Reproductive behaviour and sex trait allocations in an external fertilizer – the Charr (*Salvelinus alpinus*)

Lars Figenschou

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UNIVERSITY OF TROMSØ
Faculty of Biosciences, Fisheries and Economics
Department of Arctic and Marine Biology
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Contents:

1st supervisor:

Professor Ivar Folstad

Faculty of Bioscience, Fishery and Economy

University of Tromsø, Norway

2nd supervisor:

Dr. Sveinn-Are Hanssen

The Norwegian Institute for Nature Research

Hjalmar Johansens gate 14, Tromsø, Norway

Adjudicating committee:

1st opponent: Dr. Iain Barber, University of Leicester, England

2nd opponent: Dr. Bård-Gunnar Stokke, NTNU, Trondheim, Norway

Head of administration: Dr. Kari Anne Bråthen, University of Tromsø, Norway

1. PREFACE

Together with physical challenges, evolutionary psychology is my favorite leisure activity and discussion topic. I have “always” been fascinated by behavior - and in particular human behavior. In daily life, differences in behaviour are easy to observe. Why does one individual behave in a particular way whereas others behave differently? The most sophisticated and “charming” behaviour is associated with the struggle of presenting ourselves in a favorable light during mate search. Especially if we have a crush on somebody, it is so funny to observe our strong tendency to underestimate bad aspect of ourselves. Accordingly, in the context of courtship, so many interesting questions emerge; why do we perceive someone as “more cute and pretty” than others, and why do some males apparently have an advantage over others in competition for access to females? From my teenage years I can remember that we (the boys/comrades), used to philosophize (that is, brag), in a friendly spirit, about our own flirting abilities. We talked a lot about what “tactic” we used last Friday night, and about the outcome/non-outcome. However, which “tactic” we used to approach our mate was very dependent on the mate. Thus, there was not, at least in the late 70`s and early 80`s, one type of flirting behaviour that fitted all girls….☺

In 1997-1998, I met “serious” evolutionary thinking for the first time through a course in “Evolutionary Parasitology”. The course and the contemporary discussions with Ivar, whom is my current **super**visor, revolutionized the way I viewed life, at that time - and today. Ivar`s explanatory, informative and interesting, more or less “private” lectures, in all kinds of evolutionary topics, enlarged my growing interests. Consequently, my interest in behaviour has inevitably come to focus upon evolutionary and functional contexts, where the gene is the unit of selection. Behaviour associated with reproduction, in particular, becomes really exciting and fascinating when we remember that the organism itself is only a vehicle for self replicating molecules (genes). In an e-mail the other day, Ivar expressed this concept so well when he quoted the author J.C. Avice:

"Gametes can be metaphorically interpreted as intergenerational lifeboats for genes that must flee somatic ships, all of which are guaranteed eventually to sink"

Evolutionary biology is so fundamental and so basic to understanding the biology of organisms. It contains the biology of what makes us who we are, the biology of individual differences and the biology of our behaviour. Where do, for example, our most damaging, most frightening and inappropriate behaviours come from? I mean, from the bottom of my heart (is there, however, any bottom?), that everybody; every child and adult, should be forced to study subjects of evolutionary biology. Why? Because it is "*unfortunately still some reliquaries, that think that we, humans, are not exactly the same as all other organisms when it comes to the biology of our behaviour"* (quote from Robert Sapolsky, professor of neurobiology at Stanford University - from a lecture-series in human behaviour). Human mental function is not unique - and the way to understand human minds is by invoking processes found in minds of other animals. All animals, including humans, will necessarily behave in particular ways to maximize the genetically contribution to the generations ahead. However, when the evolutionary basis of human behaviour (and also many facets of human culture) is demonstrated, the approach is being called Darwinian or evolutionary psychology.

It is no surprise that Charles Darwin's masterpiece, "On the Origin of Species", shook society to its core on publication in 1859. Darwin was aware of it at the time; however, "*he would surely have raised a disbelieving eyebrow, that the controversy is still raging a century and a half later*" (Dawkins 2009). It is very sad that we, in 2010, still have to spend time convincing, for example, creationists and other (sorry for the expression) "mumbo-jumbo" groups, that natural and sexual selection are not just theories, but facts. The watchmaker is blind. Incredibly, world leaders still promote superstition, stories and unthinking acceptance of dogma - over scientific evidence. This is very strange, as absolutely all current and relevant knowledge, including loads and loads of genetic empirical evidence, says that all living organisms carry genes that have a common origin. Each sexually produced individual (however not identical twins) represents a unique combination of these recipes of life, affected by re-combinations of genetic material from two parents, and randomly occurring errors; mutations. Life, as an endless loop of reproduction, evolves continuously by selecting successful gene combinations, and thus their carriers, the individuals - at the sacrifice of less successful gene combinations and individuals.

The first main objective for this thesis was to find out whether male Arctic charr (*Salvelinus alpinus*) sort sperm by immunological coating, and if so, if there was a relationship between the degree of coating and the intensity of sperm competition. According to the famous “male sperm control hypothesis” from Ivar, increased sperm competition should lead to increased mutation rates and, thus, a possible measurable fragmentation in sperm DNA. The importance of these goals has, however, fortunately gradually faded due to various reasons. That is, the original aims and main goals in this thesis had a strange tendency to change during my PhD period; mostly caused by fear. For example our common fear, when we discovered that all of us (Ivar, Geir, Raine and me) were at the starting line of being founders of a whole new field in biology, namely the field of *“Evolutionary Behavioural Pipettothology”*! Especially I feared the consequences of its existence and breakthroughs…..! Thus, an agreement about emphasizing other aspects and topics of reproductive behaviour and sexual selection in Arctic charr was made. Honestly spoken; as we were not able to recognize any associations between the candidate’s brain neurons and synapse-interactions, on the one hand, and a profound understanding of micro or cell biology, and proximate mechanisms related to immunity, on the other, this thesis luckily ended up being something else.

2. ACKNOWLEDGEMENTS

There have been many contributors to this thesis. However, this thesis would never (in all my life, cross my heart!) see any fragment of light of day, if it wasn’t for two people; Ivar Folstad and Geir Rudolfsen. Therefore, in deference, I would like to thank you two, in particular. Firstly, my 1st **super**visor, my worst (and, therefore, best!) critic and reviewer, my discussion and squash- partner, easily provoked to laughter, pathfinder and guide in so many aspects of life, my life-buoy, Ivar Folstad. There have been times, Ivar, where I have been close to letting you down…. However, with amazing rapidity, your inspiring manners and encouraging talks always lifted and raised me again. Thank you so much for that (in addition to everything else, Ivar). Accordingly, my debt of gratitude to you (and Karianne as well) is enormous. So, for the future, you must promise me, that you will give me some opportunities for reciprocity. Secondly, I stand in awe to my best colleague, my private psycho-therapist, number crushing mill, study design expert and big mouthed friend from Trøndelag, Geir Rudolfsen. You are, both professionally and personally, the best a simple, common “lad” from the inner part of Troms can expect to meet. Thanks, Geir; for all your help, your pleasant and funny nature and thus for all the fun you have brought into my life. The daily life at the office will never be the same….. The contribution from these two guys to the six papers presented here has been, not only substantial, but totally invaluable. I do not dare to think about a solar system without you two guys. So, thank you both.

Moreover, I send warm thanks to my 2nd supervisor Sveinn Are Hanssen for all positive acknowledgement and constructive comments, his clear head and optimistic views during these years. Especially, when he and Philip A. Skau tried to teach me immunological laboratory techniques!! During my abroad stay in Joensuu, Finland, my good friend and college Raine Kortet played an essential role in the finishing stage. Thanks Raine - for everything! Additionally, Jukka Kekalainen and Matti Janhunen deserve honor as they also

contributed in so many ways (e.g., all the laughter-provoking jokes in Jukka`s sauna - which by the way had the best “löyly”). During fieldwork, but also during the daily aggregations at the office, there have been many helping heads and thoughts (in addition to so many tear wet and liberating laughs). Therefore I would like to say thanks to Philip A. Skau, Ståle Liljedal, Frode Skarstein, Nigel Yoccoz, Davnah Urbach, Thomas Haugland, Vidar Sørum, Jonas Sjøstrand, Bjørnar Strøm, Jonathan Vaz Serrano, Sissel Kaino, Bjørn Norman, John-Andre Henden, Rolf Ims, Marie Figenschou and Siw Turid Killengren, in addition to all of you, which I, at the moment, have forgotten. Anders Pape Møller helped me a lot with his detailed comments in the final stages of writing Papers V and VI. Thanks. Moreover, I would like to thank all the staff, and especially Anne Høydal, at the Institute of Biology, for their always encouraging support, and all my friends not associated to the institute - for letting me derive some, however not biological, advantages from you. Additionally, on the sideline, LAR has contributed with a personally “crutch”, which has helped me to concentrate on the important things – e.g., biology. Finally, Jenny Stien deserves my gratitude as she helped me with the English language in the scribbling you now hold in your hands.

Even though there have been periods when my family, included my departed mother, may have been overwhelmed with my personally “evolutionary interpretations” of daily life situations, they have always supported me - by always saying and showing that they loved me. So, all members of Fjell-Figen, Ris-Figen, Ny-Figen and Ulve-Figen have really deserved my reciprocity and gratitude. Last but not least, I would like to thank my wife, my own (pregnant) pyrotechnist from Berlevåg, my best friend, my social-life organizer and personal manager, the person in charge of the most, my dearest loving Anne Lise. Because of you; I have never been happier.

3. LIST OF PAPERS

Paper I: Lek fidelity of male Arctic charr. 2004. Figenschou, L., Folstad, I. & Liljedal, S. Canadian Journal of Zoology, 82: 1278-1284.

Paper II: Synchrony of gamete release and sperm competition in Arctic charr (*Salvelinus alpinus*). 2010. Sørum, V., Figenschou, L., Rudolfsen, G. & Folstad, I. Submitted to Animal Behaviour.

Paper III: Rapid adjustments of sperm characteristic in relation to social status. 2006. Rudolfsen, G., Figenschou, L., Folstad, I., Tveiten, H. & Figenschou, M. Proceedings of the Royal Society of London, Series B-Biological Sciences, 273: 325-332.

Paper IV: Female Arctic charr do not show apparent benefits from exposing their eggs to sperm from dominant males. 2007. Figenschou, L., Rudolfsen, G., & Folstad, I. Journal of Fish Biology, 71, 284–289

Paper V: Parasite intensities and male social status as modifiers of sperm production and sperm swimming speed in the Arctic charr (*Salvelinus alpinus*). 2010. Figenschou, L., Skau, P.A., Folstad, I., Rudolfsen, G., Hanssen, S.A., Kortet, R., Killie, J.E., Oskam, I. & Strand, H. Manuscript 2010

Paper VI: Immune activation leads to reproductive compensation in Arctic charr (*Salvelinus alpinus*). 2010. Figenschou, L., Skau, P.A., Folstad, I., Rudolfsen, G., Hanssen, S.A., Kortet, R., Killie, J.E. & Strand, H.
Manuscript 2010

4. GENERAL INTRODUCTION

”*No sensible engineer would ever propose such a process as sex when asked to design a reproduction machine*” (Sterns and Hoekstra, 2000). Sexual reproduction reduce the efficiency of gene transmission by up to 50%, disrupt favorable gene combinations, spread disease, and is energetically expensive (Wilson & Sherman, 2010). So, why do we observe asexuality in less than 1% of the animal species? Asexual species is distributed scattered, at the tips, of phylogenetic trees. This suggests that abandoning sex condemns a clade to extinction before it can radiate sufficiently to achieve high taxonomic rank (Wilson & Sherman, 2010). Moreover, according to the “Red Queen hypothesis” (Bell, 1982), asexuality is rapidly extinguished by relentlessly coevolving parasites and pathogens. In this context, behaviour associated to sexuality is the origin and source to the great and successful phylum’s.

Evolution, stripped to its barest essential, is nothing more than the temporal changes in the genetic composition of populations. Thus, this thesis is about the most important topic that exists; an evolutionary perspective on sexual behaviour. The relative ease of observing Arctic charr (*Salvelinus alpinus*) in natural populations (own observation), combined with the polyandrous lek-like mating system (Andersson, 1994; Høglund & Alatalo, 1995), make them excellent subjects for studying reproductive behaviour. Our aims have been to better understand the main evolutionary and ultimate aspects of sexual reproduction. However, the papers presented in this thesis also illuminate several of the principal proximate mechanisms involved. To demonstrate both the proximate causes, in addition to the functional and adaptive significance of various behaviours (see Tinbergen, 1963), we have made both observational and experimental studies (see Papers I – VI). We have examined traits related to male social status, sperm quality, parasite intensities, immunity, and competition for mates and fertilizations, i.e., sperm competition. More or less in sequence, we have examined:

I. charrs movement patterns associated to their lek-like mating system
II. male-male and male-female interactions at the spawning ground and the degree of (a)synchrony in gamete release
III. male adjustments of ejaculates in relation to intra-sexual competition and social status
IV. possible indirect benefits of certain mate choice
V. what modifies ejaculate quality
VI. and how an immune challenge may affect reproductive investments

Certainly, all organisms struggle to optimize their own reproductive situations. However, natural and sexual selection have designed a complex set of behavioural adaptations that coordinate and constrain individual's reproductive activity. Yet, the end products of cycles of reproductive activity are fertile offspring (i.e., vehicles), which, in turn, will replicate and thus perpetuate the self-replicating molecules (i.e., genes).

Lek-like mating system and Arctic charr

Arctic charr show no parental care, and the mating system resembles the non-resource based lek-mating as defined by Høglund & Alatalo (1995). That is mainly, (*i*): females obtain nothing more than genes from males and (*ii*) males cannot monopolize all resources to gain spawnings. In addition, the mate-search costs for females may be low and there is a relative high frequency of spawnings including several males (see Paper II), which indicate that sperm competition may be an important selective force (Taborsky, 1998). For several reasons, lek-like animals are of special interest in sexual selection theory (Andersson, 1994). As females receive nothing more from males than their sperm, such species may offer better prospects than others for identifying male characteristics by which females choose mates. As mating success often varies greatly among males on the lek, and as males are free from constraints imposed by parental behaviour, sexual selection and dimorphism are expected to be stronger in lek-like species than in other species (Darwin 1871; Payne 1984). However, even if male mating success on a lek is highly skewed in a given year, the lifetime variance in reproductive success among males may be small (Clutton-Brock 1983, 1988) as mating success is often strongly age/size dependent (Kruijt and de Vos, 1988).

The ‘lek paradox’ concept was introduced to describe mating systems in which there seems to be no material or other direct benefits of female mate choice (Borgia, 1979). The paradox is; why are females continuing to choose males based on genetic benefits for the offspring, given that directional female choice depletes genetic variation in male traits, thus precluding female choice from resulting in genetic benefits? Consequently, how can a trait value increase in a runaway fashion if, after a few generations, the variation is reduced to zero? It is not crystal-clear whether the paradox commonly exists (Kotiaho et al., 2008), yet, what is crystal-clear, is that the main factor determining the genetic contribution to the next generation in lek-like breeding systems is success in intra-sexual competition, (reviewed by Andersson, 1994; Andersson & Iwasa, 1996; Høglund & Alatalo 1995).

Sexual selection

“When the males and the females of any animal have the same general habits of life, but differ in structure, colour, or ornament, such differences have been mainly caused by sexual selection.” (Darwin, 1859)

An animal's fitness hinges on finding a suitable mate. In the majority of animal signalling systems, males are the advertising, and females the choosing sex (Holveck & Riebel, 2010). Gametes of two different sizes, or anisogamy, underlie the evolution of sex differences, in both behaviour and morphology. Contemporary and subsequent sexual selection has evolved from differences in reproductive success caused by competition over mates (Darwin, 1871; reviewed in Andersson, 1994). Sexual selection is presumed to give rise to selection pressures that favor large size, extravagant traits (i.e., often used for fighting) and endurance in struggles. There is considerable experimental and comparative evidence supporting this mechanism (reviewed in Andersson, 1994; Andersson & Iwasa, 1996; Birkhead & Møller, 1998).

There are two main processes leading to sexual selection, that is, male-male competition and choice of partners by individuals of the choosy sex, usually females. Many studies assume a tight link between female preference and male quality (Andersson, 1994; Jennions & Petrie, 1997), which is not accidental, as strong fitness-linked preferences will be selected for. Accordingly, it is suggested that the entire sexual selection process can be viewed as involving a continuous adjustment of female mate choice (Møller, 1992). In Arctic charr, the variation in number of mates within both males and females suggest that sexual selection operates in both sexes (Skarstein & Folstad, 1996).

The maintaining mechanisms

Fisherian self-reinforcing theory (Fisher, 1930) and Handicap theory (Zahavi, 1975, 1977) may both explain extravagant male ornamentation. Fisher's theory is based on females "good taste" and rest on the assumption that there is heritable variation in male secondary sexual trait, e.g., size or coloration. Female preference is thus genetically coupled to male traits and becomes self-reinforcing such that certain female genotypes will preferentially mate with certain male genotypes. The two processes build on one another and result in elaborate and often dysfunctional (in terms of natural selection) appendages in males and is often referred to as runaway selection. Popularly spoken, the peacock's tail length need serve no other purpose than a simple fashion accessory to delight the senses of the opposite sex. However, the cost of the exaggerated expression of the male sexual trait will finally prevent further exaggeration of the trait creating equilibrium between cost and benefits and putting the runaway process to rest (Fisher, 1930).

The handicap theory is based on females "good senses" - and especially for genes. Zahavi (1975, 1977) suggested that females prefer males with elaborated sexual displays because they are handicaps and therefore act as reliable signal of a male's genetic quality. Thus, costly sexual ornaments demonstrate a male`s ability to survive and reproduce, in spite of the handicap, and if any of this ability is heritable, then the tendency to survive will be passed on to the offspring.

Subsequent to the latter, (Hamilton & Zuk, 1982) suggested that the resistance to parasites may be the important genetic quality revealed trough secondary sexually characters. By choosing a well ornamented male, a female tends to acquire for her offspring those resistant genes which are at the moment important against the predominant parasites. In concordance with the handicap principle (Zahavi, 1975, 1977), less infected males in good health, are able to produce more elaborate sex characters than more infected males because these characters are costly to produce. Both observational and experimental studies support this hypothesis (Clayton, 1990; Milinski & Bakker, 1990; Møller, 1988, 1990; Saino & Møller, 1996; Taskinen & Kortet, 2002; Zuk et al., 1990a; Zuk et al., 1990b; for review see Møller & Saino, 1994).

An extension of Hamilton and Zuk`s (1982) version of the handicap theory was Folstad & Karters (1992) "immunocompetence handicap hypothesis" (ICHH), which later has been proposed as one of the main "indicator mechanisms" of sexual selection (Andersson &

Simmons, 2006). According to ICHH, the expression of sexual selected traits may be constrained through a trade-off with immune function. That is, the immune system competes for resources with sexually selected ornaments. Thus females can obtain heritable resistance to disease for their offspring from those males who can afford to invest in large sexual displays. In the ICHH, the male sexual ornamentation is a handicapping, but honest signal of male quality, due to the high levels of immunosuppressive androgens needed to produce and maintain an effective expression of sexual ornamentation. This is most likely a consequence of either adaptive (Wedekind & Folstad, 1994) or non-adaptive immuno-modulative actions of sex hormones (Hillgarth & Wingfield, 1997). An extension of the ICHH suggests, finally, that immune defenses also compromise the ability of a male to produce high-quality ejaculates (Folstad & Skarstein, 1997).

Sperm competition and sperm production

Males struggle to reproduce, and in many species this struggle continues after copulation; at the gametic level. Sperm competition is a very potent force influencing almost every aspect of male and female sexuality (Birkhead & Møller, 1998) and occurs when the sperm released by one male reduces the fertilization success of another male. Sperm competition can lead to a wide range of behavioural, morphological and physiological adaption's that enhance the success of a male's sperm relative to a rival (Parker, 1970; Smith 1984) and it is now understood that males typically differ in their competitiveness during fertilization (Arnqvist & Danielsson, 1999; Gage et al., 1995). It is also argued that "vertebrate sperm competition often operates along the principles of a raffle, or lottery, in which males inseminating the most sperm have the highest probabilities of fertilizing ova" (Stockley et al., 1997). Moreover, sperm from the first male is believed to have precedence over sperm from males attending later in the same reproductive event. This suggestion is in contrasts to observations from birds and insects, where sperm precedence of the last-mating male has been documented for several species (Birkhead & Møller 1992; Gwynne, 1984). There is comparative empirical evidence for co-evolution of female reproductive biology and ejaculate characteristics in fish (Stockley et al., 1996) and, moreover, evidence for female choice of sperm provided by particular males in sperm competition situations (Bishop, 1996; Bishop et al., 1996; Olsson et al., 1996). This latter type of mechanism may also be present in Arctic charr (Urbach et al., 2005).

In fish, where sperm competition often has a major role in reproductive output (Taborsky, 1998), larger males are believed to have an advantage over smaller males because of greater sperm volume (Gjerde, 1984; Kazakov, 1981) and dominance (Ball & Parker, 1996; Fleming et al., 1996; Parker, 1993; Parker & Begon, 1993; Parker et al., 1990) with dominance allowing proximity to and synchrony with individual spawning female. Theoretical models predict that males mating in a more disfavored role, (i.e., subordinate males employing sneaker tactics) should invest more in their ejaculates than males mating in a favored role (Burness et al., 2004; Leach & Montgomerie, 2000; Liljedal & Folstad, 2003; Neff et al., 2003; Rudolfsen et al., 2006; Vladic & Jarvi, 2001). That is, males experiencing high sperm competition risk should invest more in sperm production than males experiencing low risk of sperm competition. There is now ample evidence that sperm production is costly (reviewed in (Wedell et al., 2002), thus males are expected to strategically allocate resources to sperm production according to mating opportunities (Gasparini et al., 2009). Moreover, in the context of life history theory, there are trade-offs between ejaculate investments and other life sustainable processes, such as defense against pathogens. Thus, it may pay males to differ their investments in sperm production (sperm quality and quantity) depending on age, social status and infection levels.

Male social status; context dependant reproductive tactics

Different solutions to maximize male fitness may result in alternative reproductive tactics between males (Taborsky et al., 2008). Alternative reproductive tactics are more likely to appear when there is a large asymmetry in competition for reproduction, leading to high variance in fitness between males (Wade et al., 2003). More specifically, it is expected that when few territorial males monopolize female access in addition to defending a territory against other territorial males, sneaker males will appear (Andersson, 1994; Wade et al., 2003). Sneaker`s reproductive tactics are based on nonaggressive behaviour rather than behaviour such as guarding, territory defence or weaponry (Gross, 1996; Wade et al., 2003). Such alternative behaviour is successful in terms of producing matings in order to explain its evolutionary maintenance (Brockmann & Taborsky, 2008). Consequently, variation among male reproductive tactics can be largely adaptive, and in dynamic, role-flexible species, males may shift tactic depending on context, adapting to the reproductive phenotype giving the highest genetic contribution to the next generation (see Taborsky, 2001). These status dependent shifts in reproductive tactics are common, especially in fish species (Taborsky, 2001) and outnumber fixed reproductive tactics (Gross, 1996).

Dominant male Arctic charr may allocate resources to investments in direct defense of mates and/or in courtship behaviour and secondary sexual signals of male quality. Subordinate male`s on the other hand, seem to invests more in sperm density and sperm velocity, and in the lek-like spawning system of charr, subordinates seems to exploit at least some of the reproductive investments of dominant males by darting swiftly into the spawning site to break the “monopolization” of mates by dominant males (own observations).

Life history and reproductive compensation

If it was possible to maximize all fitness-related traits in an organism, we may end up with a, so-called “Darwinian demon”, which would live forever and reproduce constantly. Yet, organisms must balance investments in different activities to allow them to maximize fitness in the environments they inhabit. Arctic charr therefore must also balance their reproductive effort between present and future reproduction, something which may have lead to fixed physiological trade-offs in charr`s life history traits. Trade-offs exist at many levels, and in reproductive situations many strategies and tactics exist to maximize own fitness. Fish are exceptional because of their unparalleled variability of reproductive patterns (Gross, 1996; Oliveira & Almada, 1998; Oliveira et al., 2001; Taborsky, 1998). Predictions from life-history theory, i.e., the “terminal investment hypothesis”, suggest that individuals should invest more in current reproductive output if the chance of surviving to reproduce again is low (Cluttonbrock, 1984; Williams, 1966). Thus, individuals investing too much in current reproduction may risk to be punished with, for example, a shorter life or fewer resources for later investments in reproduction.

Parental status and offspring fitness

One of the key assumptions in the theory of sexual selection is the heritability of traits associated to reproductive success (Andersson, 1994). Numerous studies have identified heritable traits associated to mating success and, in general, traits directly connected to fitness and reproductive success have lower heritabilities than traits less relevant to fitness (Sterns & Hoekstra, 2000). A summary of 1120 experimental estimates from Mousseau & Roff (1987) showed that mean heritability varies between the trait categories; life-history (0.262), physiology (0.330), behaviour (0.302) and morphology (0.461). Thus, paternal traits such as body size, courtship vocalizations, territoriality and male ornamentation are, in part, transferred between generations via parental genes. In charr, male social status is a plastic trait associated with mating behaviour. Although selection through female choice may acts on traits underlying increased social status (e.g., size), only a few studies have shown that successful fathers sire successful sons (see however Wedell & Tregenza, 1999).

Arctic charr and sexual behaviour

With the exception of paper VI, all studies in this thesis are from Lake Fjellfrøsvatn, situated at 69 ° N, northern Norway. The Arctic charr population in this lake has its spawning period in the autumn, and during the last two decades, we have discovered 4 spawning grounds in the lake. In the first part of September, males start to aggregate at these (lek) sites. The male-male interactions are vigorous before and during the arrival of sexually mature females and depending on the social context at the spawning ground, males may shift between dominant and subordinate reproductive roles. Arriving females, which seem to have certain spawning site preferences, are closely guarded by one of the larger, more aggressive and dominant males which aggressively chase and bite other males approaching the female (Fabricius & Gustavsson 1954; Sigurjonsdottir & Gunnarsson, 1989; own observations). Males court females by gliding alongside them while quivering with high frequency, low amplitude waves (Fabricius 1953; Sigurjonsdottir & Gunnarsson, 1989; own observations) and on rare occasions this courting behaviour leads to spawning and release of gametes (Fabricius 1953; own observations). Size differences between males may be large within a spawning population (Paper I) and in sperm competition spawning events, the nearby, subordinate males dart into the spawning site, which offers no protection against sneakers, and release their own milt (own observations). Moreover, in a moment of inattention from the guarding male, i.e., when the guarding male chases away other competitors, a subordinate male may stimulate the female to spawn without the presence of the guarding male. Sperm velocity may play a key role in fertilization (Liljedal et al., 2008; Paper IV) and charr males have the capacity to rapidly adjust own sperm velocity and density in response to hierarchical position (Paper III). The different male mating tactics in charr seem to be conditional and very plastic, with relative body size as the most important determinant of tactic choice (Sigurjonsdottir & Gunnarsson, 1989).

Immunity and sperm quality

Examining the individual's immune response to pathogens offers potential insights into mechanisms of life history trade-offs, sexual selection and parasite-mediated selection. The term immunocompetence is often used to refer to the ability of an individual’s immune system to resist and control pathogens or parasites. The energetic expenditure of producing and maintaining components of the immune system may have a major effect on condition, thus creating a link between immune system and condition dependent sexual advertisement (Wedekind, 1992; Wedekind & Folstad, 1994). Thus, immune function seems to be important in sexual selection and in the context of reproduction. For example, there is much empirical evidence that females often prefer parasite free males or males with low parasite burdens, and male ornamentation has been shown to be affected by parasites (Møller, 1990; Møller, 1991; Von Schantz et al., 1997; Zuk et al., 1990a). Moreover, host parasite co-evolution may explain why heritable fitness is maintained and hence the evolution of male ornamentation (Hamilton & Zuk, 1982). The costs of the evolving handicaps have to a large extent relied on energetic explanations. Yet, cost associated to both secondary sexually development and to primary sexually development, i.e., traits indicative of sperm quality may also be viewed from an immunological perspective (Folstad & Karter, 1992). Haploid sperm cells are targets of immunological attacks, and therefore influenced by a male`s ability to down-regulate immune responses during the production of ejaculates (Folstad & Skarstein, 1997). As the immunosuppression during spermatogenesis is most likely dependent on the level of infections, parasitic infections and pathogens may negatively affect sperm quality. Consequently, parasite resistant males will be at an advantage during sperm production and may produce better ejaculates than non-resistant males. As stated in the article “Is male germ-line control creating avenues for female choice”, this male trade-off between immune activity and sperm quality may be the common denominator which combines theories of sperm competition and the evolution of secondary sexual traits (see Folstad & Skarstein, 1997).

In internal fertilizers, anti sperm antibody is directed against several different antigens (Mathur et al., 1988; Primakoff et al., 1990; Shetty et al., 1999), each expected to have different effects on sperm function (Bronson, 2000; Chamley & Clarke, 2007). Sperm which are antibody coated over most of their surface are probably unable to enter cervical mucus (the lower, narrow portion of the uterus where it joins with the top end of the vagina), yet they remain completely motile in semen (Wang et al., 1985). Thus, the chance that they will reach the environment of the egg and fertilize it is low. Comparing internal fertilizers with those of external fertilizers may seem inappropriate, but the recent findings of an important effect of ovarian fluid on sperm swimming speed in external fertilizers (Turner & Montgomerie, 2002; Urbach et al., 2005) is interesting in this respect. Thus, in external fertilizers, the effect of Ig on sperm surfaces may, as in internal fertilizers, be more prominent during interactions with female fluids (Ayvaliotis et al., 1985; Bronson, 2000). Indeed, strong male-female interaction effects on sperm swimming speed in ovarian fluid are documented in charr (Urbach et al., 2005).

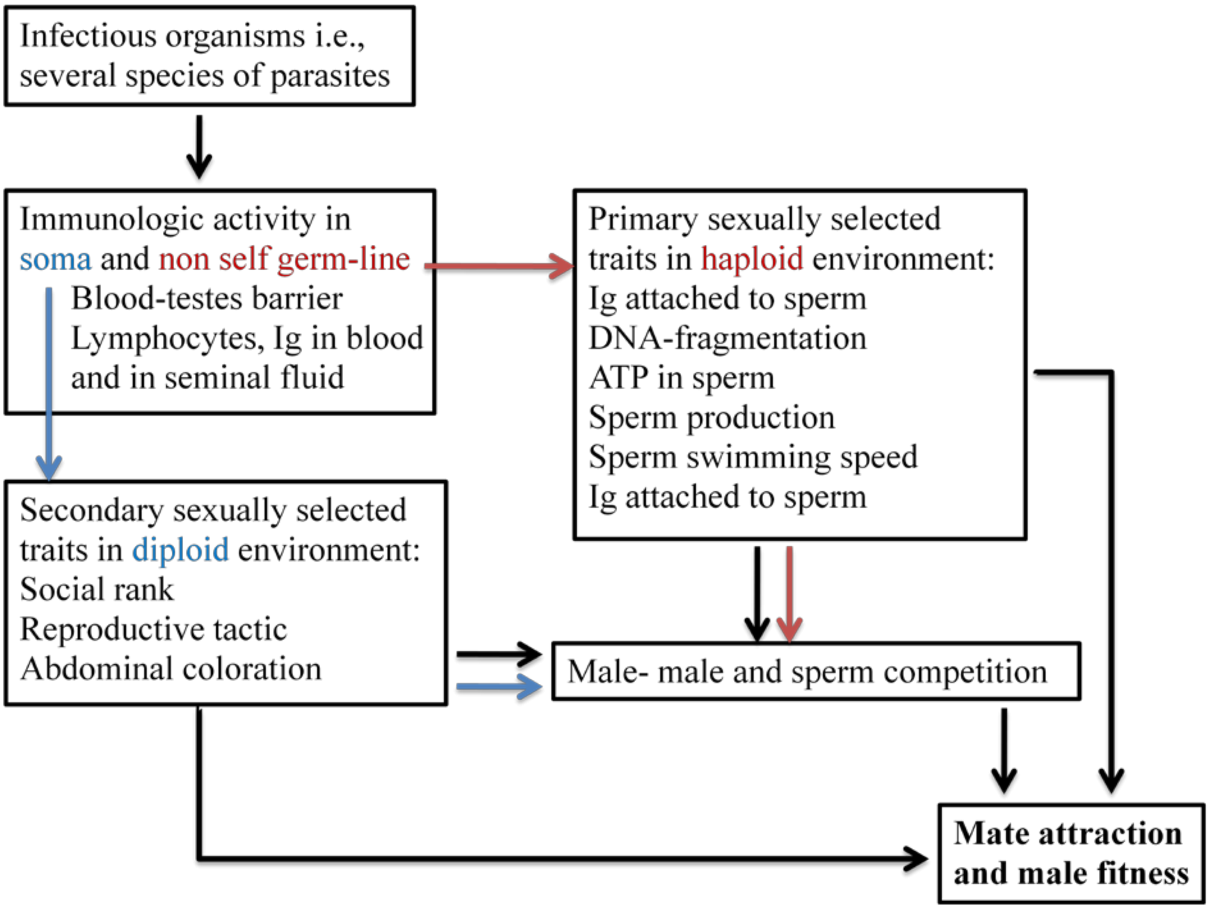

Figure 1

Illustration of factors influencing male attraction and fitness in Arctic charr. The chain of cause and effect is, off-course, likely to be much more complex than the one-way influence shown here.

5. PRESENTATION AND SUMMARY OF PAPERS

Paper I

A basic and fundamental understanding of the actually mating system is essential when examining reproductive behaviour. Previous to our examination of the Arctic charr population in Fjellfrøsvatn, we inferred charr reproductive behavior from knowledge about fish in general, salmonides in particular and one study of charr reproductive behavior in Thingvallavatn, Island (Sigurjonsdottir & Gunnarsson, 1989). Still, population density at different leks, migratory patterns between the leks and the possible degree of lek fidelity was unknown to us. By observing tagged males during the spawning period at three different lek sites, we examined fish density and movements between leks. Although, movement between two of the leks was substantial, individuals from the third lek seemed to be comparatively isolated. However, traits indicative of focal reproductive success (i.e., fish length and spermatocrit, were not associated to whether or not individuals moved between leks. The lek fidelity documented in Paper I may be important for production of local genetic differences between subpopulations of Arctic charr, and be related to sympatric speciation.

Paper II

Even though Paper I gave us a better understanding of the charrs mating system in Lake Fjellfrøsvatn, little investigation has occurred concerning actual spawning situations, i.e., which females and males releases gametes (see however (Sigurjonsdottir & Gunnarsson, 1989). Dominant and subordinate males may differ in spawning synchrony with the female and, in sperm competition, the relative time difference in point of ejaculation between the dominant and the sneaker(s) may affect paternity. Therefore, we used underwater video recordings to estimate the synchrony of gamete release between the female and the male and the possible time delay experienced by sneaker males. We captured 85 milt releases from dominant and subordinate males during 45 recorded spawning events; 25 events with sperm competition and 20 events where only one male released milt. Most of the ejaculates (76.5%) were released in sperm competition and the mean number of males releasing milt in each spawning was 2.6. In sperm competition, dominant males spawned more in synchrony with females than the subsequent subordinate males. Yet, when males spawned alone with the female, subordinate males released their gametes more in synchrony with females than dominant males. Results from this study provide essential information for designing studies regarding the importance of spawning synchrony and sperm traits for fitness (Egeland et al., 2010 in prep.).

Paper III

In Paper III we examined effects of rapid changes in social status on ejaculate investments during experimental trials. Here we demonstrate that males which become dominant produce less sperm, with lower velocity but had higher sex steroid concentrations than subordinate males. The differences in sperm characteristics originated from a decreased investment in ejaculates among males that became dominant. These adjustments of sperm production and sperm velocity do not appear to be traded against sperm longevity. Thus, males forced to mate in disfavored roles seem to invest more in ejaculate quality than males in favored roles. Moreover, dominant males had the highest concentrations of plasma sex steroids but the slowest swimming sperm cells. Thus, immunosuppressive steroids alone are unlikely to control sperm characteristics in charr. Yet, this was the first study to report that males, in a species with status dependent shifts in reproductive tactics, have evolved rapid tactic specific adjustments of sperm production and sperm velocity - corresponding to that predicted from their reproductive roles.

Paper IV

Differences among dominant and subordinate male charr mating tactics are associated with behavioural differences in dominance, mate guarding and courtship. It is now recognized that there is often pronounced variation in female preference for different male phenotypes and many empirical studies initially derived the prediction that females should prefer to mate with the highest quality male available (Jennions & Petrie, 1997; Qvarnstrom et al., 2000). We showed in Paper III that dominant males produced less dense sperm, with lower velocity than subordinate males. Empirical evidence for a genetic basis for offspring viability (Barber et al., 2001; Rideout et al., 2004; Wedekind et al., 2001; Welch, 2003) and evidence that offspring fitness may be related to parental status (Wedell & Tregenza, 1999), initiated a study to evaluate whether differences in male traits may be translated into differences in offspring traits, i.e., paternal effects. In this experiment we fertilized eggs with similar amount of sperm from size-matched dominant and subordinate males and monitored resulting egg and larvae development. Eggs fertilized by subordinate fathers resulted in more offspring being produced than eggs fertilized by the same amount of sperm from dominant fathers. The subordinate male's higher investment in sperm density (see Paper IV) and also in sperm swimming speed (see Paper III) suggests that subordinate males may be selected to pay the associated cost to ensure at least some success in the transfer of genes from one generation to the next. However, the results from paper IV, indicate that paternal status had no significant effect on measurements of offspring, i.e., larvae total length, yolk area and yolk red intensity.

Paper V

Parasites exploit hosts as a resource for their own reproduction. As parasites and hosts co-evolve, hosts have evolved a wide range of adaptations to prevent parasitic infections. These adaptations range from the complexity of antigen-specific cell-mediated responses to adaptive behaviors that may reduce the likelihood of an individual becoming exposed to pathogens. One should expect the immune response of a host to be optimized to the extent that low intensity infections will be tolerated if the costs of complete removal of parasites outweigh the benefits. Accordingly, optimal resource allocation to immune function will depend on other demands for resources, and their associated benefits (Sheldon & Verhulst, 1996). The existence of potential trade-offs like this lead us to examine possible modulators of traits indicative of sperm quality in Paper V and to measure possible effects of immunostimulants in sperm traits in Paper VI. Males that are forced to fight infection by up-regulating immune function pay a cost of reduced sperm quality (Andersson & Simmons, 2006). Thus, males with genetic resistance against parasites may have an advantage as they may be able to lower their immune activity during spermatogenesis and produce ejaculates of higher quality (Bronseth & Folstad, 1997). Thus, parasite intensity and immune responses may not only be related to the development of secondary sexually selected traits and to male social dominance, but also, to primary sexual selected traits, i.e., sperm quality and sperm characteristics. In paper V, we therefore sought correlational evidence for the impact that (*i*) male social status, (*ii*) parasite intensities and (*iii*) immunity may have on primary sexually selected traits. Parasite intensities and traits associated to male social status were the most significant effect modulators on sperm quality and quantity in charr. Male social status strongly predicted both sperm swimming speed and the amount of ATP in sperm cells, whereas parasite intensity was the best predictor of sperm production. Indeed, all captured variance in parasite intensities in male charr was related to their sperm production. It seems that parasites shift the host's reproductive investment towards investments in sperm production at the cost of investment in social dominance. Indeed, high spermatocrit levels are typically associated with individuals of low social status.

Paper VI

One of the indicator mechanisms of sexual selection (Andersson & Simmons, 2006), the "immunocompetence handicap hypothesis" (Folstad & Karter, 1992), suggests that the immune system competes for resources with sexually selected ornaments, and that variation in ornamental display might reflect variation in immunocompetence. Similarly, variation in ejaculate quality might also reflect differing levels of immune activity as sperm cells are perceived as "non-self" by the male and are exposed to immunological attacks in the testes and epididymus (Friberg, 1982; Hogarth, 1982; Roitt et al., 1993). This attack, which may reduce male fertility (Skau & Folstad, 2005) is often manifested as high levels of immunoglobulins (Ig) on sperm cell surface (Chamley & Clarke, 2007). The notion that a trade-off exists between immunity and reproduction is now a central concept in theories of sexual selection (Andersson & Simmons, 2006; Folstad & Karter, 1992; Folstad & Skarstein, 1997; Simmons & Roberts, 2005). However, as the proximate mechanisms controlling the associations between immune response, parasite intensities and traits indicative of sperm quality are not straightforward, we examined the effect of increased immunological activity on sperm traits. In our experiment, one third of the males given immunostimulants "chose" not to invest in reproduction, i.e., sexual maturity, compared to only 4.3% among control males. However, among the males that did mature, immunised males invested more in sperm production and had lower amounts of Ig attached to their sperm cells than males in the control group. Thus, a subtle effect seems to occur; if infected males "choose" to mature they invest more in sperm production. We believe that this is an adaption to a (more) subordinate mating strategy.

6. SHORT CONCLUSION

This thesis illuminates several aspects of mate choice and sexual selection in a species with external fertilization and no parental care. We have contributed to the basic framework within evolutionary biology and ecology and at a more specific level we have added some new pieces to the Arctic charr “jig-saw puzzle”. For example, we have established the significance of male social status, and in the two inter-disciplinary studies, showed that parasitized and infected males seem to invest more in sperm quality, less in ornamental development and adapt to sperm competition (i.e., subordinate mating tactic). Thus, as reproductive decisions in charr seem to be strongly influenced by parasites and antigens, host parasite co-evolution may have been a significant mechanism in the maintenance and evolution of both male sexual behaviour and ornamentation.

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Paper I

Lek fidelity of male Arctic charr

L. Figenschou, I. Folstad, and S. Liljedal

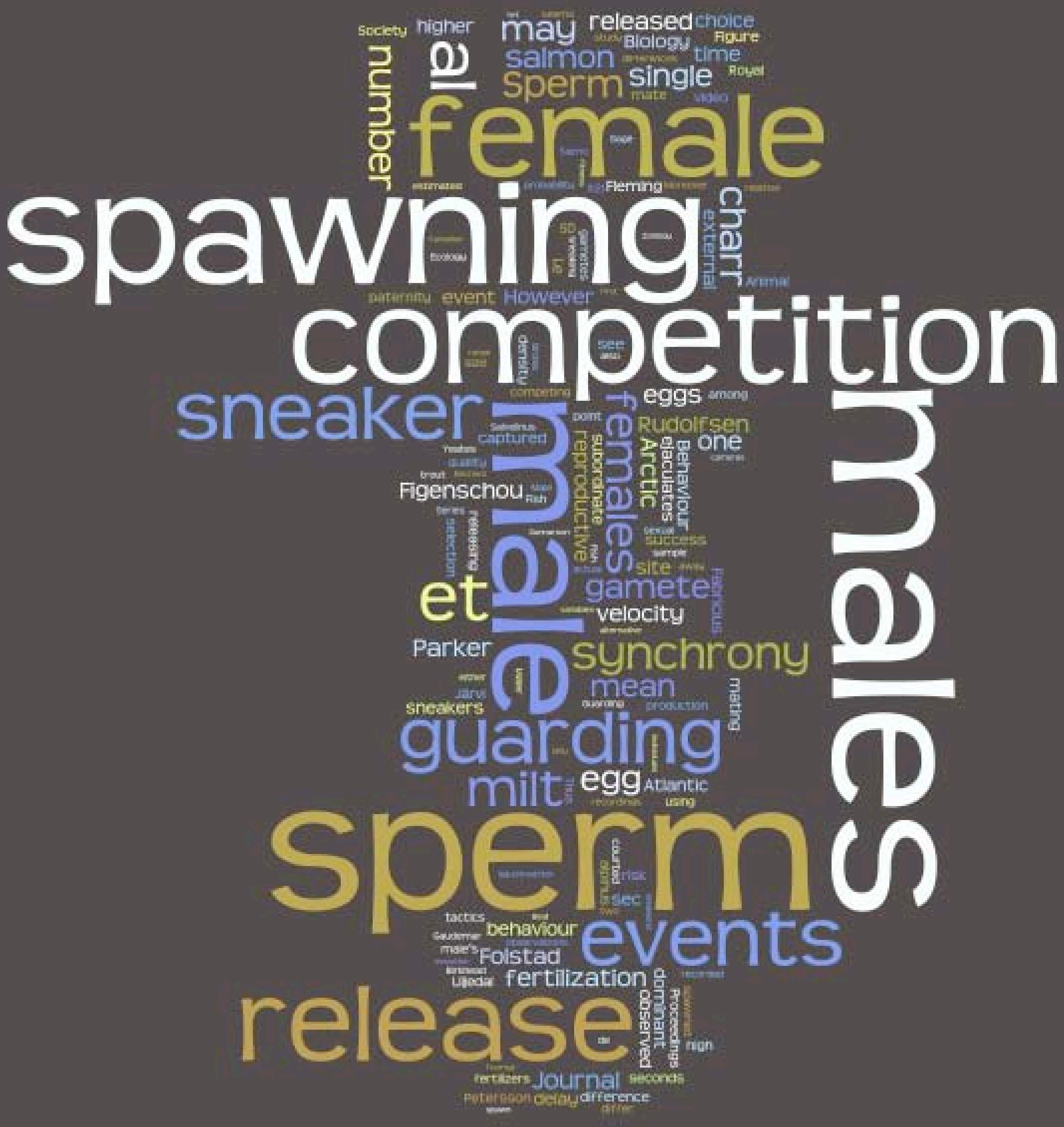
Can. J. Zool. **82**: 1278 -1284 (2004) doi: 10.1139/Z04-106 © 2004 NRC Canada

Paper II

Synchrony of gamete release and sperm competition in Arctic charr (*Salvelinus alpinus*)

Sørum V, Figenschou L, Rudolfsen G and Folstad I

Submitted to Animal Behaviour 2010



Paper III

Rapid adjustments of sperm characteristics in relation to social status

Geir Rudolfsen, Lars Figenschou, Ivar Folstad, Helge Tveiten and Marie Figenschou

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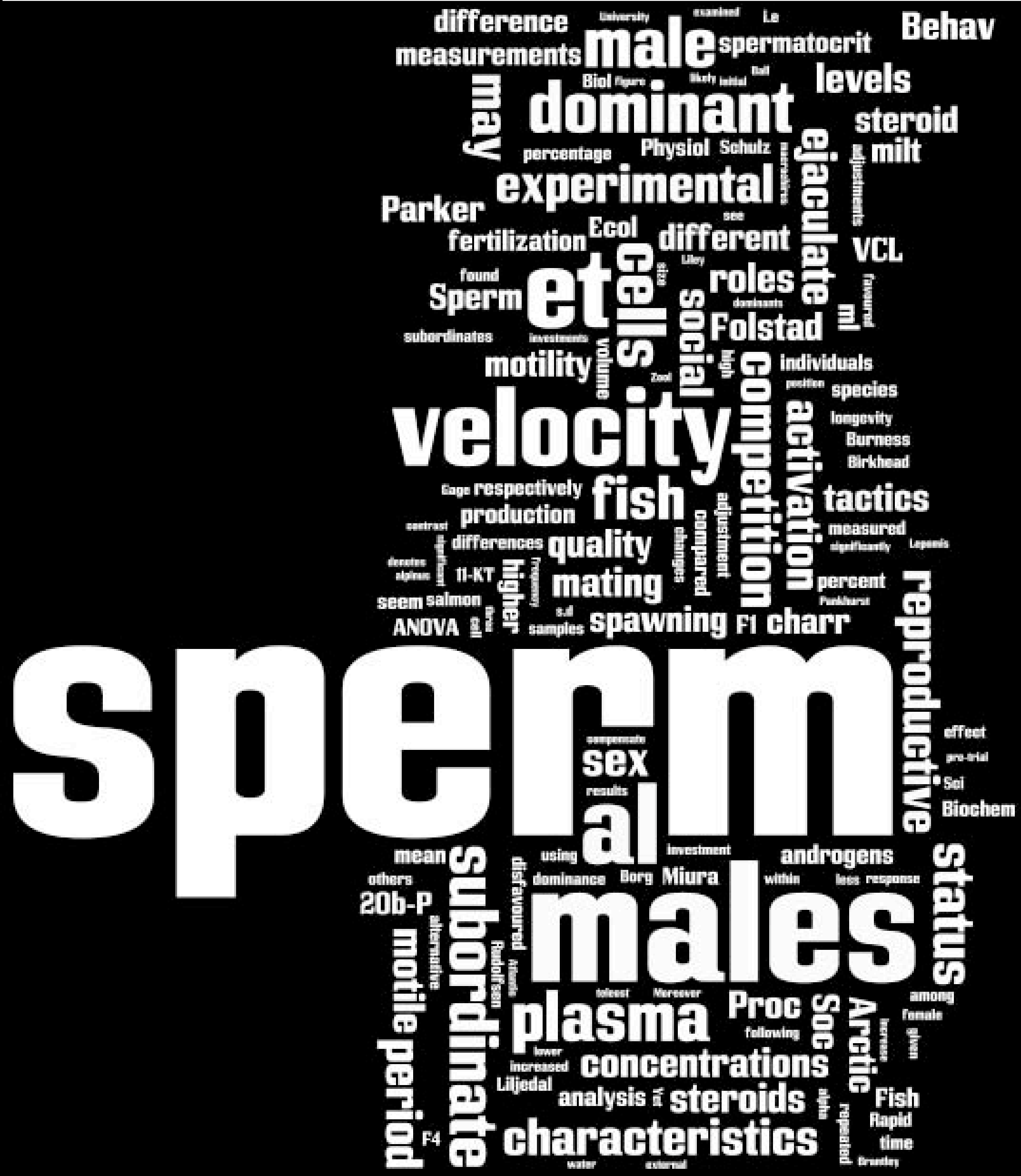


Paper IV

Female Arctic charr do not show apparent benefits from exposing their eggs to sperm from dominant males

Lars Figenschou, Geir Rudolfsen and Ivar Folstad

Journal of Fish Biology (2007) 71, 284 -289 doi:10.1111/j.1095-8649.2007.01477

Paper V

Parasite intensities and male social status as modifiers of sperm production and sperm swimming speed in the Arctic charr (*Salvelinus alpinus*)

L. Figenschou, I. Folstad, G. Rudolfsen, S.A. Hanssen, P.A. Skau, R. Kortet, J.E. Killie, I. Oskam, and H. Strand

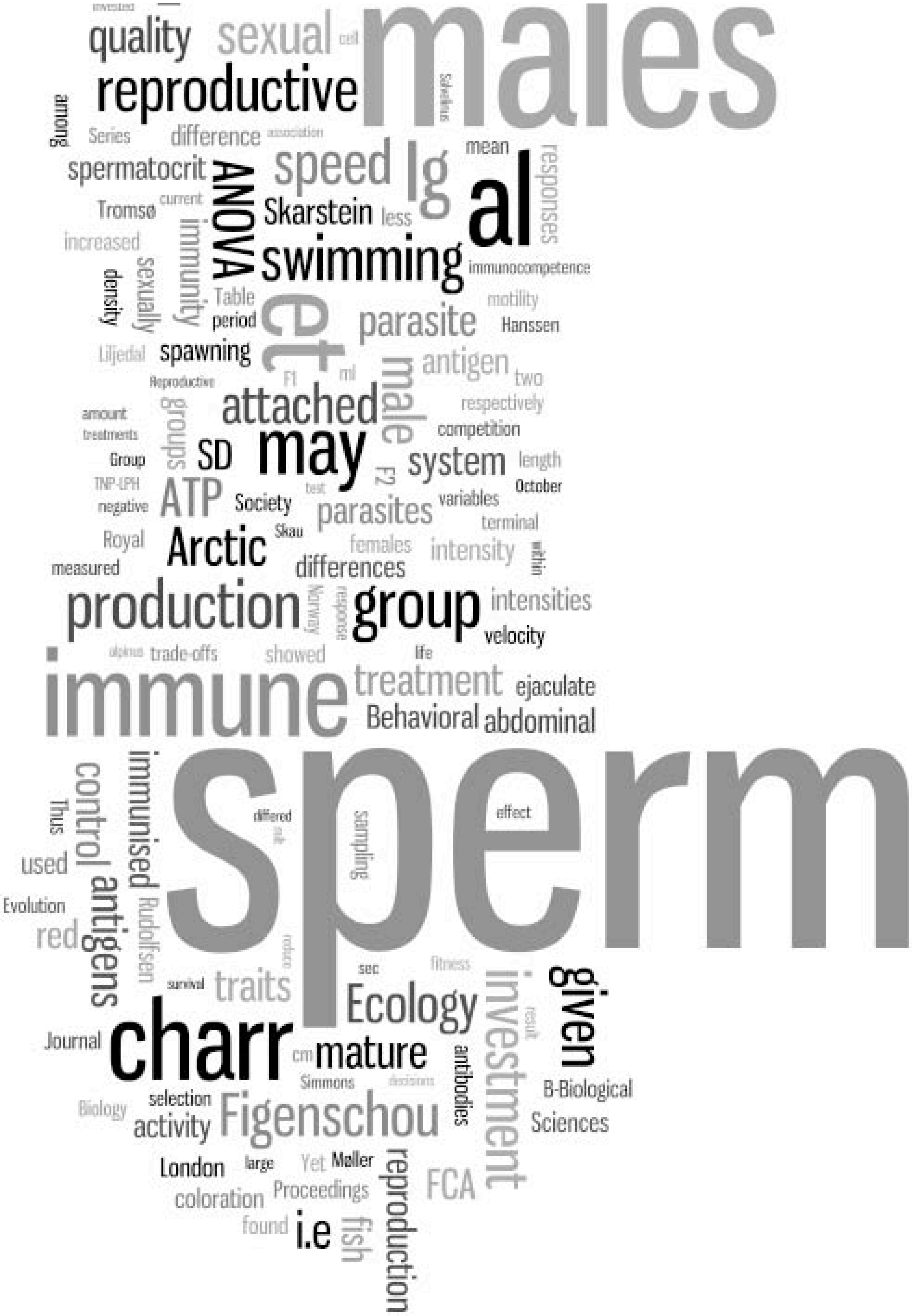
Manuscript 2010

Paper VI

Immune activation leads to reproductive compensation in Arctic charr (*Salvelinus alpinus*)

L. Figenschou, I. Folstad, G. Rudolfsen, P.A. Skau, S.A. Hanssen, R. Kortet, J.E. Killie, and H. Strand

Manuscript 2010



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