or morphological adaptation that aid in rejection of unwanted mates and of a male to withstand such rejection response of a female during the premating struggle (Rowe *et al.*, 1994; Arnqvist & Rowe*,* 1995; Arnqvist & Rowe, 2002; Rowe & Arnqvist, 2002). According to these hypotheses, the outcome of premating conflicts is determined, in part, by the relative sizes of the two interacting individuals (Fairbairn 1988, 1990; Sih & Krupa, 1992; Arnqvist *et al.*, 1996; Rowe & Arnqvist, 1996). From the female perspective, morphological traits that would increase their efficiency at rejecting harassing males is expected to be body size and antennules length (paper II & IV). This is because, the strength of mobility and escape ability are known to be associated with body size and/or prolonged organs such as antennules that are involved in detection of stimulus and powering fast jumps (Dusenbery & Snell, 1995; Hedge & Krishna, 1997; Mauchline, 1998; Maly & Maly, 1999; Biedermann, 2002). Therefore, my findings are most consistent with the hypothesis that females evolved the rejection response to avoid mating, which presumably carries a cost. Additionally, large females and females with longer antennules (paper II & IV) seem more powerful and hence, able to reject males more easily in the premating struggle and consequently, mate less frequently than do small females or females with short antennules. There is no comparable evidence of such relationships from closely related species in copepods, but observations in *Coelopa ursine* with similar mating behavior have revealed that large females reject males more easily than small females and that male mating success is negatively associated with female size (Crean & Gilburn, 1998).

Moreover, as expected the length of the female furca and the genital double somite are directly correlated with the mating status of females/male mating success. These two morphological traits of females are regions of importance for male precopulatory clasping behaviors (Mauchline, 1998; Berger & Maier, 2001). Hence, any female trait/behavior that make it more difficult for males to achieve a firm furcal and genital somite grip should be beneficial for females thereby decreasing the frequency of superfluous and costly mating (Rowe *et al.*, 1994; Arnqvist & Rowe, 1995; Arnqvist & Rowe, 2002; Rowe & Arnqvist, 2002). While such traits are unlikely to be furca and genital length per se, a reasonable hypothesis is that size of such traits is one of the possible factors that influence directly the efficiency of males' grip on them. Consequently, females with relatively short furca and short genital somite are able to dissociate the males grip more easily during the struggle thereby making it more difficult for them to secure mating position and impede access to the genital field where males attach their spermatophores. It could still be argued that other female behaviors, such as (the degree of) downward curling/tilting of the urosome (Yen 1988; per. observation) that comprises the genital somite and the furca, in conjunction with the influence of their size, might also contribute to the observed non-random mating pattern with respect to these female traits. In support of this suggestion, the effect of such female abdominal curling during mate-rejection behaviors in influencing males grip and in resulting non-random mating with respect to certain male phenotypes have been shown in many insects exhibiting inter-sexual conflict over the mating rate and possession of flexible female abdomens (e.g., Weall &

Gilburn, 2000). In sum, the results of my studies are more consistent with predictions and assumptions of the female reluctance hypothesis, which holds that the efficacy of females resistance, and hence their mating activity, is related to the morphological characters that effect such behaviour, which function as arms in premating conflicts (Arnqvist, 1989b; Rowe *et al*., 1994; Allen & Simmons, 1996; Arnqvist, 1997; Holland & Rice, 1998; Blanckenhorn *et al*., 2000; Gavrilets *et al*., 2001).

My previous and current results do also provide a basis for assessing the important assumptions of sexual selection hypothesis via quality-based mate choice. Such model assumes that choosiness in either sex is favored by high variance in quality among individual of the other sex (Parker, 1983; Andersson, 1994). From the male perspective, the key factor in such mate quality is expected to be female fecundity, which determines a male's expected fertilization success from the mating. Thus, male mate choice is expected where there is a considerable variation in potential female fecundity. Female size is known to be positively associated with fecundity in diaptomids, including *E. graciloides* (e.g. Smyly, 1968; Maly, 1973, 1983; Paper II). Therefore, if large females are more valuable to males (by virtue of their increased fecundity), then this hypothesis predict that male mating success would be an increasing function of female body size, where males mate disproportionaly with larger and more fecund females. However, the observed inverse association between female size and mating success do not conform to this hypothesis and hence, I have no support for quality-based models of male mate choice.

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Paper IV

No apparent fitness benefits of polyandry in female *Eudiaptomus graciloides* (Copepoda: Calanoida)

Abstract

The search for adaptive explanations of polyandry is one the most active areas of research in evolutionary biology. I examined the adaptive significance of polyandry in *Eudiaptomus graciloides* (Lilljeborg), from Takvan Lake, Northern Norway (69°07'N, 19°05'E). Previous studies on the natural mating frequency of females from the same population have revealed considerable variation in female mating rate, measured as number of spermatophores per mated female (average of 2.4, range 1 to 7). In this study, I compared egg production, clutch size, fertility, hatching rate of eggs, hatchling body size and early hatchling survival from females that had been mated once or twice. Double-mated females showed no larger fitness than single mated females. Yet, body size of females explained much of the variation in clutch size regardless of whether mating had been single or double. Consistent with previous studies, my results suggest that double-mated females had relatively shorter antennules and longer furca than single-mated females. Therefore, a more likely explanation for the occurrence of polyandry in *E. graciloides* is that females are manipulated by males into mating at a rate beyond their optimum as a result of selection on males to copulate as often and with as many females as possible.

INTRODUCTION

Understanding the adaptive significance of polyandry, i.e., the occurrence of mating with multiple males by females, is one of the most active areas of research in evolutionary biology. It is an interesting problem because, unlike for males where the advantage of mating as often as possible is obvious, for females the adaptive basis of polyandry is less clear and not in accordance with the conclusion drawn from the potential reproductive rates (Andersson, 1994; Arnqvist & Nilsson, 2000; Hosken & Stockley, 2003). Moreover, mating is a costly activity, resulting in loss of energy or foraging time (Wilcox, 1984; Bailey *et al.*, 1993; Clutton-Brock & Langley, 1997; Watson *et al.*, 1998), increasing risks of predation (Arnqvist, 1989; Fairbairn, 1993; Magnhagen, 1991; Rowe, 1994; Ward, 1986) and increasing disease transmission (Daly, 1978; Hurst *et al.* 1995). Yet, females commonly mate with multiple males, a pattern that is nearly universal in nature (Arnqvist & Nilsson, 2000; Jennions & Petrie, 2000; Hosken & Stockley, 2003).

Several hypotheses have been put forward to explain the evolution and maintenance of polyandry. These hypotheses can broadly be categorized into those that are based on direct (material) benefits, or indirect (genetic) benefits to females following multiple matings (Andersson, 1994; Arnqvist & Nilsson, 2000; Hosken & Stockley, 2003). In the former case, polyandrous females may derive benefits such as nutrients received from spermatophores or seminal fluids, an adequate sperm supply securing fertility or parental investment that cause female reproductive rate to increase with the number of matings (Thornhill & Alcock, 1983; Arnqvist & Nilsson, 2000; Wiklund *et al.*, 2001; Hosken & Stockley, 2003). Additionally, females may gain indirect genetic benefits that increase offspring fitness (Jennions & Petrie, 2000). Such indirect benefits might arise from improving on earlier matings (good genes models; Thornhill & Alcock, 19883; Simmons, 1987; Olssen, *et al.*, 1996), reducing the risk of genetic incompatibility (Zeh & Zeh, 1996, 1997), inbreeding avoidance (Tregenza & Wedell, 1998, 2002), and increased genetic diversity (Baer & Schmid-Hemel, 1999). The different hypotheses have been empirically examined for a variety of organisms (see review: Arnqvist & Nilsson, 2000; Jennions & Petrie, 2000; Hosken & Stockley, 2003). However, the extent to which these hypotheses might apply across taxa is challenged because there are increasing number of cases in which benefits to females from multiple matings could not be found (Kolodziejczyk & Radwan, 2003; Brown, *et al.,* 2004; Maklakov & Lubin, 2004; Patricia & Graeme, 2004)

An alternative hypothesis based on sexual conflict has been proposed to address the occurrences of polyandry. This hypothesis invokes that polyandry may evolve as a result of selection on males to copulate as often and with as many females as possible (e.g., Parker, 1979; Rowe *et al.,* 1994; Holland & Rice, 1998; Arnqvist & Nilsson 2000). As males benefit more from higher mating rate than do females (Bateman, 1948), they may seduce or coerce females to mate at a rate beyond their optimum, while females are expected to resist mating attempts to reduce direct costs associated with superfluous mating (Parker, 1979; Arnqvist & Nilsson 2000; Gavrilets *et al.,* 2001). However, pre-mating struggle or resistance is also a costly behaviour to females in terms of increased energy consumption (Watson *et al.*, 1998), reduced foraging time (Wilcox, 1984) and increased predation risk (Rowe, 1994), and in the face of repeated male harassment, these costs may outweigh the cost of mating itself. Under such condition, female might be better off by accepting superfluous matings, in a strategy of 'making the best of the bad job'. This phenomenon, termed as 'convenience polyandry', has been proposed as an explanation for the occurrence of multiple matings among females of many species (Thornhill & Alcock 1983; Arnqvist, 1989, 1992; Rowe, 1992; Rowe *et al.,* 1994; Watson *et al.*, 1998; Weigensberg & Fairbairn, 1996).

The present study attempts to assess the effect of female mating history on fitness measures of females and their offspring in the copepod *Eudiaptomus graciloides* (Lilljeborg). To do so, I compared egg production, clutch size, fertility, hatching rate of eggs, hatchling size and early hatchling survival from females that had been mated once or twice. The experiment was performed under laboratory conditions using wild-mated females that had been brought to the laboratory.

Study species

Eudiaptomus graciloides used for the study came from Takvan Lake, Northern Norway (69°07'N, 19°05'E). The population in this lake is uni-voltine. Its mating and reproductive season is in the winter under ice cover at an average temperature of 1.7^{o} C (Paper I). The sex ratio is usually slightly skewed towards females (mean male proportion ± SD: 0.44 ± 0.07). However, the following two points suggest that the operational sex ratio of the population might be highly skewed towards the males. First, a male can produce many spermatophores during the breeding season. For example in a preliminary study, a male produced up to 5 spermatophores during 10 days. Second, after mating and fertilization of eggs, females normally carry their eggs in egg-sacs for many days until hatching of the larvae. At a temperature close to that *in situ* (average temperature 3±1° C; 18:6 h light: dark cycle), the time from egg production until hatching is about 26 days (see results).

Males usually search and chase receptive females and they frequently attempt to grasp them. Females respond to this with a vigorous escape reaction and try to dislodge the males (Watras, 1983). During this premating struggle, the males attempt to secure first a furcal and then a genital double somite grasp of the female with its right antennule and right fifth leg, respectively (Berger & Maier, 2001). These two points of attachments are critical to subdue escaping females, and males possess geniculated antennules and a modified fifth leg that are adapted to grasp females during mating (see Mauchline, 1998). After securing the right position, the males transfer spermatozoa and associated seminal secretions contained in a spermatophore to the females (Blades &Youngbluth. 1980; Dussart & Defaye. 1995). Spermatophores are simple, tube-shaped flasks that narrow into a neck towards their open ends. Males use a cement-like material to externally attach spermatophores on the genial opening of female. During mating, a male can attach only one spermatophore and the average time between the productions of two spermatophores is 43 hours (SD ± 27.12, n = 18; 3±1°C; 18:6 h light: dark cycle).

Unless detached and/or used by the females, the spermatophores normally stay in the attached position on females. This allows counting of spermatophores on the genital opening of females under a dissecting microscope and makes diaptomid copepods well suited for studies of the natural mating frequency. Previous studies using this approach have revealed considerable variation in female mating rates between and within populations. For example, in the Takvatn population, the average number of spermatophores during the breeding period is 2.4 (SD = ± 0.42), suggesting a moderate level of polyandry. Yet, some females have been observed carrying up to seven spermatophores (Paper I).

Females lack seminal receptacles to store sperm (Cuoc *et al.*, 1989a, b) and hence, re-mating is necessary to produce a new set of fertile eggs (Watras & Haney, 1980; Watras, 1983; Chow-Fraser & Maly, 1988; Berger & Maier, 2001). Spermatophores discharge their contents over the genital area of females where they form an attached spermatophoral mass (Cuoc *et al.*, 1989a, b). Females usually detach spent spermatophores with their modified fifth legs before extrusion of egg sacs. The eggs are extruded into a sac and carried for some days until the nauplii hatch (Chow-Fraser & Maly, 1988; Berger & Maier, 2001).

Diaptomid copepods have complete metamorphosis. The eggs hatch into a larva called Nauplii. There are six naupliar stages, usually abbreviated N1 to N6. N1 has no oral apparatus, lives on its vitelline reserves and barely move. This suggests that the amount of reserves, which is a function of N1 size, can have important implications for the survival of this non-feeding stage (Cooney & Gehrs, 1980; Wyngaard, 1986; Guisande *et al.*, 1996). After moulting into N2, the nauplii starts to feed. N6 moults to the first of the five copepodid stages, abbreviated C1to C5 and finally C5 moults to adults (Dussart & Defaye, 1995; Muchline, 1998).

MATERIAL AND METHOD

Sampling and Experimental Procedures

Live *E. graciloides* were collected in March 2007 from Takvatn Lake, Northern Norway (69°07'N, 19°05'E). Copepods were sampled at three stations in the pelagic area of the lake from 30 m depth to the surface using a 30 cm diameter conical net fitted with a 50 μm mesh. Samples were diluted and kept in lake water in 30 l plastic tanks and transported to a controlled light-temperature room (average temperature 3 ± 1° C; 18:6 h light: dark cycle, provided by 20 watt incandescent lamps). Here, mated females carrying externally attached spermatophores were sorted under a dissecting microscope and kept separately according to their number of spermatophores for each sampling station. In diaptomid copepods, successful egg production after mating, or after being with a male, is only 50% (Watras & Hanery, 1980; see results). Therefore, to get a sample size required for the experiment, I used only females with either one or two spermatophores. As females with more than two spermatophores were rare, they where not included. From the three stations, I randomly selected 150-mated females, 75 of which were single mated and the rest (75) double-mated. Until egg production, females from each sampling station carrying the same number of spermatophores were kept mixed in 2 l beakers containing filtered lake water (i.e., 25 animals per bottle). I also isolated several hundred adult males from the field samples and kept them separately in maintenance aquaria for later use. The remaining animals were transferred to 25 l maintenance aquaria. Copepods were kept in the above-mentioned light-temperature regime and fed *ad lib* (see below) with lab cultured *Scenedesmus gracilis* at the concentration of about $5x10^4$ cells ml^{-1} (as measured by hemacytometer counts). After 3 days of separation, egg-producing females were transferred to individual chambers containing 20 ml of filtered lake water. As diptomid females needs re-mating to produce a new clutch of fertile eggs (e.g., Berger & Maier, 2001), I introduced a male to each chamber on the same day the females were individually separated. The male remained present until the death of the female. If the male died before the female, it was replaced by a new male. During the 3 days period after separation, a total of 62 females (26 single mated and 36 double mated) produced egg sacs, 10 female died (4 single mated and 6 double mated) and the rest (78) failed to produce eggs.

Animals in the individual chambers were fed with the alga *S. gracilis* along with 1 ml of aerated, pre-filtered lake water three times per week. Their digestive tracts were examined under a dissecting microscope throughout the experimental period. At all times it appeared full, indicating an *ad lib* feeding regime (Watras & Hanery, 1980). Sediment materials from the bottom of the chamber were removed with a pipette and 10 ml of water was replaced weekly in each chamber with fresh, pre-filtered lake water.

Females were examined daily for survival and other fitness parameters. Their clutch size and their number of fertilized and unfertilized eggs were noted. Differentiation between the latter two types of eggs were made based on their distinct characteristics; unfertilized eggs are amorphous in shape and appear as a gray mass in the clutch sac (they finally disintegrate), whereas fertilized ones remain distinct and dark (Watras & Hanery, 1980). Moreover, the date of hatching, the number of eggs hatched and the number of live and dead offspring were recorded. Additionally, females were checked for any change in reproductive phases, mating and additional clutch production. To minimize disturbance, observations were made under a dissecting microscope while the animals swam freely in their individual chamber. All observations, that is, from sorting to follow-up routine observations and photographing (see below) were performed at approximately 3°C. The experiment lasted for two months, when all females had died.

As a control for the effect of routine observations and handling on the possibility of additional clutch production by the experimental animals, 40 females carrying viable and comparable clutch size (3 – 10 eggs) were sorted out from the maintenance aquaria and kept individually with a male in a similar sized chamber as the experimental animals. They were also kept at the same light-temperature regime and fed the same amount of food at the same interval as the experimental animals. Unlike the experimental animals, control animals were checked only once for the possible production of a second clutch.

Morphological trait measurement.

A photograph of live adult females and nauplii were taken using a light microscope mounted with a digital camera (Sony Model DSC – P10). For photographing, each animal was placed in a small confining area (3x3x1mm^3 for adults and 0.5x0.5x0.3mm^3 for nauplii) made on excavated slides using Scotch tape. The amount of water was adjusted to the top level of the confining area with the aid of a pipette (3 – 30 µl) under a dissecting microscope. The confining area were large enough to let an animal freely move to a distance of about one body length as long as it maintained the same orientation. In this way, pictures were taken for 48 randomly selected females that failed to produce egg sacs and for all 62 egg-producing females. For the former group of females, pictures of only body length were taken, but for the latter, pictures of additional morphological traits were taken (Table 1). Moreover, whenever eggs hatched, 3 nauplii were randomly selected, photographed and placed in separate chambers filled with filtered lake water.

Pictures of nauplii and the genital double-somite and furca of adult females were taken at 200 □ magnifications, while for body length, body width and antennule length 100□ magnifications were used. To estimate the repeatability of measurements (Table 1), all traits were photographed twice in all cases. The measures of morphological data of the digital images of animals were later obtained using CorelDraw 11 (for all linear measures) and Canvas 8 (for antennules) digital image analysis software. All trait measurements were made to the nearest 0.001 mm. For the statistical analysis of bilateral traits (antennule and furca), the mean of the left and right side measurements were used.

Table 1. Traits included in the measurements and their repeatability estimate as the proportion of the total variations attributed to between-individual variation.

Traits	R^2
Maternal	
Body length	0.986
Prosome width	0.975
Genital somite width at spin	0.974
Right antennule	0.989
Left antennule	0.989
Right furca	0.978
Left furca	0.979
Nauplii	
Length	0.988
Width	0.981

Statistical Analysis

Female size was not different between the three sampling stations in the pelagic zone of the lake ($F_{1,\,100} = 0.231$, $P = 0.63$) and hence, sampling stations were not accounted for in the subsequent analysis. Measures of morphological traits and fitness components used as dependent variables were tested for normality, and measures of proportions, such as proportion of unfertilized eggs, proportion hatching and surviving, were arcsine transformed to meet the assumption of normality. All morphometric measurements were standardized to have a mean of zero and a unit variance before the analyses were carried out. All statistical analyses were performed with StatView 5. The data were analyzed using general linear models. The estimators of reproductive output were compared between the group using ANCOVA procedures with groups as fixed factor and female size as a covariate, as the latter strongly influences egg number in *E. graciloides* (paper I & II). Additionally, simple and multiple logistic regression analyses of female mating rate (single mated, double mated) were run on the different measures of female body size components, employing separate analysis to each trait and measures of all traits together. Significances of the parameter estimates (for both simple and multiple logistic regressions) and for the model (for the multiple logistic regression) were determined by likelihood ratio chi-square tests.

RESULTS

Nearly half (55%, excluding dead) of the experimental females did not produce egg sacs during the 3 day time period after separation. Female size was not different between the two groups ($t = 0.551$, $df = 100$, $P = 0.58$) and hence, it did not influence the probability of producing egg sacs. Moreover, logistic regression analysis, using mating rate as a categorical variable and female size as a covariate, revealed no significant difference between treatments (log likelihood = -70.2, $df = 2$, $X^2 = 0.53$, $P = 0.77$). Additionally, during the entire period of the experiment, neither the experimental nor the control females produced another clutch despite the constant presence of a male. This indicates that routine observation and handling had not dramatically altered the reproductive capacity of experimental animals. Additionally, when comparing the survival of nauplii that were photographed with nauplii that were not photographed, no significant difference were found ($t = -1.482$, $df = 55$, $P = 0.14$), suggesting that the light of the compound microscope did not affect the survival of the former nauplii.

The 62 clutches produced were investigated for clutch size, number of fertilized eggs, proportion of unfertilized eggs, proportion of individuals hatching and surviving up to nauplli two (N2). The mean and standard deviations of these variables of female reproductive fitness are shown in Table 2. Although it would appear that clutch size is higher for double-mated females, female body length explained a significant amount of the variation in clutch size (standardized regression coefficient ± SE = 0.539 ± 0.174; $P < 0.0001$). Thus, the data were analyzed using an analysis of covariance, using body length as the covariate. This showed that body size had a significant effect on egg production ($F_{1,50} = 15,8$, $P < 0.001$) but there was no effect of mating rate ($F_{1,50} = 3439$, $P = 0.98$).

Table 2. Mean and standard deviation of morphological traits and estimators of reproductive success.

Traits	Single mated	Double mated	Total
Maternal			
Morphological traits (mm)			
Body length	0.837 (0.026)	0.858 (0.032)	0.846 (0.030)
Prosome width	0.287 (0.012)	0.303 (0.019)	0.294 (0.017)
Genital somite width at spin	0.112 (0.013)	0.115 (0.019)	0.113 (0.016)
Antennules	1.206 (0.037)	1.204 (0.049)	1.205 (0.041)
Furca	0.064 (0.003)	0.068 (0.004)	0.066 (0.004)
Fitness measures			
Clutch size	6.25 (1.296)	7.231 (1.632)	6.661 (1.514)
Fertilized clutch size	5.917 (1.857)	7.077 (1.998)	6.403 (1.987)
Proportion unfertilized eggs*	0.059 (0.215)	0.030 (0.157)	0.047 (0.192)
Days for 50% hatching	25.787 (2.522)	25.000 (2.611)	25.464 (2.565)
Proportion hatched**	0.826 (0.310)	0.930 (0.118)	0.870 (0.252)
Proportion survived up to N2**	0.603 (0.348)	0.551 (0.285)	0.591 (0.315)
Longevity of females in days***	35.042 (8.483)	26.70 (7.875)	31.250 (9.140)
Nauplii (N1)			
Length	0.176 (0.005)	0.176 (0.007)	0.176 (0.006)
Width	0.108 (0.016)	0.107 (0.017)	0.108 (0.016)

*The proportion unfertilized eggs is the proportion of eggs calculated from the total clutch.

**The proportion that hatched and survived up to N2 is calculated from the fertilized clutch.

***Longevity of a female is the number of days from the start of the experiment until the death of the female.

Mating rate, i.e., the number of spermatophores (Table 2) had no detectable effect on the number of fertilized eggs (ANCOVA with female size as a covariate: $F_{1,\ 50}$ = 3382, P = 0.94 for mating rate and $F_{1,\ 50}$ = 8.77, P = 0.005 for female body size), on the proportion of eggs hatched (t = -1.07, df = 59, P = 0.29) or on the proportion of larvae surviving up to N2 (t = 0.65, df = 58, P = 0.52). Neither were proportions of unfertilized eggs (t = 0.66, df = 60, P = 0.52) nor offspring size (t = 0.33, df = 52, P = 0.74 for length and t = 0.045, df = 52, P = 0.96 for width of N1) significantly different when comparing clutch produced by single mated and double mated females. In all analyses where female size was used as a covariate, none of the interactions were significant. Therefore, there is no evidence to suggest that multiple mating enhances female fitness.

Moreover, both simple and logistic regression of morphological data using mating rate as a binary dependent variable (1 = single mated, 0 = double mated) suggested that female size (body length and width), antennules and furca length influenced relative mating rate (Table 3). In univariate analyses, mating rate was positively related to body length, prosome width and furca length. However, when analyzing all measured traits in a multivariate model, the effect of body size was non-significant, antennule lengths were negatively related, while those of furca length was positively related to spermatophore numbers. These analyses suggest that variation in the size of antennules and furca among females were the most important traits explaining the variation in female mating rate.

Table 3. Standardized univariable and multivariable regression coefficients for measures of female body size as predictors of female mating rate. Significances of the parameter estimates and of the model were tested by ordinary least squares regression analyses. SE = Standard Errior.

Traits	Univariate (±SE)	Multivariate (±SE)
Body Length	**0.753 (0.315)***	0.411 (0.672)
Prosome width	**1.149 (0.391)****	1.039 (0.708)
Genital somite width	0.228 (0.285)	0.028 (0.419)
Antennules	-0.071(0.283)	**-1.137 (0.605)***
Furca	**1.420 (0.455)****	**1.295 (0.515)***
		LR = - 21,667***
		Df =5

*$P<0.05$, **$P<0.01$, ***$P<0.001$

DISCUSSION

Female mating rate, measured as number of spermatophores, had no effects on several components of female fitness. Double mated females did not differ from single mated females in fitness measures, that is, in egg-sac production, clutch size, fertility, hatching rate, body size and survival of early stage offspring. However, body size of females explained much of the variation in clutch size among females regardless of whether females had been mated once or twice. So, why then do female *E. graciloides* mate multiply when there is no significant benefit from the behavior?

At the most basic level, female fitness is dependent on fertilization of the eggs she produces (Hosken & Stockley, 2003). Multiple mating might therefore function as fertilization insurance, simply by making ample amount of sperm available (Arnqvist & Nilsson, 2000; Hosken & Stockley, 2003). Fertility benefits of this nature may occur when females fail to store sufficient sperm (Hosken & Stockley, 2003). Yet, as females of *Eudiaptomus* lack organs to store sperm (Cuoc *et al.*, 1989a, b), this hypothesis may not apply. Another possibility for fertility benefits from polyandry to occur is when male ejaculates are insufficient to fertilize the whole clutch (Hosken & Stockley, 2003; reference therein). However, single mated females seem not sperm limited, as their fertility and hatching rate did not differ from that of double mated females. Therefore, fertility insurance is unlikely to be the primary mechanism promoting polyandry in female *E. graciloides.*

Hypotheses of polyandry based on direct benefits to females also predicts that females' fitness should be elevated with the number of matings as this provides females with more resources (Thornhill & Alcock, 1983; Arnqvist & Nilsson, 2000; Stjernholm & Karlsson, 2000; Hosken & Stockley, 2003). Such benefits are suggested to occur in mating systems where males transfer spermatophores that contains both sperm and nutritious accessory gland product, which females, in some species, use to increase their longevity and reproductive output (Pitnick *et al.,* 1997). In diaptomid copepods, males transfer spermatophores that contain both spermatozoa and associated seminal secretions during mating (Blades &Youngbluth. 1980; Dussart & Defaye. 1995). However, to my knowledge, there is no information as to whether the secretions function as nutrients or not. The lack of association between spermatophore numbers and clutch size, after controlling for the effect of female body size, suggests that the accessory seminal secretions of spermatophores are unlikely to have a nutritional function. Therefore, reproduction in *E. graciloides* does not seem to be constrained by substances derived from spermatophores that increase the number of eggs produced.

Moreover, the accessory substances of ejaculates are also known to have numerous complex effects on female reproductive performance. One of these is stimulation of egg production. Additionally, the act of mating itself have been suggested to have positive effects on female fitness as it may directly trigger female egg production (Thornhill & Alcock, 1983; Arnqvist & Nilsson, 2000; Hosken & Stockley, 2003. According to these hypotheses, double-mated females should have higher probabilities of egg production than single-mated females. However, I found no significant difference between the two groups of females in egg production.

A number of indirect genetic benefits have also been proposed for the evolution of polyandry (e.g., Thornhill & Alcock, 1983; Simmons, 1987; Olsson, *et al.,* 1996; Jennions & Petrie, 2000). Under these scenarios, female fitness should be elevated with the number of matings as a result of increased offspring viability or reproductive success. Such benefits could be expressed at any stage in life and could take many forms, for example, in embryo viability as indicated by egg hatching success, in offspring survival to adulthood, and in subsequent reproductive successes. These, and numerous other fitness parameters might be positively associated with multiple matings, and hence the indirect benefits from multiple matings might be notoriously difficult to reject. As I recorded only the more immediate measures of reproductive success, namely the size and the survival of offspring during the early stages, I may not be able to reject indirect benefits. However, as survival to adulthood is a function of offspring size (e.g., Cooney & Gehrs, 1980; Wyngaard, 1986; Guisande *et al.*, 1996), these measures could at least be indicative of mother's reproductive success. Therefore, although every possible benefit of polyandry to female *E. graciloides* from Takvan is impossible to rule out, the obvious and immediate benefits were not evident in this study.

Given that mating is a costly activity (Wilcox, 1984; Bailey *et al.*, 1993; Cordts & Parttridge, 1996; Clutton-Brock & Langley, 1997; Watson *et al.*, 1998; Arnqvist, 1989; Fairbairn, 1993; Magnhagen, 1991; Rowe, 1994; Ward, 198; Daly, 1978; Hurst *et al.* 1995) from which females receive no appreciable benefit, why then do females mate multiply? A hypothesis more consistent with the results of this and previous studies (paper II & III) is that females are manipulated by males into mating at a rate beyond their optimum (e.g., Parker, 1979; Rowe *et al.,* 1994; Holland & Rice, 1998; Arnqvist & Nilsson 2000). As superfluous mating is costly to females, they may attempt to escape male harassment. (Parker, 1979; Arnqvist & Nilsson 2000; Gavrilets *et al.,* 2001). However, this avoidance tactic, which involves antennules, are particularly costly in terms of energy expenditure (Mauchline, 1998), and in this population energy expenditure largely rely on stored energy that is also used for egg-production (Paper I). Therefore, both multiple mating and escape from mating, in the face of male harassment, are costly for females. Under such condition, females simply may make the "best of a bad job” by going for the less costly option “convenience polyandry” (Thornhill & Alcock 1983; Arnqvist, 1989, 1992; Rowe, 1992; Rowe *et al.,* 1994; Watson *et al.*, 1998; Weigensberg & Fairbairn, 1996). According to this hypothesis, females on average are predicted to switch between resisting and accepting superfluous mating at a certain harassment threshold that renders the least costly behavior the optimal option. That is, when the costs of mating to an average female are extremely high relative to costs of resistance, the model predicts little or no multiple mating. On the other hand, when the costs of mating to an average female are sufficiently small, then there will be a threshold were switching to submission to male coercion is optimal. Moreover, the perceived male harassment rate may vary among females due to their variability in behavioural and morphological traits. Therefore, under any given average harassment rate, such variation will generate variation among females in mating propensity and mating rate (Watson *et al.*, 1998). For example, for a given body size, a female with relatively longer antennules may have an advantage and hence, require less energy to reject a male suitor than a female with shorter antennules. Under such condition, the least costly option for the former female might be to resist superfluous mating while for the latter to accept mating. Another important female trait related to reproductive behaviour is caudal furca, as it is a region of importance for male pre-copulatory clasping behaviours. For a given body size, a female with relatively longer furca might have less mechanical advantage to dissociate the male grip than a female with a shorter furca, and an adaptive female behavior in this case could be to accept multiple matings. These mechanisms, rather than benefits to females from multiple mating, could explain the observed intermediate level of multiple mating under natural condition in female *E. graciloides* from Takvatn Lake.

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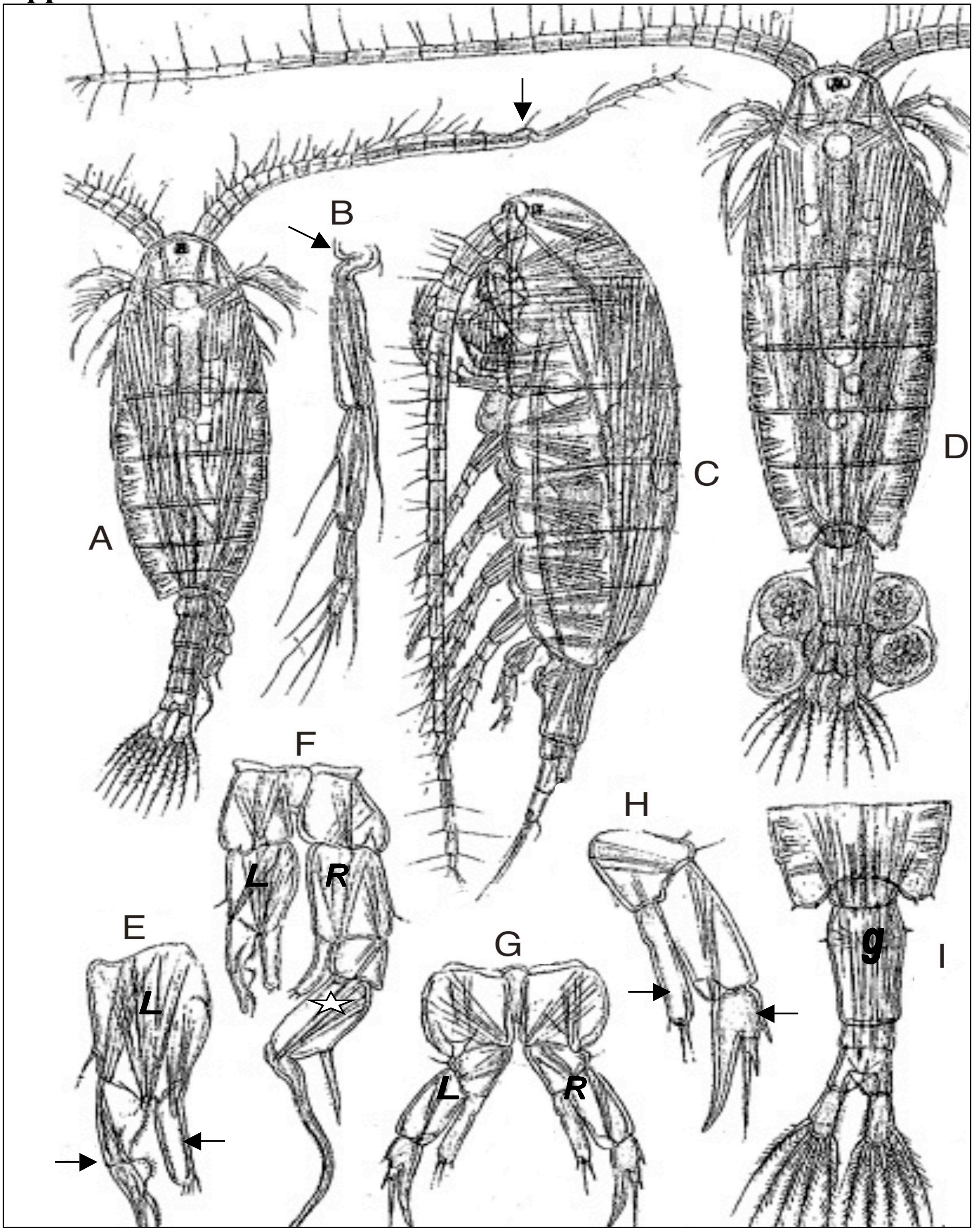
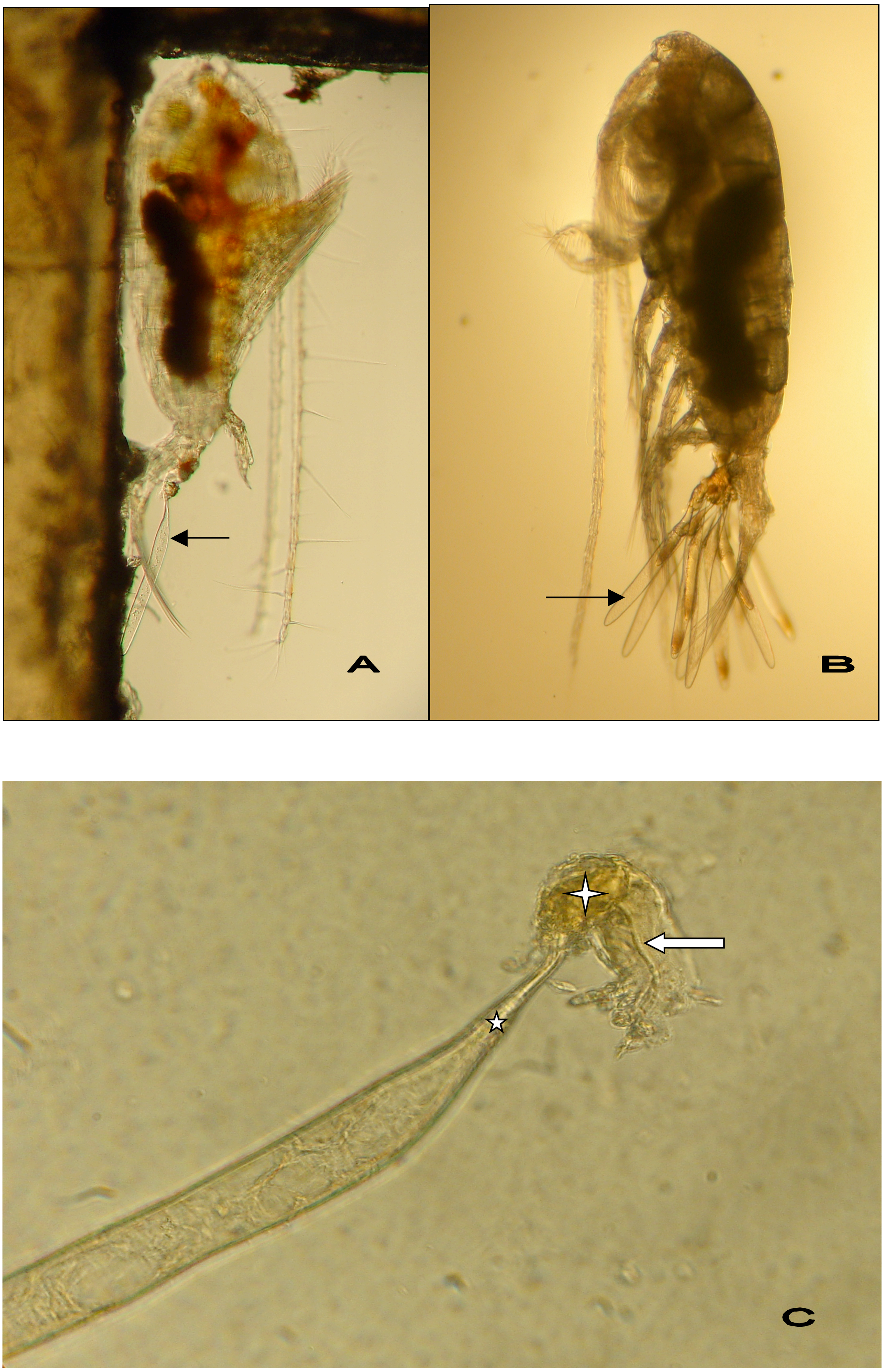
Appendixes



Appendix 1. *Eudiaptomus graciloides*. Diagrammatic illustration of adult male and female and their secondary sexual characteristics. As: **A**. Male and its geniculated right antennule. **B**. Closer view of male geniculated right antennule and its hinge (arrow). **C**. Female with out egg and with out spermatophores, side view. **D**. Female with attached egg sac, top view. **E – F**. Male fifth leg. **E**. Closer view of left leg (*L*) showing the details of exopod (right arrow) and endopod (left arrow). **F**. Fifth pair of male legs, showing the large chela (5-point star) of right leg (*R*). **G**. Fifth pair of swimming legs of female. **H**. Closer view of female right leg, showing the details of exopod (left arrow) and endopod (right arrow). **I**. Female urosome, comprising of genital double somite (*g*), urosomites and ending with two furca with rami. Figures from Sars (1903). Drawing not to scale.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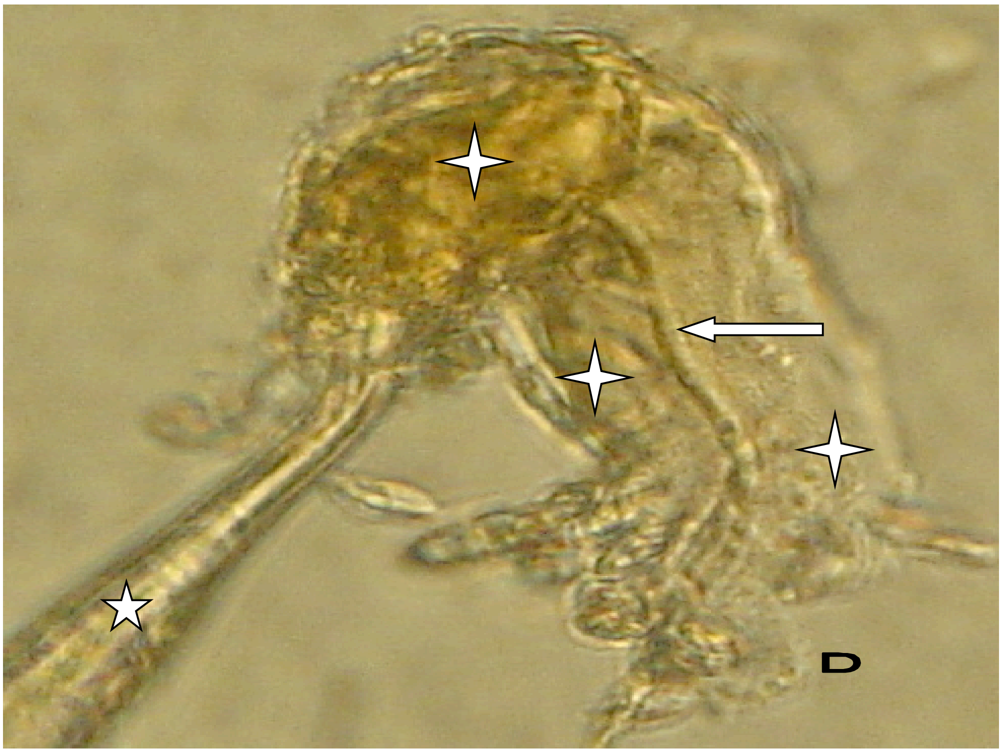
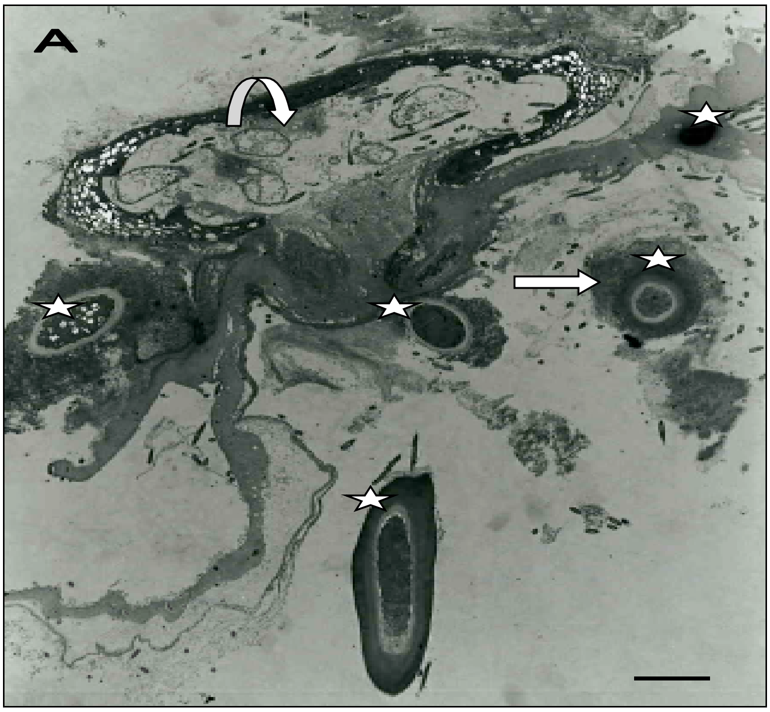
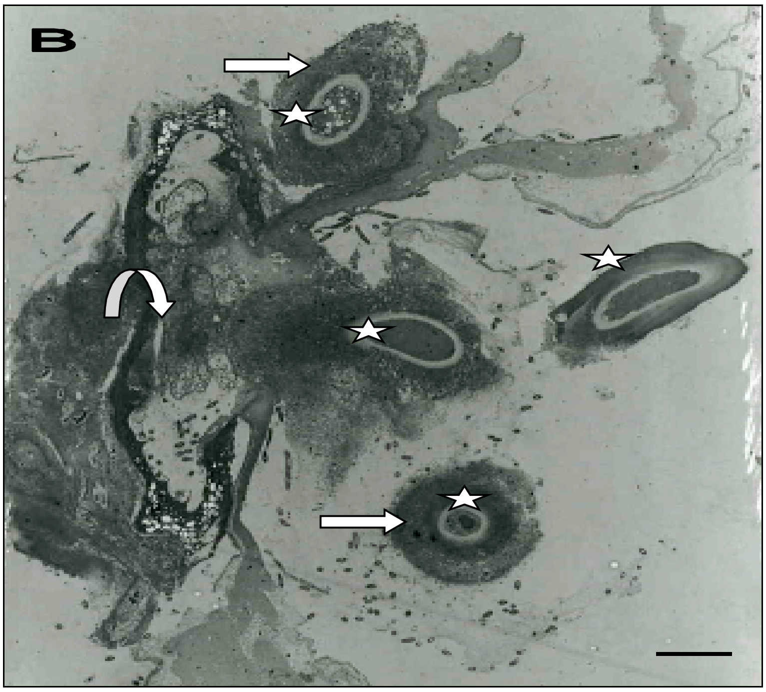


Appendix 2. Photographs of female *Eudiaptomus graciloides* with one (**A**) and nine (**B**) externally attached spermatophores around the genital area. **C.** Empty spermatophore with fertilization tube (arrow) detached with fine needles from the female genital area. **D**. Closer view of spermatophore neck (5-point star) with fertilization tube (arrow) and adhesive substance (4-point stars).

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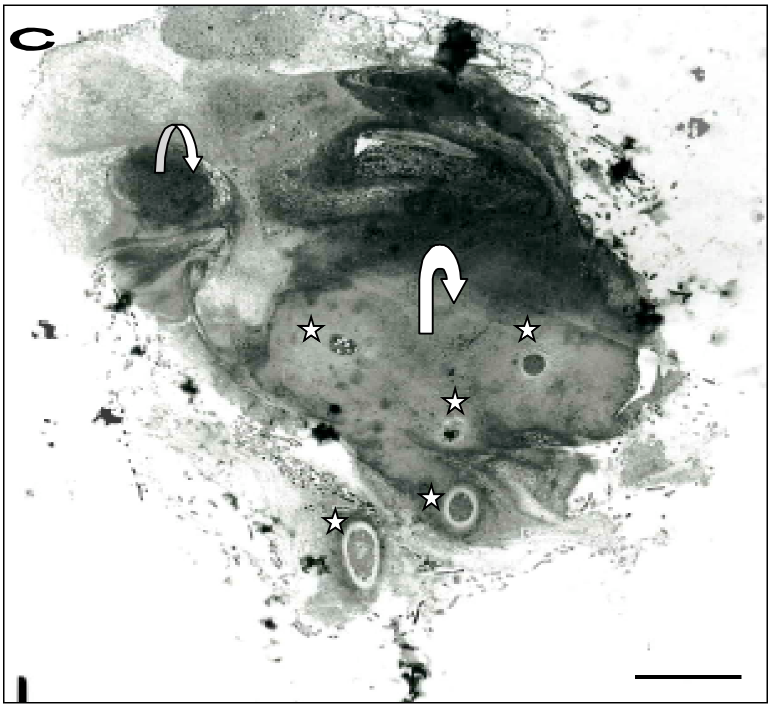
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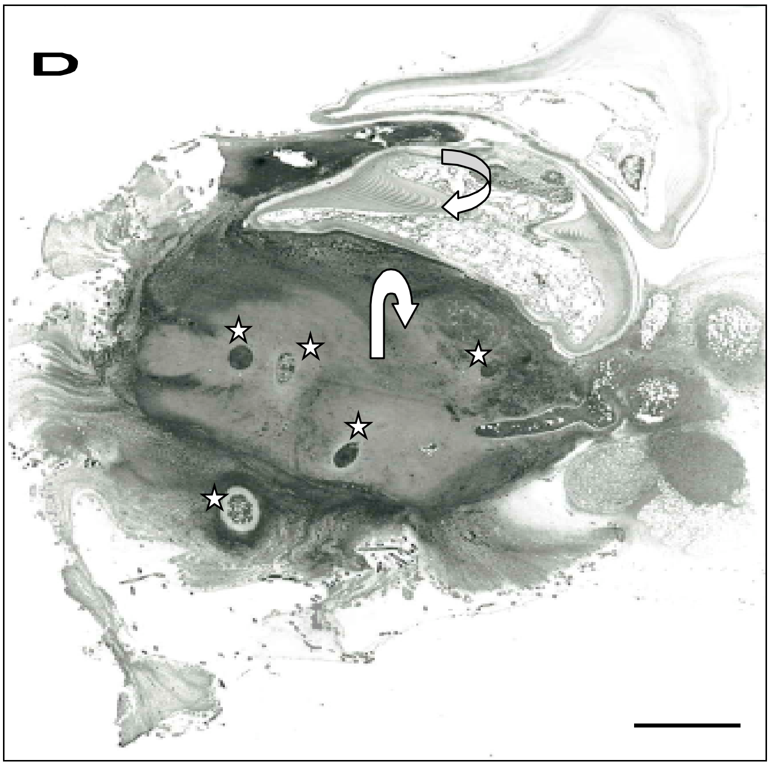

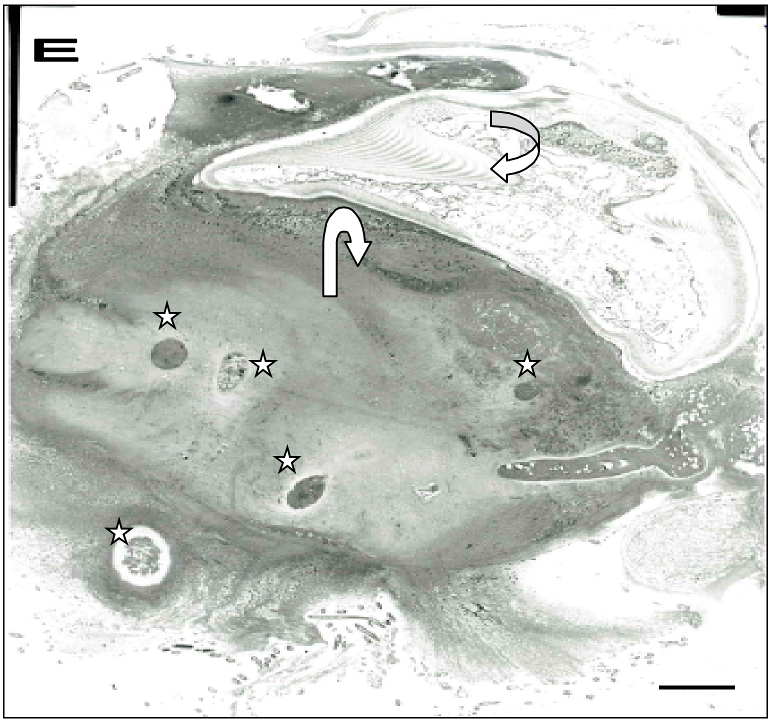

Appendix 3. Photomicrographs of serial sections from genital area of female *Eudiaptomus graciloides* with five externally attached spermatophores. Sections (**A**) starting from the ventral face, along the horizontal plan, of genital area up to the genital atrium (**E**). **A – B.** note the 5 spermatotphores (5-point stars) fixed on the genital area and note also adhesive substance surrounding the spermatophores (right arrow). Curved down arrow indicate the genital operculum position and a small part of it eliminated. **C – E.** Note the 5 spermatotphores (5-point stars) and note also the total elimination of genital operculum (curved down arrow). U-turn arrows indicate the genital atrium. Note spermatophores just at genital atrium. Note muscle of the genital operculum (curved left arrow, in **D** & **E**). Scale bars: **A**, **B** & E 7µm; **C** & **D** 10 µm.

ISBN: 978-82-92461-80-8