N° d'ordre :

THÈSE Transfrontalière UPPA / UPV

UNIVERSITÉ DE PAU ET DE PAYS DE L'ADOUR

ECOLE DOCTORALE DE SCIENCES EXACTES ET DE LEURS APPLICATIONS (ED 211)

Zoé GAUTHEY

EN VUE DE L'OBTENTION DU DOCTORAT
DE L'UNIVERSITE DE PAU ET DES PAYS DE L'ADOUR
Spécialité :
Biologie et Physiologie des Organismes

EFFECT OF ENVIRONMENT ON SEXUAL SELECTION IN BROWN TROUT (*SALMO TRUTTA*)

Soutenue le : 9 décembre 2014
Devant la commission d'examen formée de :

Katja Rasanen	Chargé de recherche, EAWAG Zurich	Rapporteur
Neil Metcalfe	Professeur, Université de Glasgow	Rapporteur
Tommaso Pizzari	Professeur, Université d'Oxford	Examinateur
Arnaud Grégoire	Maître de conférence, Université Montpellier 2	Examinateur
Arturo Elosegi	Professeur, Université UPV	Directeur de thèse
Jacques Labonne	Chargé de recherche, INRA/UPPA	Directeur de thèse

Acknowledgments

Je tiens tout d'abord à remercier mes trois encadrants de thèse Jacques Labonne, Cédric Tentelier et Arturo Elosegi pour m'avoir accompagnée tout au long de ce travail. Chacun d'entre eux a su m'apporter diverses connaissances dans des domaines quasi inconnus à mon arrivée. Merci pour leur patience, la transmission de leur savoir et aussi leur disponibilité et leur écoute. Que ce soit sur le terrain, au labo, par mail, la journée ou la nuit, ils ont toujours été là pour me prêter main forte et m'aiguiller dans les divers choix qui se posaient à moi. Jacques, je te remercie particulièrement pour tout le temps que tu as consacré que ce soit pour me former à la modélisation, pour de multiples corrections et ton soutien de manière très générale. Cédric, merci de m'avoir fait découvrir le monde de l'enseignement supérieur, de t'être penché avec moi sur des scripts incompréhensibles et d'avoir été présent tout au long de la thèse. Arturo, merci pour m'avoir accueillie à Bilbao dans ton laboratoire et d'avoir été disponible aussi souvent que possible, merci pour tous tes précieux conseils concernant la mise en place des protocoles et tes critiques constructives concernant mon travail. Je sais que vous avez tous les trois beaucoup investi en temps et en énergie et vous en suis extrêmement reconnaissante. Néanmoins, vous pouvez être sûrs que pendant cette thèse j'ai appris énormément grâce à vous et j'en retire de multiples bénéfices.

Merci à Stéphane Panserat pour toutes les discussions sur les métabolites que nous avons pu avoir. Merci pour les précieux conseils que tu m'as apportée pour la rédaction des articles.

I would like to thank Andrew Hendry for having invited me to Mc Gill University for one month. I really appreciated the possibility of working in an "Anglo- Saxon lab". Andrew, thank you for your time and all your new and fresh ideas which have greatly contributed to my work.

Merci à Olivier Lepais, co-voitureur mais surtout généticien très à l'écoute. Je te dois tout mon savoir sur les analyses de parenté, la prise en main de divers logiciels. Merci pour tous les petits coups de main que tu as pu m'apporter, tes réponses à mes questions « R », à n'importe quelle heure de la journée même le vendredi en fin d'après-midi.

Merci Aurélie Manicki. Travailler avec toi a été très agréable. Tu as su être à la fois pédagogue et j'ai apprécié ta positive attitude. Je garderai un très bon souvenir de cette période qui aura quand même duré quelques mois.

Merci à Jacques Rives, les 3 années consécutives sur le terrain avec toi ont été un pur bonheur. Merci pour ta joie de vivre, ta bonne humeur, ton écoute. La sirène du Bastan s'en va, j'espère que tu t'en remettras.

Merci à Pascale Coste, Jean Christophe Aymes, Stéphane Glise et François Guéraud qui m'ont aidée lors de mes différentes manipes, notamment sur le terrain, au Lap et pour la mesure d'œufs. Sans vous, tout le travail que j'ai fait n'aurait pas pu être réalisé. Merci pour votre temps, vos explications et vos précieux conseils.

Merci à tous les stagiaires qui ont participé à la réalisation de ce travail, Alexandra, Laura, Elie et Vincent !

Merci à FX Dechaume-Montcharmont, Emmanuel Desouhant et Agnès Bardonnet pour leur participation à mes comités de thèse et pour leurs remarques constructives sur mon travail.

Plus généralement, je remercie le labo et particulièrement Agnès Bardonnet et Valérie Bolliet pour m'avoir accueillie pendant 3 ans, au sein du laboratoire ECOBIOP INRA/UPPA à Saint-Pée sur Nivelle. Merci à Pantxika pour sa disponibilité et son aide concernant toutes les fameuses tâches administratives. Merci à toutes les personnes du labo que j'aurais par mégarde oublié de citer.

Mael, Marine et Seb, je vous remercie, vous avez été la famille qui me manquait au Pays Basque. Je me souviendrai des nuits d'hiver pluvieuses passées chez vous, toujours un bon petit plat préparé et un lit qui m'attendait et m'évitait de repartir jusqu'à Bayonne. J'espère rester votre Joey.

Merci à tous les amis que j'ai pu rencontrer ici. Merci à Veronaze, Julie, Vivi, Ikram, Lulu ; Karine ma pépita d'or sans qui l'aventure n'aurait pas été tout à fait la même, Benjamin, Jordi avec qui on a partagé les mêmes galères et même sauté dans la Nive, Julie pour ton aide, ton écoute, ton soutien et nos fameux mails « Help » ; Lisa pour ta girl power attitude et ton coaching au quotidien. Merci Loïc pour ces derniers mois avec toi, tu as su m'apporter ton aide et surtout tu as su supporter mon stress. Vous avez tous contribués à mon bien être pendant ces trois années.

Merci à tous ceux qui étaient loin mais qui je sais étaient toujours de mon côté : la team dijonnaise, je parle bien sûr des dragons et de Matthias, merci à Nico ainsi qu'à mes cops Margaux, Aurèle, Sonia et Laure qui ont su me rebooster dans les moments difficiles et me supporter. Ça n'a dû toujours être facile pour vous alors MERCI encore! Merci à toute la famille, grands-parents, parents, ma sœur Juliette…Vous m'avez tous été d'un très grand soutien.

Résumé

La sélection sexuelle est une composante de la sélection naturelle qui génère des différences de succès reproducteur entre les individus par le filtre de la reproduction, et influence donc la transmission intergénérationnelle des gènes. Dans le cadre de cette thèse, l'effet de la variabilité de l'environnement hydraulique sur la sélection sexuelle chez la truite commune a été étudié à différentes échelles : intra- et inter-populationnelle. Des méthodes nouvelles permettant de mieux appréhender l'investissement reproducteur, ainsi que de décomposer l'effet des traits sur la fitness des individus en fonction des différentes étapes de la sélection sexuelle, ont été mises au point. Les expériences réalisées en milieux naturel et semi-naturel indiquent que la variabilité environnementale n'affecte pas le choix d'habitat de reproduction par les femelles, mais peut affecter l'investissement reproducteur dans la compétition par exemple, ainsi que les flux de gènes entre des populations génétiquement distinctes. Ces résultats permettent une première projection de l'évolution de la sélection sexuelle dans le contexte du changement climatique qui prédit l'augmentation de la variabilité hydrologique en zone tempérée.

Abstract

As a component of natural selection, sexual selection produces variation in reproductive success throughout the reproductive period, and therefore impacts genes transmission between generations. During this PhD, the effect of variation in hydraulic environment on sexual selection in brown trout was investigated at both within and between populations scales. New approaches to improve estimation of reproductive investment, as well as models to decompose the effect of traits on individual fitness at each stage of sexual selection, were developed. Experiments in natural and semi-natural environments indicate that environmental variation does not impact reproduction habitat choice by females, but it can modify reproductive investment in some populations, as well as it can control gene flow between genetically distinct populations. These results help to understand the evolution of sexual selection in the broad context of increasing stochastic variations of river systems hydrology as predicted by climate change models in temperate areas.

Resumen

Como componente de la selección natural, la selección sexual produce variación en el éxito reproductor a lo largo del periodo de reproducción y, por tanto, afecta a la transmisión de genes entre generaciones. En esta Tesis doctoral se ha investigado el efecto que tiene la variabilidad en el medio hidráulico sobre la selección sexual en la trucha común, a escala intra- e interpoblacional. Se han desarrollado nuevas aproximaciones para estimar la inversión reproductiva y modelos para descomponer el efecto de los rasgos biológicos en el fitness individual a cada estadio de la selección sexual. Experimentos realizados en ambiente natural y seminatural indican que la variación ambiental no afecta a la selección del hábitat reproductor por parte de las hembras, pero que puede modificar la inversión reproductiva en algunas poblaciones, además de controlar el flujo génico entre poblaciones genéticamente diferenciadas. Estos resultados ayudan a comprender la evolución de la selección sexual en el contexto del incremento de variabilidad estocástica en la hidrología fluvial, prevista por los modelos de cambio climático de áreas templadas.

Laburpena

Hautespen naturalaren atal gisa, hautespen sexualak ugal arrakastaren aldakortasuna dakar ugalaldian zehar, eta beraz, belaunaldien arteko geneen barreiadurari eragiten dio. Doktoretza Tesi honetan errekako hidraulikaren aldakortasunak amuarrain arruntaren haustepen sexualean duen eragina aztertu da, populazio barneko zein populazio arteko eskalan. Ugal-inbertsioa estimatzeko hurbilketa berriak garatu dira, eta ezaugarri biologikoek sexu-hautespenaren urrats bakoitzean banakoen dohipenean duten eragina zatitzeko ereduak ere. Ingurune natural eta erdi-naturaletan eginiko esperimentuek erakusten dute ingurumen-aldakortasunak ez diola emeen ugal-habitataren aukeraketari eragiten, baina populazio batzuetan ugal-inbertsioan eragin dezaketela, bai eta genetikoki desberdindutako populazioen arteko fluxu genetikoa kontrolatu. Emaitza horiek laguntzen dute hautespen sexualaren eboluzioa ulertzen klima-aldaketarako ereduek eskualde epelerako iragartzen duten aldakortasun hidrologiko estokastiko handituaren harira.

Introduction générale

La sélection sexuelle est un processus central de l'évolution qui génère des différences de succès d'appariement (nombre de partenaires sexuels) et de succès reproducteur (nombre de descendants) entre les individus d'une population, affectant de fait la fitness des individus et donc la transmission des gènes d'une génération à l'autre. Différents mécanismes, ainsi que leurs interactions, peuvent être à l'origine de la variation du succès reproducteur, comme la compétition intra-sexuelle, la préférence intersexuelle, les soins parentaux. Ces mécanismes ont un coût que l'on qualifie d'investissement reproducteur (allocation de l'énergie dans l'investissement gamétique et l'activité comportementale). Chez la truite commune (*Salmo trutta* L.), une intense compétition intra-sexuelle se déroule chez les mâles pour s'accaparer les femelles, et les femelles expriment une préférence sexuelle apparente pour différents phénotypes de mâles, tout en procédant à la construction du nid (soins maternels), ce dernier étant extrêmement important pour la survie des descendants. Ces mécanismes sont tous deux sous l'effet direct de l'environnement, et notamment de l'environnement social mesuré par le sex-ratio opérationnel (OSR, ratio du nombre de mâles en activité sur le nombre de femelles en activité) : le coût de la compétition dépend de l'OSR, et les soins parentaux sont liés à la prédation et au cannibalisme (eux aussi sous dépendance de l'OSR). Cependant la variabilité de l'environnement physique comme les changements de débit peuvent aussi affecter ces coûts ainsi que la survie des descendants.

Dans le cadre de cette thèse, j'ai cherché à approfondir la compréhension des effets de l'environnement sur la sélection sexuelle chez la truite commune. Pour ce faire, j'ai travaillé le problème à plusieurs échelles : individuelle (mécanismes endogènes), interindividuelle (groupe social et plus particulièrement la prise en compte du phénotype du partenaire sexuel), inter-populationnelle. Pour chacune de ces échelles l'effet de la variation des conditions hydrauliques a été étudié, ces conditions étant facteur extrêmement structurant du déroulement du cycle de vie chez cette espèce. Par ailleurs, la variabilité des paramètres hydrauliques est au centre des questionnements concernant les effets du réchauffement climatique : les prévisions des modèles théoriques indiquent un accroissement des évènements extrêmes (crues et sécheresses) en zone tempérée.

Afin de traiter cette question, des méthodes originales à trois niveaux. Premièrement, des indicateurs de concentration de métabolites énergétiques dans le plasma sanguin pour

mesurer la variation d'une partie de l'investissement reproducteur (celle liée au comportement de reproduction) au cours de la période de reproduction ont été utilisés. Deuxièmement, de nouveaux modèles ont été développés permettant de mesurer l'effet séparé des traits des mâles et femelles sur chacune des différentes étapes de la sélection sexuelle : recherche de partenaires, succès d'appariement, succès reproducteur. Ces modèles se basent sur une décomposition des matrices de succès reproducteur et des matrices d'observation du comportement, pour séparer explicitement les effets des traits sur chaque étape de la sélection, tout en permettant une ré-estimation du succès d'appariement, généralement sous-estimé par les méthodes existantes. Enfin, des expériences originales ont été mises en place dans un chenal expérimental permettant d'observer et mesurer la sélection sexuelle tout en faisant varier l'origine des géniteurs (effets populationnels) et le débit (effet environnemental).

CHAPTER I:
INTRODUCTION

Sexual selection is a component of natural selection described by Charles Darwin in *The Descent of Man, and Selection in Relation to Sex* (1871) as an evolutionary process by which limited access to opposite sex may lead to variations of both mating success (number of partners) and therefore reproductive success (number of offspring produced). Sexual selection acts during reproductive period through two mechanisms known as the agents of sexual selection: 1) intra-sexual competition in which member of one sex compete to access to mates of the other sex and 2) inter-sexual selection in which traits confer an increased probability to be selected by the opposite sex. These two mechanisms, as well as parental care, generate potential variations in reproductive success between individuals and therefore can affect individual fitness, which in turn leads to the structure of populations through generations. Reproduction involves costs for parents since individuals have to partition their time and energy to different functions of reproduction such as competition, gamete production and parental care. **Reproductive investment** is therefore described as the allocation of time and energy for reproduction. The costs and benefits associated to overall reproductive investment may vary depending on environmental contexts, at different scales: 1) the social environment that can be described by the availability of sexual partner and the local density of competitors, 2) the physical environment that may impact directly individual condition, offspring survival, and may also affect for social environment. In semelparous species, overall reproductive investment is directly influenced by individual condition: differences in body mass will result in different abilities to invest in reproduction. In iteroparous species, individual condition may also constrain reproductive investment, however individuals may display various patterns of reproductive investment between reproduction seasons, leading to various life-histories, since current reproductive investment will also affect future survival and therefore future opportunities of reproduction.

In this introduction, I will detail concepts such as energy allocation, reproductive investment, agents of sexual selection, and measures of cost and benefits. I will also describe some measures of sexual selection, and the underlying assumptions that could be improved. I will then evoke how environment may influence all these evolutionary landmarks. In a second part, I will focus on the case of sexual selection in brown trout, and on the suspected effects of environmental change, with a focus on effects of climate change. I will finish by presenting the different chapters composing this Philosophical Dissertation.

I. A general background for sexual selection

1) Energy allocation

During their entire life, organisms allocate their time and energy to different essential functions distributed into categories such as "growth", "maintenance" and "reproduction". The principle of allocation enounced by Williams in 1966 implies that resources are limited within an environment leading each individual to optimize their resource allocation to these different functions in order to maximize their own fitness (Stearns, 1992). Thus, the principle of allocation is directly related to the notion of tradeoff since energy (or time) invested in a function is no longer available for another one. The concept of cost takes an important role in many fields of evolutionary biology and cost-benefits analysis have been deeply studied to better understand evolution of life history traits (Levins, 1968; Roff, 1992; Williams, 1966).

The most prominent tradeoff that iteroparous organisms have to face is the choice between current versus future investment in fecundity or parental care (Trivers, 1972).

Then, a reproductive strategy can be viewed as a tradeoff maintained in a balance between the benefit of reproductive effort (increased fecundity, see Table 1) and the cost of reproductive effort (increased mortality, missed future opportunities of reproduction). Accordingly, individuals must select tactics within this strategy to balance the tradeoff between investment in reproduction and survival (Abrahams, 1993). In semelparous species, investment in the reproduction is only concentrated in the current reproduction. The consequence of the tradeoff between survival and reproductive effort is that individuals may choose to maximize their reproductive success for a given reproductive season and to invest less in another reproductive season. Therefore, fitness is logically best estimated by the lifetime reproductive success (Fisher, 1930), although even nowadays it remains difficult to obtain, since it requires to have access to the total number of offspring produced by each individuals during its life.

Growth, gametic investment andsurvival are important fitness traits, and their variation is expected to be the outcome of natural selection, where only optimal values of traits are selected conditional on environmental variation. Observed trait values are therefore expected to increase overall fitness of individuals once an evolutionary optimum is reached. But as Darwin noticed, a large part of trait variation in many species in the wild cannot only be explained by means of natural selection alone. To improve his theory, Darwin (1871) coined that sexual selection could promote significant variation in traits, between sexes and sometimes within sexes, and that such variation would sometimes appear to be not adapted and reduce individual survival. In the same, time, these extreme traits may increase access to sexual partners and may be involved in mate choice.

Therefore, sexual selection is also a process that explains a wide range of diversity of traits between sexes such as differences in appearance, size, physiology, life history and behaviours.

Table 1. *Definitions*

Definitions	
Reproductive investment	Energetic budget invested in the current reproduction by an individual (Williams 1966) The proportion of resources available devoted to reproduction (Reznick, 1985)
Operational sex ratio (OSR)	Ratio of receptive males during the reproduction out of receptive females (Emlen & Oring, 1977)
Reproductive success (RS)	Number of juveniles produced to the next generations by an individual (Clutton-Brock, 1988)

2) How to measure the costs of reproduction

The cost of reproduction has received particular attention because it plays an important role in evolution of reproductive tactics (Roff, 2002). Investment in reproduction relies on various components, such as gametic investment, intrasexual competition and parental care and it is ultimately conditioned by the tradeoff between current *versus* future reproduction. The consequences of the costs of reproduction have been broadly measured as well as its relationship with future survival and fecundity. Empirical evidence of cost of reproduction may be measured through different approaches reviewed by Reznick (1985):

- Phenotypic correlations studies are the most common studies: they correlate an index of reproductive effort with a potential cost traits such as parental mortality, parental growth or future reproduction. For example Clutton-Brock et al. (1982) showed a reduction of production of offspring the year after calving in red deer.

- Experimental studies test how reproductive effort is affected by manipulated environment. This kind of studies have been mostly realized in birds by manipulating the number of eggs in the nest and looking at the potential effect on survival. Other studies tend to manipulate food availability or temperatures.
- Genetic correlation studies investigate relationship between life history variables (e.g. Rose & Charlesworth, 1981a).
- Responses to selection studies tend to perform selection experiments (e.g. Rose & Charlesworth, 1981b).

However, although it seems pretty clear how to measure reproductive cost in theory, the actual link with energy allocation is not often provided, whereas this principle is at the core of life history evolution. By knowing the actual cost of a tactic in various environments, it should become possible to make robust predictions about the possible direction of evolution under putative environmental scenarios. Such relationship between reproductive tactics and energy is difficult to establish directly in wild populations for field biologists: a lot of factors are interacting, sampling in open populations can lead to strong statistical bias, and in many cases, killing animals is ethically problematic and nowadays precluded. Therefore, the cost of reproduction has been also approached in other ways, including gamete production (Hayward & Gillooly, 2011; Vézina & Williams, 2005), hormonal regulation, immune functions, proteins, and resistance /tolerance to stress and toxicity (Harshman & Zera, 2007). Another widely-used surrogate for energy expenditure is the decrease of weight or condition during reproduction (Schulte-Hostedde, Millar, & Hickling, 2001; Stevenson & Woods, 2006, McElligott et al.2003). All these measures are useful proxies of reproductive investment to study evolution in natural or near natural conditions, as they all propose different but non-exclusive point of view of the costs associated to reproduction.

3) Agents of Sexual selection

The relationship between phenotypical traits and fitness is a key of evolutionary biology and allows to estimate the strength of selection on a trait across the generations. Sexual selection has been described by Darwin (1871) as a driving evolutionary force leading to select traits maximizing mating access due to a high variation in fitness between individuals. As stated previously, he clearly made a distinction between two processes of sexual selection 1) intra-sexual competition, in which members of one sex compete to access to mates of the other one and 2) intersexual selection in which some individuals possess traits that confer an advantage for mating access.

Somehow these two processes are rarely equilibrated between sexes (Andersson, 1994). One of the most cited explanations relates to anisogamy between sexes: because gamete size greatly varies between sex, the sex investing more in gamete size is also expected to be more choosy, whereas the other sex attempts to take benefits from smaller but more numerous gametes by mating as many times as possible to enhance its reproductive success. Under this assumption and in most species, variation in reproductive success and therefore sexual selection tends to be stronger in males than in females since the latter are categorized as the "limited resource". Females are therefore predicted to increase their reproductive success by choosing mates of good quality either via good genes or mates that will contribute best to providing resources such as spawning territories (Bateman, 1948; Trivers, 1972). Intense intra-sexual competition is often observed in males (contest competition and scramble competition). Additionally, in many species, male fitness is correlated with the size of weapons, body size, and other extravagant traits such as colourful patterns in birds or fish. Therefore "the coveted" sex should be more selective for choosing mates, preferring to some extent quality over quantity of sexual partners, in

order to maximize the survival of their offspring, which will increase its own fitness. While many studies in sexual selection have focused on sex roles and sex differences, and have generally accepted this general picture about sex roles, intra-sexual competition and inter-sexual selection happen in both sexes (H. Kokko & Jennions, 2008) and "reversed" sex roles are also commonly observed (Kvarnemo & Ahnesjö, 1996; Vincent, Ahnesjö, Berglund, & Rosenqvist, 1992; A. B. Wilson, Ahnesjo, Vincent, & Meyer, 2003) thereby somewhat contradicting the anisogamy explanation. Agents of sexual selection are therefore also controlled by other factors, such as the adult sex ratio (ASR), operational sex ratio (OSR), mating rate and mortality rates (H Kokko & Monaghan, 2001).

Thus individuals should show some preferences for attractive member of the other sex that may provide various sorts of benefits. Some sexual partners may provide good parental care, such as nest defense and offspring feeding, or can provide nuptial gift, or can increase the probability of fertilization (gametes quality); all these benefits are labelled as "direct benefits", because they are the direct consequences of the sexual partner's actions. However, a sexual partner is also expected to provide "indirect benefits", that benefit offspring through genetic transmission (the good genes hypothesis: Williams, 1966): individuals could carry good genes that they will transmit to their progeny which will in turn enhance offspring's growth or survival rate. Additionally, the preference may also target sexual partner with respect to ones own genotype or phenotype: genetic compatibility of offspring is also an important component of their fitness. This is exemplified by inter-sexual preference expressed on the major histocompatibility complex (MHC) structure in many taxa (Forsberg, Dannewitz, Petersson, & Grahn, 2007; Milinski, 2006; C Wedekind, Seebeck, Bettens, & Paepke, 1995).

The effective mate choice is the outcome of the interplay of these two agents of sexual selection in a group of breeders. The effective mate choice is therefore not an evolutionary optimum: it hence reflects the outcome of intra-sexual competition in each sex, the possible divergence of intersexual selection between sexes, and it is conditioned by the phenotypic availability (who is present during a mating episode). This divergence of evolutionary interests between males and females is known as the sexual conflict. Therefore, evolution of traits and preference, and who mates with who has a major importance on individual fitness and evolution of reproductive isolation and relies *de facto* on environmental context such as social environment (OSR, phenotype availability) and may additionally be influenced by physical environment.

4) How to measure sexual selection: Bateman gradient and other indices

Numerous measures of sexual selection exist in the literature to predict and quantify patterns of sexual selection (A.G. Jones & Ratterman, 2009; Klug, Heuschele, Jennions, & Kokko, 2010; Mills, Grapputo, Koskela, & Mappes, 2007; Shuster & Wade, 2003). Here I only present the most popular measures.

The OSR might be the most used proxy of sexual selection: it is the ratio of the number of males on number of females available at any given time for mating, and it is often used to predict the strength of competition over mates (Clutton-Brock & Parker, 1992; Emlen & Oring, 1977; Kvarnemo & Ahnesjö, 1996; Reynolds, 1996). Intra-sexual competition in the most numerous sex is predicted to increase with an increase of the OSR, and it can potentially in turn increase the overall sexual selection. However, the use of OSR as a measure intensity of sexual selection has been criticized (Klug et al., 2010). The thought that a male (or female) biased OSR may enhance variation in sexual selection for a given

sex relies on the fact that intra-sexual competition will increase with a greater monopolization resources (Emlen & Oring, 1977). Monopolization is here described as the capacity of some individuals of one sex to dominate mating opportunities by monopolizing the resource leading to an exclusion of other individuals competing for the same resource. However, for a same value of OSR, mean and variance in mating access may vary according to the ability of one individual male among competitors for the opposite sex to monopolize several females, in this case the mean and the variance of mating success is predicted to be high. And yet, for a same OSR value, if there is no difference in the ability of competitors to monopolize the opposite sex, which leads to polygamy, mean and variance in mating success will be lower (J. Collet, Richardson, Worley, & Pizzari, 2012).

Other indices based on the mean and variance of both mating success and reproductive success, as well as on the covariance between these two variables, are also widely used to estimate intensity of sexual selection. One of the most common is the opportunity for sexual selection (*Is*) based on the relative variance in mating success of a given sex (ratio of variance in mating success and the squared mean mating success, (Wade & Arnold, 1980, Table 2). The strength of sexual selection within a sex is expected to increase as I_s increases, the greater the variance of mating success, the stronger the selection. Additionally the measure of the selection of specified traits suggested by Lande & Arnold (1983) and called "standardized selection gradient" (β) is focused on the covariance between the standardized selection differential of mating success and the standardized trait identified. The selection gradient thus indicates how much the relative fitness (mating success or reproductive success) will vary as a function of the variation of the focal trait. The change in the mean of the trait before and after selection can be compared

in order to see the direction of sexual selection after one or several generations using the selection differential index (*S*).

Another measure widely used is the Bateman gradient (β_{ss}) which is a particular type of selection gradient measuring the relationship between number of mates and reproductive success (Bateman, 1948). The Bateman gradient assumes that a relationship may exist between reproductive success and number of mating success that will give rise to the opportunity for sexual selection to act. Bateman argued the intensity of sexual selection should depend on variation in the number of mates and reproductive success in both sexes. To highlight his thought, he performed an experiment using *Drosophila melanogaster* as a biological case of study and looked at the variance of reproductive success of each male and female. Results showed that males had a higher variance in number of mates, reproductive success variation was higher in males and that the slope of the linear regression of reproductive success on the number of mates was also higher in males than in females. These three assumptions are known as the 3 Bateman principles in the literature. He concluded, as Darwin, that sexual selection is more intense in males than in females. Bateman gradient has been frequently used to study evolution of sex roles and sexual dimorphism in natural populations (Jones, Rosenqvist, Berglund, Arnold, & Avise, 2000).

It is noteworthy that the "mating success" concept is at the core of each of these measures: Opportunity for selection (*I*), Selection gradient (β) and Bateman gradient (β_{ss}). "Mating success" is in fact used as a trait in order to estimate the strength of sexual selection. This concept is therefore of prime interest for any evolutionary biologist interested in sexual selection.

Table 2. *Classical measures used to quantity the strength of sexual selection and that will be used in the present manuscript**

Measure of sexual selection	Description
Operational sex ratio (OSR)	The average ratio of males to females who are ready to mate at any given time in a given place (Andersson, 1994; Emlen & Oring, 1977; Kvarnemo & Ahnesjö, 1996).
Opportunity for sexual selection (I_s)	A standardized measure of intra-sexual variation in mating success; measured as the relative variance in mating success for a given sex (σ^2 represents the variance in the mating, $\bar{X}^2$ is the square of the mean of mating success) to provide an upper limit to the strength of directional sexual selection (Arnold & Wade, 1984; Jones & Ratterman, 2009; Lande & Arnold, 1983; Shuster & Wade, 2003; M.J. Wade, 1979) (Arnold & Wade, 1984; Jones, 2009; Lande & Arnold, 1983; Shuster & Wade, 2003; Wade, 1979) $$I_s = \frac{\sigma^2}{\bar{X}^2}$$
Selection gradient (β)	The slope of the regression of relative fitness (e.g mating success, reproductive success) on the phenotypic value of the focal trait. If several traits are examined, the partial regression coefficient for each trait is equivalent to its selection gradient. When calculating the selection gradient with respect to sexual selection, the relative mating success of a given sex is used in place of relative fitness. This measure is often referred to as the strength, intensity or force of selection on a given trait (Andersson, 1994; Arnold & Duvall, 1994; Arnold & Wade, 1984; Jones, 2009)
Bateman gradient (β_{ss})	Specific selection gradient which measures the slope of the regression of reproductive success on mating success for a given sex. An estimate of the strength of selection acting on mating success (Arnold & Duvall, 1994; Jones, 2009)

*Modified from Klug et al. 2010

5) Definition of mating success and its consequences

In the literature, a wealth of definitions for individual mating success during one reproductive period can be found (Arnold, 1994; Bateman, 1948; Jones, 2009; Parker & Tang-Martinez, 2005; Uller & Olsson, 2008).

(1) the number of mating events (or number of copulations)

(2) the number of different mates with whom a focal individual has copulated with

(3) the number of mating events that bear or sire of progeny

(4) the number of different mates that bear or sire of progeny (or mates with whom a focal individual copulated and produced offspring).

Since sexual selection is defined as selection that arises from variation in both reproductive and mating success, each of these definitions of mating success has its importance. Indeed, the two first definitions are the more global definition of mating. They measure mating events or number of mating without consideration for offspring production. Therefore, they implicitly integrate potential costs of reproductive behaviours acting before copulation, since the mating success can lead to a null fitness in some cases, and a positive fitness in some other cases. Mate sampling and intra-sexual competition are partly but poorly described by mating events. Still, these two mechanisms are of major interest in order to estimate costs. However, the first definition fails to account for mate acquisition since the number of different sexual partners is not measured (Arnold, 1994) whereas number of mates may positively influence individual fitness. At the opposite, the two latter definitions only inform on benefits without integrating costs that are essential to understand evolution of sexual selection. These two definitions are thus conditioned only by processes acting after copulation, such as fertilization and/or good genes effects. But it

is now accepted that both pre-copulatory and post-copulatory processes may affect sexual selection (Arnold & Wade, 1984a; Pélissié, Jarne, Sarda, & David, 2014; Pischedda & Rice, 2012).

Interestingly enough, the studied biological species, or the methods used will usually influence the decision of which definition of mating to use to analyze sexual selection. As an example, Arnold (1994) delivers his own definition sexual selection:

"*Sexual selection is selection that arises from differences in mating success (number of mates that bear or sire progeny over standardized time interval)*".

In the same paper, he also suggestss that this definition would be widely favored in the decades to come because he foresaw the rise of molecular methods for parentage assignment. If possible, it would be handy to have access to estimates for each of these definitions, in order to extract the maximum of information on both costs and benefits of reproductive strategies.

6) Methodological bias in the use of sexual selection indices

Mating success measurement can be assessed directly by behavioral observations or indirectly from genetic assignment (parentage analysis based on molecular markers), or sometimes by the combination of the two (Coltman et al., 1999; Garant, 2001; Garant, D., Dodson, J. J., & Bernatchez, 2001; Adam G. Jones & Ardren, 2003; Serbezov, Bernatchez, Olsen, & Vøllestad, 2010). However, with the rise of molecular biology, the fourth definition of mating success became the most commonly used. Indeed, reproductive success estimated from parentage analysis results in a matrix of number of

offspring produced between all possible pairs of males and females. The number of different mates producing offspring is estimated by counting the number of "positive elements" (> 0) on a single line or column of the matrix. However, such use of these matrices leads to an important bias in the understanding of sexual selection (Collet, Dean, Worley, Richardson, & Pizzari, 2014; Snyder & Gowaty, 2007). This bias revolves around the problem of zero values in the matrices: a zero value can be the outcome of different processes linked to reproductive mechanisms such as pre-copulatory mechanisms and post-copulatory mechanisms, or it can be due to sampling. A zero can therefore represent: 1) No mating success (or copulation) between a given pair of individuals, 2) a mating success (copulation) that never produced offspring due to pre-copulatory (lack of parental care) and post-copulatory mechanisms (sperm quality, cryptic choice), 3) a mating success that produced offspring who died before sampling due to good (or bad in this case) genes effect (Williams, 1966), 4) successful mating resulted in offspring but who failed to be sampled (Arnqvist, 2013).

Behavioural observations may also generate matrices of mating success with the same structure (McDonald, James, Krause, & Pizzari, 2013). While such approach allows to partly integrate some of the costs of reproduction (because all mating success will not result in offspring production for all individuals), it will nevertheless also generate a bias that will underestimate mating success in the population. This is so simply because it is usually not possible to observe all interactions among individuals within a population, hence generating spurious zero values in the matrix. Of course, and in most cases, there is also no certainty that a mating success also produced offspring.

All these spurious zero values may highly diminish our grasp of actual strength of sexual selection: because all indices of sexual selection (Is , β and βss) are calculated with this

kind of matrices, a big proportion of zeros will lead to an overestimation of the variance in mating success, and a underestimation of the mean of the mating success.

The reader may have noticed that the two approaches (behavioural observations, genetic assignation) shed light on different parts (or stages) of sexual selection. In an ideal situation, one could be tempted to take advantage of both sources of information in a single unified framework. And in a changing world, one would very much like to have access to various definitions, notably the ones that integrate costs, since costs are expected to scale with environment variations.

7) But is individual reproductive success the sole results of individual investment?

Another overlooked yet tremendous pitfall in sexual selection analyses is that, despite dealing with matter of pairs of sexual partners, all approaches fail to account for a simple founding truth: it takes two to tango. This is intuitively simple: the reproductive success and the quality of offspring is obviously most of the time the consequence of both parents' reproductive investment and genetic quality. Some theoretical models, although not directly addressing the scales of a pair of sexual partner, already accounted for that effect (Kokko & Monaghan, 2001). And yet, reproductive success is usually analysed for each parent without taking into account the effect of the sexual partner. This might at least generate two biases. First, because in many cases scientists study sexual selection in both sexes, there is therefore unhandled pseudo-replication in the analyses. But even when pseudo-replication is partly controlled for, for instance by using mixed-models to account for random-effects, it remains possible to conclude a given strength of sexual

selection in one sex whereas reproductive success might be influenced by the other sex and non-random mating rules.

8) Fitness decomposition

The direction of evolution is controlled by the evolution of fitness. Fitness is the combination of survival, fecundity and offspring number produced. While measuring indirectly the combinations of traits that yield the highest fitness gain is clearly informative on the current direction of evolution, understanding how this gain is built through the different mechanisms that compose sexual selection is of major interest if one wants to make predictions on future evolution under different environmental change scenarios. It should be of interest to study both points of view in a single framework to estimate sexual selection indices, especially for iteroparous species that may vary reproductive investment between reproductive seasons depending on their age or on environmental variation (Jones, 2009; Pélissié et al., 2014)

Arnold and Wade (Arnold & Wade, 1984a, 1984b) suggested decomposing fitness into different components taking into account the relative importance of both pre and post-copulatory components of sexual selection. To do so, they partitioned the opportunity for selection by disentangling effect of sexual traits on hierarchized components affecting fitness (Lande & Arnold, 1983). For example, fecundity and survival are the two components affecting directly lifetime fitness but are themselves affected by other components of mating systems such as parental investment, nuptial gifts or mate acquisition. Then, the authors used a series of models to analyze the statistical relationships between a particular trait and fitness components and to evaluate potential tradeoff between fitness components. Other authors such as Rose, Paczolt, & Jones (2013) and Pischedda & Rice (2012), have also demonstrated that both pre and post-

copulatory mating have their importance in estimating fitness by quantifying their contribution of sexual selection. For example Pischedda & Rice (2012) showed that without taking into account male rank as a pre-copulatory component, variance in reproductive success was overestimated, leading the conclusions towards an important role of sperm competition and cryptic female choice, two mechanisms that finally do not play a major role for opportunity for sexual selection in *Drosophila melagonaster*. Also, Pélissié et al (2014) recently developed an approach based model on behavioural observations of the snail *Physa acuta* to measure mating success and show that the effect of pre-copulatory stages are underestimated in the study of sexual selection. However, their model requires observing all copulations, which is often difficult into the wild. Adapted statistical models integrating both behavioral and genetic data on mating success, by disentangling pre-copulatory and post-copulatory would be therefore an improvement to measure sexual selection (Arnold & Wade, 1984a, 1984b; Pélissié et al., 2014; Pischedda & Rice, 2012).

Decomposing fitness to sub components associated to different stages of reproduction seems to be an appropriate method to estimate costs of mating success: at least mate sampling, mating success (in its various definitions) and reproductive success. Mate sampling indeed can help to estimate costs (the estimates of the costs of sampling in populations (Backwell & Passmore, 1996), the various determinants of mating success would inform on both the ability of individuals to access sexual partners but also on their ability to actually sire offspring (because mating is costly, parade, competition), which is another vision of the costs of reproductive investment, and simultaneously provides information on benefits of reproductive investment(in the case of matings leading to offspring production). And finally, variation in offspring production per successful

mating informs on mate quality (direct and indirect benefits) and allows quantifying benefits of reproduction.

9) Effect of environment on sexual selection

Environment may vary across time (Chaine & Lyon, 2008; Kasumovic, Bruce, Andrade, & Herberstein, 2008) and across space (spatial heterogeneity), and may act at different spatial scales: environment can differ between populations that are geographically isolated, but it can also display heterogeneity at local scale thereby proposing a range of ecological variation within populations (Jann, Blanckenhorn, & Ward, 2000). Environment is known to be the main agent of natural selection. It first acts through processes not linked to sexual selection, such as survival until reproduction. However, this selection will potentially affect phenotypic availability for subsequent reproduction. Second, environment will also influence growth and metabolic status of individuals, conditioning their future reproductive investment. The environment can also influence sexual selection directly, by biasing adult sex ratio in the population (due to differential mortality), by biasing local operational sex ratio (spatial heterogeneity of sex distribution in the population), by changing costs and benefits of mating tactics (Head, Wong, & Brooks, 2010), or by changing the relationship between mates phenotype and offspring survival, therefore changing the benefits of mate choice.

While it seems intuitively logical that costs and benefits can be environment dependent, it has also been formally described in analytical models. Kokko & Jennions (2008) assume that selective pressures influence individual tactics by acting differentially on costs outside and inside the mating pool (differential mortality of rate), and that an optimal

strategy within a population can be found where all individuals of both sexes achieve maximal fitness. They also show that the benefits inside the mating pool depend on OSR (Table 1), Adult Sex Ratio (ratio of number of mature males on number of mature females in a population at any time, ASR) and survival of individuals. Therefore survival and reproductive success in each group (inside and outside of the mating pool) govern sexual selection. It is predicted that if survival changes (in or out of the mating pool) for one of the sexes, then the evolutionary equilibrium that describes the optimal strategy for both sexes changes. Kokko and Jennions (2008) predict that evolution of sex roles relies on a tradeoff between 1) providing parental care and being no longer available for mating, and 2) avoiding parental care, therefore staying in the mating pool to improve mating prospects. Therefore, mortality rate – which is generally highly environment dependent-, OSR and ASR (that represent social and demographic environment) may lead to the evolution of sexual selection.

10) Can human induced environmental change affect sexual selection?

Many studies have demonstrated that human induced global change can act on ecological processes (Kerley et al., 2002; Relyea, 2001). A good example is provided by the effects of the alteration of climatic patterns in recent decades (IPCC 2013) that have dramatically shifted the migration dates in birds therefore affecting phenology in these species (reviewed in Gordo, 2007). Several studies showed for example that increase in temperatures may affect food availability reducing chicks survival and therefore have direct consequences on population size (Both, Bouwhuis, Lessells, & Visser, 2006). Additionally, temperatures may have considerable effect on sex ratio at hatching or birth in species with environment-sex determination depending on temperature. Urbanization,

deforestation and habitat fragmentation are other examples that may lead to different behavioural responses that will condition population dynamics, evolutionary processes and ultimately biodiversity. Specific causes of behavioural responses can be multiple (*ie.* inducing changes in the sensory environment, changes in habitat size, habitat structure and connectivity and changes in density of conspecific). Increases of temperature and global change more generally can affect individual (offspring and parents) survival (Angilletta Jr, Niewiarowski, Dunham, Leaché, & Porter, 2004; Hance, van Baaren, Vernon, & Boivin, 2007), and reproductive output (Winkler, Dunn, & McCulloch, 2002). While a lot of focuses on the consequences of human induced changes on survival and individual status, far less work has been devoted to study their consequences on sexual selection (Blanckenhorn, Stillwell, Young, Fox, & Ashton, 2006; Moller, 2004). For example, plastic behavioural responses can influence mating patterns and physiological processes (reviewed in Tuomainen & Candolin, 2011). For instance, alterations in mating behaviour and mate choosiness can for example affect gene flow between populations and generate reproductive isolation as seen in cichlids (Maan, Seehausen, & Van Alphen, 2010; Seehausen, 1997). Likewise, in sticklebacks, eutrophication in the Baltic Sea increases growth of algae, which in turn increases the time and energy spent by sticklebacks on courtship and mate choice: this variation in energy budget has direct consequences on the cost of mating (Candolin, Salesto, & Evers, 2007). The effects of human-induced environmental change can therefore also directly affect sexual selection.

II Brown trout, sexual selection, and environmental variation

1) Brown trout as a biological model for sexual selection

Darwin (1871) often reflected on salmonid astonishing life histories, either because of their life cycles, or because of their intersexual differences in traits behavior or phenotypical traits. Salmonid fishes are indeed an appropriate system for studying evolution of sexual selection facing environmental conditions. First, they are renowned for their tendency to show a wide range of variable behaviours during reproduction and these behaviours can be now be measured in natural and or experimental environments (Esteve, 2005; Freychet, 2011; E Petersson, Järvi, Olsén, Mayer, & Hedenskog, 1999; Schroder, 1981). Second, in salmonids, the environment can vary spatially and temporally leading to a possible evolution of costs and benefits of reproductive strategies which are closely linked with biotic and abiotic pressures.

The genus *Salmo* is one of the most studied within the family of Salmonidae, along with *Salvelinus* and *Oncorhynchus.* The salmonid subfamily *Salmoninae* exhibits about 30 species well described in the literature (Klemetsen et al., 2003). In the present manuscript I will describe only *Salmo trutta* L. (brown trout), because I used it as a case study throughout my thesis project. Brown trout is indigenous to Europe, North Africa and western Asia (Klemetsen et al., 2003). Brown trout is present in many regions of Europe from north of Iceland, Scandinavia and Russia to South of the Mediterranean Sea. After many introductions, brown trout has now reached a world-wide distribution (Elliott, 1994) because of its impressive capacity to spread and colonize new areas with ecological variability (Lecomte, Beall, Chat, Davaine, & Gaudin, 2013). *Salmo trutta* is defined as an anadromous fish which can have two reproductive strategies: the migratory strategy and the resident strategy. In the former, juveniles migrate to the sea to maturate with a period of smoltification and come back to their birth river or a different river for spawning (respectively “homing” and “straying”), whereas residents trout perform both their development and reproduction period in river: the present manuscript will focus only

on resident brown trout. Accordingly, river connectivity can affect dispersal in this species and environmental contrast varies greatly from upstream mountain torrents to lowland plain rivers. Thus, local conditions such as population density, ASR, OSR and phenotypic distribution may be strongly affected by these environmental contrasts.

In brown trout, females compete for spawning sites and spawn on gravel bars where they excavate a series of depressions called "nests" where they lay their eggs (Greeley, 1932). The availability of these spawning sites is structured by the variation of particle size. Particle size can notably condition oxygen availability in the redd (Acolas, 2008) and can provide a good protection for the eggs. To access females, a fierce competition between males occurs with a display of agonistic behaviours, such as chases, bites and lateral display (Keenleyside & Dupuis, 1988). Interactions between males are often hierarchized as a function of their reproductive status, *i.e.* dominant or peripheral (Blanchfield & Ridgway, 1999; Erik Petersson & Järvi, 2001). Larger males have been described as more advantaged in comparison with smaller males during contest competition in different species of salmonids (Fleming & Gross, 1994; Schroder, 1981). Females have been reported to exhibit preference for adiposis fin size (Petersson et al., 1999) and for relative individual body size (Labonne et al., 2009). As a result of strong preference and competition, sexual selection is expected to be relatively strong in brown trout, and recent analyses confirm this view, while also mentioning the role of environmental uncertainty in the maintenance of plasticity in sexual behaviours (Serbezov et al., 2010). Although this thesis will not focus on the genetic basis of traits involved in sexual selection, it is of interest to note that the salmonid genome underwent a polyploidy event some tens of millions years ago (Allendorf & Thorgaard, 1984; Hoegg, Brinkmann, Taylor, & Meyer, 2004), and that the current genome might be highly influenced by this event: former copies of genes may have evolved to code for different functions, whereas some others

may still code for similar functions. Second, this polyploidy event *de facto* erased the sex chromosome. Recent research suggest that a Sex locus is now present in many salmonid species, but at various stage of degradation, and very little is currently known regarding the genes that might be physically linked to this locus (Yano et al., 2012, 2013).

2) Environmental change and brown trout reproduction

In addition to changes in land use, water use and river channelization that may affect the brown trout life cycle at various stages and levels, the effects of climate change since the late 19th century (IPCC 2013) also threatens river ecosystems. This is particularly theoretical models predict an increase of the rainfall perturbation in frequence and intensity (Dankers & Feyen, 2008; Milly, Dunne, & Vecchia, 2005; R. J. Stevenson & Sabater, 2010; Vitousek, 1994). Indeed the increase in the frequency of extreme rainfall events is expected to directly influence water discharge in rivers, thereby potentially affecting the suitability of reproduction habitats for brown trout. Stream flow is predicted to increase in the western areas of Europe (Stahl et al., 2010; Stahl, Tallaksen, Hannaford, & van Lanen, 2012) such as in the Pyrénées mountain range. Moreover an increase of water temperature in rivers is also predicted with an increase of air temperature (IPCC 2013) which can affect metabolic rate of individuals and therefore their allocation in biological activities such as reproduction (Charnov & Gillooly, 2004; Gillooly, Brown, West, Savage, & Charnov, 2001).

An increase of water discharge may have direct consequences on resource availability especially in freshwater food webs (Perkins, Reiss, Yvon-Durocher, & Woodward, 2010). Therefore energy stores are affected which will in turn modify the allocation of energy to

the different functions (*e.g.* reproduction, survival, maintenance…) and will ultimately modify condition survival. This would in turn shuffle the initial conditions at the onset of reproductive season, by changing density, ASR and OSR of populations. Increased stochasticity in river water flow could also impact the energetic budget of spawners during reproduction, by impacting directly the cost of competition or parental care. Droughts and large floods may also have direct impacts on habitat structure. They may impact significantly survival in redds which, by providing protection against predation for the embryonic stage, are at the center of this species' reproductive system and life cycle. Because the adaptive value of behaviours associated to sexual selection mechanisms is modulated by offspring survival, the evolution of reproductive system in brown trout is probably linked to variations in selective pressures on offspring viability.

For all these reasons, it is logical in this thesis to investigate the evolution of populations and sexual selection in relationship with environmental change, and specifically with climate change.

3) How to read this manuscript

The general objective of this work is to investigate the effects of environment (at different scales) on the evolution of sexual selection in brown trout, to better understand how environmental factors can shape the evolution of traits and behavioral responses. Environment was therefore considered at different scales: 1) individual scale 2) inter-individual scale, taking into consideration phenotypic traits of sexual partners 3) inter-population scales. To address those scales, two groups of experiments were designed: in natural and in semi natural conditions, which is a prerequisite to measure effects of

selection in a realistic context. I also developed specific experimental and statistical methods to improve the measure of reproductive investment and to increase our insight into fundamental components of sexual selection such as mating success. Using these new methods, as well as the general background of behavioural and evolutionary ecology, I then studied the effects of environmental variation on the costs and benefits of each individual strategy involved in reproduction. I particularly focused on one expected trend in environmental modification: increased stochasticity of water flow.

The present manuscript is composed of **six chapters**, the **first** one being this introduction. The **second chapter** describes the different experiments conducted in order to answer to the general objectives of this thesis. The reader will also find there some technical developments regarding several aspects such as the measure of reproductive investment. Often, in the following chapters, references will be made to this methodological chapter. Sometimes though, methodological details will be revealed later in each chapter, because they do not concern the whole document. This should avoid unnecessary page browsing. The **third chapter** is based on an experiment and describes how individual status affects components of sexual selection. This individual status is investigated through traits, such as weight and its variation, but also through metabolic condition (as revealed by the study of energetic metabolites dynamics in the plasma) over the reproductive season, or behavioural activity during reproduction. The **fourth chapter** will then replace the individual in its social context: I will there investigate interactions between individuals, as seen by OSR and phenotypic availability variation, for instance, and some aspects of intra-sexual competition. A special focus will then be made on the fundamental dependency between sexual partners to analyse mating and reproductive success, and to that end, I will propose a new statistical model to decompose sexual selection stages, account for phenotypes of both sexual partners, and improve the use of various source of

data in a unified framework. I will also propose a comparison with the classical approaches to estimate selection gradients in sexual selection. The **fifth chapter** will bring environment into action, benefiting from the previous chapters and developments to improve our grasp on environment effects on sexual selection. I will here study how environmental stochasticity may affect post-zygotic selection through habitat selection by females and how environmental stochasticity may condition reproductive investment, mating success, and reproductive success. These multiple potential effects of environmental stochasticity will each time be investigated in two populations, in order to check if environmental variation has a uniform impact on populations, or if each population may react differently to this selective pressure. I also placed individuals originating from different populations in sympatry, which will allowed testing reproductive isolation (and therefore gene flow) due to sexual selection between populations, depending on environmental contrast (here, the stochasticity of environment).

In each of these five chapters, some elements of discussion will be provided. The last and **sixth chapter** proposes a more general discussion. Here, I will then try to synthesize my findings, review the progresses made and the obstacles encountered, point at areas where more investigation is needed, and finally provide a general perspective for the effect of environment on sexual selection in brown trout.

Finally, some **Supplementary Informations** are provided afterwards. The first four elements of these **Supplementary Informations** represent projects of scientific articles either submitted to review or still in progress. They are not necessary for the reader, but they may sometimes provide additional details, or propose a different point of view on my work.

CHAPTER II.
Experimental approach and methods

I. Context

Several experiments were conducted during this work either in a semi natural environment (A, B1, B2) or in a natural environment (C) in order to address different aspects of reproductive success at different scales.

Experiments in the semi-natural environment (A, B1, B2) were built as monitor reproductive behaviours of known individuals over the course of a whole reproductive season in an artificial channel beside the Lapitxuri stream, a tributary to the Nivelle River in south-western France (+43° 16' 59", -1° 28' 54"). The setup of these experiments will be developed in the first part of this chapter.

The experiment led in natural environment (C) was built in order to track females' choice for spawning site, and to relate habitat choice to offspring survival, accounting for individual egg size. This experiment will be described in the second part of this chapter.

II. Experiments in semi-natural environment

1) Semi-natural conditions: the Lapitxuri spawning channel

Study of reproductive behavior in the wild is informative, although it is a challenge: it is usually performed on open populations with little (and costly) possibility to have access to both individual identification during mating behaviours and reproductive success over a whole reproductive season. Additionally, many factors can be confounded and hard to interpret in a context of changing environmental conditions that may prevent efficient monitoring. Alternatively, reproductive behavior of brown trout can be studied in

controlled conditions (Petersson et al., 1999) which howaver oversimplify environmental influences. For example, individuals are generally constrained to a limited number of mates, which may modify intra-sexual competition and inter-sexual preference compared to natural conditions.

Here I targeted a specific objective: to be able to monitor reproductive activity and reproductive success of a whole group of individuals, without interfering with mate choice rules**.** To do so a first **experiment A** (fully described in the paragraph II.II.7.a) was conducted in 2010-2011 and constituted a first test of wild brown trout reproductive behaviour in the artificial channel of Lapitxuri. I inform the reader that this experiment (experiment A) was undertaken by the lab just before the beginning of my PhD (Freychet, 2011). Back in 2010, we had no evidence that this approach would succeed, but relying on the lab's experience of reproductive behavior in wild populations, we had some precise ideas of what to expect in terms of behavioural patterns (Garcia-Vazquez et al., 2001; Labonne et al., 2009; Tentelier, Larrieu, Aymes, & Labonne, 2011). In the following paragraphs (from II.II.2 to II.II.6), I detail common methods and information for experiments A, B1 and B2. The differences between these experiments are explained in paragraph II.II.7.

The Lapitxuri channel is a derivation of the Lapitxuri stream, a tributary to the Nivelle River in south-western France (+43° 16' 59", -1° 28' 54", Fig. 1). It has already been used for many experiments focused on Atlantic salmon reproduction (A. Hendry & Beall, 2004). Because the experimental channel is a derivation from a natural river, food is readily available by drift from incoming water. The channel (total length = 130 m) consists of 13 communicating and linear sections, each measuring 10 meters long and 2.80 meters wide. Upstream and downstream exit from each section can be prevented

with grids, and net traps can be placed downstream of each section to catch drifting individuals. The whole channel is covered by nets to prevent avian predation, as well as to protect from disturbance.

Several environmental features can be manipulated in the experimental channel, making it a quite flexible tool to test predictions about the effect of environment on fish reproduction. Riverbed can be modified by adding, removing and arranging different substratum size. Likewise, water depth can be managed at the scale of each section by placing planks of a chosen height at the downstream limit of the section, and at a finer scale by adding or removing substrate. Moreover, woody debris can be placed anywhere to build hiding places for fish. Hence, one can easily arrange favourable zones for spawning or resting. Water discharge can also be manipulated by controlling the quantity of water derived from the Lapitxuri stream. Thanks to both an outlet and a supply pipe plugged between the seventh and the eighth sections, water discharge can be manipulated independently (to some extent) in the upstream and downstream halves of the channel. Additionally, and importantly for studies on reproduction, the density and sex ratio of groups of fish can be manipulated in each section, since sections can be isolated from each other with grids.

Finally, the artificial channel provides advantages for monitoring reproductive behaviour and reproductive success. The net protecting the channel against predation also serve as a hiding fence for observers, and the power outlets along the bank allow plugging video cameras and spotlights to record behaviour on a long term. The precise estimation of reproductive success is facilitated by the possibility to collect virtually all juveniles at the end of the spawning period, either by electrofishing or in drift nets downstream each section.

Figure 1*. Lapituxuri experimental channel. Nets are deployed to prevent birds from fishing. Passage between sections can be prevented if required. Substratum can be modified, as well as water depth and shelters availability. Additionally, water discharge can be controlled by tuning the in-flow from the natural river, independently for sections 2 to 7 and 8 to 13 respectively (thanks to a by-pass) and can also be regulated using a water pump (lower left corner of the picture).*

2) Experiments timeline

For all three experiments A, B1 and B2, male and female spawners were sampled in the wild (exact places are given later), then acclimatized during 48h in tanks without food and released in the artificial channel (Fig. 2). All fish were diagnosed as mature to semi-mature through the presence of sperm (males) and the presence of eggs (females) (with different maturity degrees for females), assessed by gentle pressure on the fish's abdomen. After acclimatization, they were individually anesthetized (0.3mL/L of 2-phenoxyethanol), measured, weighed and photographed for individual recognition. A

blood sample (500 µL) was also taken from the caudal vein of each fish, for further analysis of plasma metabolites. Fish were then released in the artificial channel where they were free to move and reproduce. Behavioural activity was video recorded during all the reproductive period including night period using some lights. Aerial and sub-aquatic cameras were adequately placed each time reproductive activity was detected. Video records were analysed each day to remove non-essential data and to free recording space.

After the last reproduction (usually middle of January), trout were removed from the experimental channel, anesthetized, identified (from pictures taken before reproduction), measured, weighed, and a small piece of their caudal fin was cut and placed in absolute ethanol for genetic analysis. A blood sample was again taken from each fish, in order to monitor variation of plasma metabolites between the beginning and the end of the spawning period. Males and females were stripped to assess if there was any remaining eggs or sperm. They were then kept in a tank and released 48 hours later in their original river.

After the removal of adults, traps were checked every day to capture the emergent juveniles (Argent & Flebbe, 1999). At the end of the experiment (800 degree.days and about two months after the last reproduction), all remaining juveniles were captured by electrofishing. A subsample of the total juveniles was kept for genetic analysis: 20 individuals were taken randomly each day from the traps irrespective of the number of juveniles trapped and 20% of the electrofished individuals were kept randomly. Bigger juveniles were sampled for a piece of caudal fin after being anesthetized. Other juveniles were killed with a lethal dose of 2-phenoxyethanol and placed individually in a tube of absolute ethanol (90°). The remaining juveniles were released in their river of origin (experiment A only).

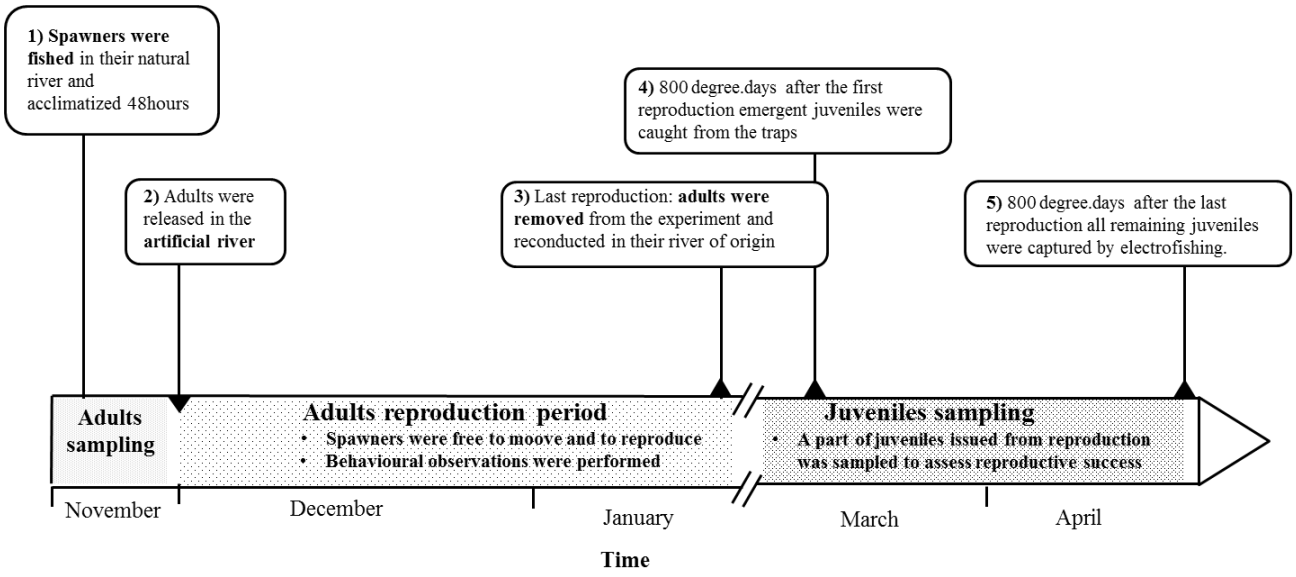


Figure 2. *Timeline of the experiment conducted in the artificial river (semi natural environment, EXP A, B1 and B2) through time. Boxes indicate the five different steps of the experiment. Arrows pointing downwards indicate introduction of fish in the experiment whereas those pointing upward show the removal of spawners and juveniles from the experiment.*

3) How to recognize fish during reproduction

One of the key factors in such experiments is to be able to identify individuals, to relate their behaviour, traits (body size and colourness) and fitness at individual scale. Individuals were thus measured, weighed and photographed for recognition before and after reproduction. No tagging of any sort was used, so to avoid interference with either survival or behavior (trout is thought to use visual cues in both intra-sexual competition and inter-sexual preference (Petersson et al., 1999). Fish recognition was possible before and after reproduction from inter-individual phenotypic variation: the density and the position of both black and red spots vary consistently from one individual to another and do not change over the reproductive season (example in Fig. 3). This phenotypic

consistency not only ensured individual recognition on pictures taken at the beginning and the end of the experiment. But also allowed individual recognition on underwater videos sequences shot during reproduction (Fig. 4).

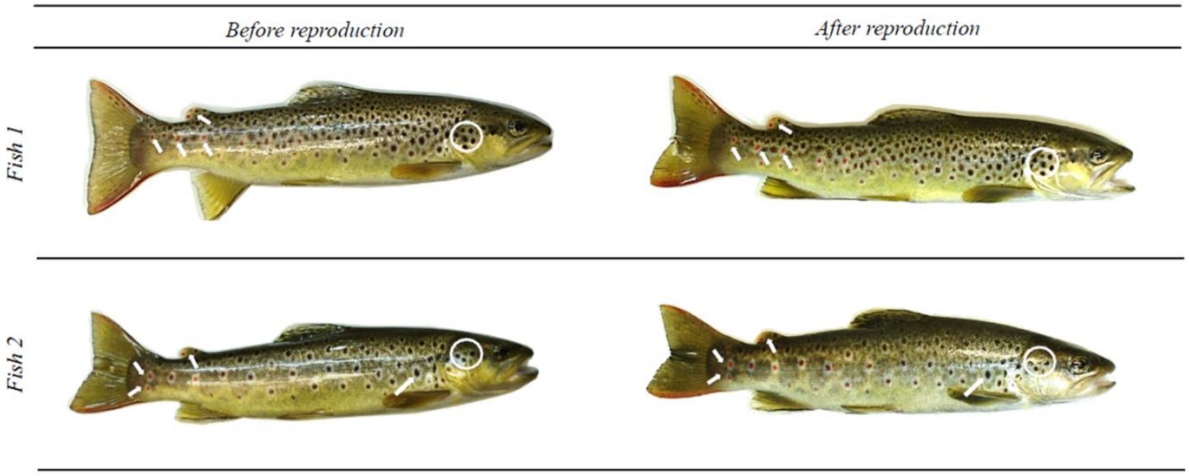


Figure 3. *Pictures of two fish before and after reproduction .The number and the color of spots vary from one individual to another one but stay constant for one individual. The white arrows and open circles show specific spots in specific areas determinant for fish recognition.*

Figure 4. *Pictures of a specific female a) before the reproduction and b) during a reproductive event. The white arrows and open circles show specific spots in specific areas useful for fish recognition during the reproductive period. Each individual possesses its own specific spots that make us able to be distinguished from other individuals.*

4) Behaviour recording

Video recordings were performed during the period of reproduction (Fig. 2) in order to acquire behavioural data such as digging behaviours in females and competition between males. To do so, individuals were observed each day from the river side. When reproductive behaviours indicating that a female and one/or several male(s) were close to spawning (female digging , males chasing), subaquatic video camera were placed in the river and aerial digital video cameras were placed on the bank (Aymes, Larrieu, Tentelier, & Labonne, 2010; Tentelier et al., 2011). Subaquatic view was mainly used to identify

fish and ascertain behavioural item, while aerial view allowed estimating the operational sex ratio.

For each mating episode defined as one female lays her eggs and at least one male releases sperm), 3 hours of videos were analyzed. This consisted of 90 minutes before gamete release and 90 minutes thereafter in order to identify individuals that participated to the encounter process (individuals present during this mating episode) followed by the copulation process (individuals releasing gametes during this mating episode, Fig. 5) and to record behavioural items. This is hereafter referred to as an Observation Unit (OU)[1]. The pre-copulation period corresponds mainly to the intra-sexual competition between males, whereas the post-copulation was expected to yield informations on parental care (explained later). Different behaviours were recorded such as the number of diggings for each female during an OU, the number of chases emitted by each male, the presence of each individual and the release of gametes (Table 3).

Observation units were also space-limited: a zone of one meter around the female's nest construction was defined visually. Individuals were considered as "present" when they entered the zone. They were considered as "absent" when they were outside of the zone. Any male and female pair present (not necessarily simultaneously) during a given OU was noted as having encountered each other. The total number of observed encounters was stored in a male x female matrix. A copulation event was defined as the simultaneous gamete release of a male and a female. The total number of observed copulations over the experiment was also stored in a male x female matrix (see Supplementary Informations 7). Finally, in some occasions, some individuals, despite being present in the spawning zone, were too far from the subaquatic camera to be unambiguously identified. These

[1] Note that an Observation Unit is a mating episode that was observed, and in which copulation occurred.

individuals were therefore not directly taken into account for the encounter observations, but we accounted for that bias in some of our analyses (see § IV.V).

Operational sex ratio (OSR) was estimated at the scale of the OU based on video recordings. It was determined as the total number of identified and unidentified males engaged in the intra-sexual competition around the focal female during the OU. Whenever one or possibly more unidentified males were present, we incremented the OSR numerator accordingly. Indeed, some males could remain unidentified simply because they were not seen close enough from the subaquatic camera. However, the aerial camera usually allowed to estimate the maximum number of males simultenaously present around the redd.

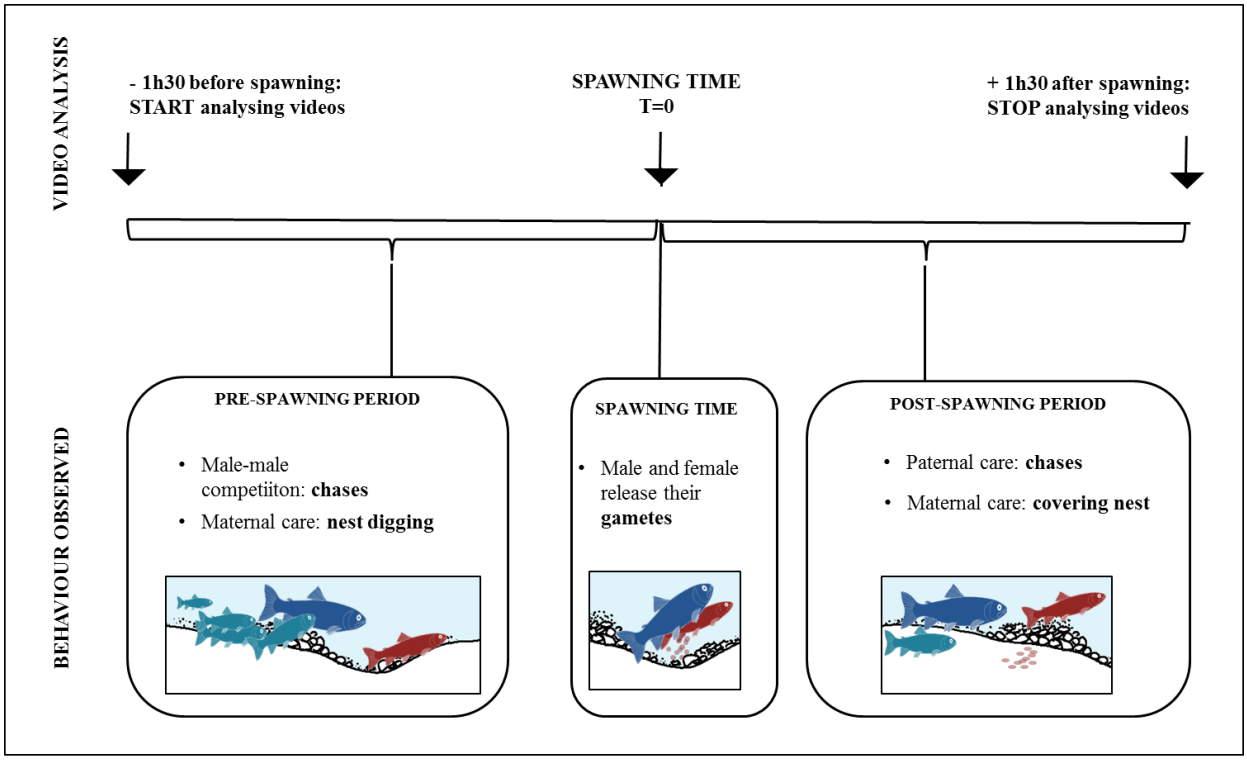


Figure 5. *Timeline of the different behaviours occurring during the video analysis. In the present study, only the number of digging of females and the number of chases between males were recorded (during both pre-spawning and post-spawning periods).*

Table 3. *Ethogram of the different behaviours measured during a reproductive bout*

Behaviour	Behaviour description
Chase	An individual male towards acompetitor, resulting in either the competitor fleeing, or a bite from the darting male.
Digging	Laterally oriented movement from the female to excavate substratum from the ground.
Female gamete emission	Female quivering with jaws open, followed by eggs expulsion. NB: false orgasm is an option, and it is usually spotted because females do not cover the redd afterwards.
Male gamete emission	Male quivering with jaws open, followed by sperm expulsion.
Presence state	An individual entering the 1m buffer zone around the nest.
Absence state	An individual leaving the 1m buffer zone around the nest more than 10 seconds.

5) Reproductive success estimation

In order to estimate reproductive success of each individual, a genetic assignment method based on microsatellites was used. Four steps were needed to accomplish this work: DNA extraction, microsatellites multiplex PCR, genotyping and parentage analysis.

a) DNA extraction

DNA was extracted with a modified NaCl / chloroform based protocol (Müllenbach, Lagoda, & Welter, 1989) to use 96 wells plates allowing extracting high quality DNA from 192 samples per day at low cost: 0.5 cm² of fin clip was lysed with 200 µL of buffer (NaCl 75 mM, EDTA 25 mM, Sulfate Dodecyl Sodium 1%, pH 8) containing 10 µL of proteinase K at 20mg/µL in a 1.2 ml microtube. Samples were then incubated at 55°C

overnight. 100 µL of NaCl 5M was added, tubes were gently shaken, and 300 µL of chloroform were added. After gently mixing for 10 minutes, samples were centrifuged at 2000 rpm for 10minutes, the upper phase removed to a new microtube. DNA was precipitated with 250 µL of isopropanol, and after 5 min of mixing samples were centrifuged for 5 minutes at 4100 rpm. The supernatant was removed and the DNA pellet washed with 500 µL of 70% ethanol for one hour. After a centrifugation step of 5 minutes at 4100 rpm, ethanol was discarded, the DNA was dried at ambient temperature and pellet was finally re-suspended in 100 µL of TE 1X buffer.

b) Microsatellite multiplex PCR

Amplification of eight microsatellites was carried out in a 5 µL final volume using Qiagen Type-it Microsatellite kits. Each reaction contained 1X PCR Master Mix, 0.2 µM of each unlabeled reverse (Eurofins MWG Operon) and labeled forward primer (6-FAM: Ssa85, Str73INRA, Ssa410Uos, HEX: Str60INRA, Ssosl417, Ssa408Uos (Eurofins MWG Operon) or NED: SsoSL438, Sssp2216 (Life Technologies)) and approximately 25 ng of template DNA. The amplification reaction was carried out using a Applied Biosystem 2720 thermal cycler (Life Technologies) and consisted first in an initial denaturation at 95 °C for5 min, followed by 30 cycles of denaturing at 95 °C for 30 s, annealing at 57°C for 3 min, extension at 72 °C for 30 s and a final extension step at 60 °C for 30 min.

Eight microsatellites previously developed for salmonids were selected: *Str60INRA*; *Str73INRA* (Estoup, Presa, Krieg, Vaiman, & Guyomard, 1993); *SsoSL438* (Slettan, Olsaker, & Lie, 1995)*; Ssa85* (O'Reilly, Hamilton, McConnell, & Wright, 1996)*; SsoSL417* (Slettan et al., 1995)*; SSsp2216* (Paterson, Piertney, Knox, Gilbey, & Verspoor,

2004); *Ssa410Uos* and *Ssa408Uos* (Cairney, Taggart, & HOyheim, 2000). We used a multiplex protocol allowing amplification of the eight loci in one polymerase chain reaction (multiplex PCR, Fig. 6) following Lerceteau-Köhler & Weiss (2006).

c) Genotyping

Amplified fragments were sized on a ABI 3100-Avant (Life Technologies) using a GeneScan 500 LIZ internal size standard (Life Technologies), scored twice to check error rateusing STRand software (Toonen & Hughes, 2001) and raw allele sizes were binned into discrete allele classes using MSatAllele package (Alberto, 2009) for R version 2.13.0 (R Development Core Team 2011).

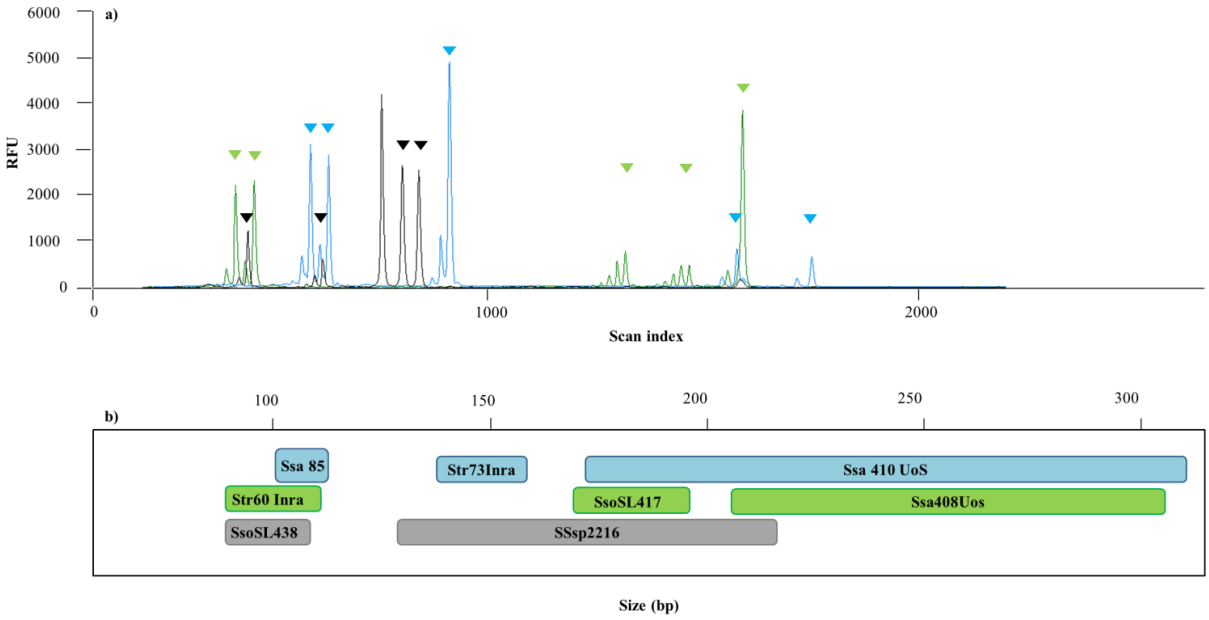


Figure 6. *Examples of microsatellite electrophoregram profile with the multiplex PCR for one individual (a) and diagram showing allele size range of each microsatellite (b). Triangles indicate alleles at each locus. In (b), rectangles represent the potential allele size range known from the literature.*

d) Parentage analysis

Parentage analysis was performed using Cervus software (version 3.0.3, Kalinowski 2002) to assign parents to each sampled offspring, using allele frequencies computed from the genotypes of the candidate parents. The following simulation parameters were used: 10 000 cycles, a number of candidate mothers and candidate fathers depending on the experiment, a mistyping error rate of 1%, a genotyping error rate of 1%. We used the "parents pair analysis, sexes known" option in Cervus to assign juveniles to parents. All juveniles with more than one locus missing were removed from the analysis. We accepted parentage assignment at confidence level of 80% and only when the juvenile was assigned to two parents. Hardy Weinberg equilibrium and linkage disequilibrium between loci were tested using Genepop 4.2 (Rousset, 2008) with Bonferroni correction for multiple comparisons.

6) Measures of plasma metabolites concentration in blood samples

a) Why plasma metabolites?

Reproductive investment is often estimated through weight variation in many species (see § III.II). Here I simply describe an alternative approach using plasma metabolites to investigate reproductive investment during the reproductive season (*i.e.* after gamete maturation) in brow trout. On the one hand, plasma metabolites analysis is based on simple blood tests and therefore could be used as a non-lethal method; on the other hand they can inform about the energetic status of individuals. When the energy obtained

through food by an animal surpasses the metabolic expenditures, excess energy is stored in form of lipids in adipose tissues and in muscles. On the contrary, when the levels of energy readily available fall, lipids (such as triglycerides) are released into blood to provide energy in the form of adenosine triphosphate and will be later degraded into fatty acids in an energy-yielding process called muscle lipolysis (Sargent, Tocher, & Bell, 2002). Under relative fasting, the dynamic of triglyceride concentration in blood globally decreases steadily (Kakisawa, Kaneko, Hasegawa, & Hirano, 1995). By contrast, fatty acids first show a plasma peak before decreasing (McCue, 2010). If fasting lasts, animals turn to use muscle proteins (proteolysis), thus leading to increased concentrations of plasma amino acids (Black & Skinner, 1986; McCue, 2010). High muscle proteolysis denotes a poor metabolic condition of individuals since they consume amino acids when fat reserves are spent. Finally, glucose is not used as a main energy provider in fish. Plasma glucose level during fasting tends to be rather stable, but an increase in glucose concentration denotes a physiological stress that fish may undergo over the course of reproductive season (Schreck, Contreras-Sanchez, & Fitzpatrick, 2001; Silbergeld, 1974). Therefore, plasma metabolites can be analyzed to infer energy expended by organisms in period of intense activity, such as reproduction. For instance, the concentration of triglycerides in plasma is a good indicator of bird health and reproductive success (Masello & Quillfeldt, 2004; Merilä & Svensson, 1995).

In species in which gametogenesis occurs before the reproductive season, such as brown trout, the measure of plasma metabolites during the reproduction does therefore not account for gametic investment (probably more explained by weight variation upon gamete release). Additionally, in many species, food intake is reduced during the reproduction because animals allocate preferentially their time and energy to different

reproductive activities such as looking for mates or defending territories (R. A. Anderson & Karasov, 1988; Barboza & Jorde, 2001; Cherel et al., 1988; Doucett, Booth, Power, & Mckinley; Esteve, 2005). The variations of plasma metabolites may therefore reflect a specific part of reproductive investment, largely independent from gametes production.

b) Method description

A blood sample (500 µL) was taken from the caudal vein of each individual with a disposable heparinized syringe at the beginning and at the end of the reproductive period. Blood samples were centrifuged for 5 min at 3500 rpm, 300 µl of plasma were removed and placed in a new tube, and immediately frozen at -20°C and then at -80°C. As previously measured in other studies (Kamalam et al., 2012; Panserat, Perrin, & Kaushik, 2002), the concentration of plasma glucose (Glucose RTU™ kit, bioMérieux, Marcy l'Etoile, France), triglycerides (Sobioda kit, bioMérieux) and free fatty acids (NEFA HR kit, Wako Chemicals, Neuss, Germany) were determined using commercial kits adapted to a microplate format. Total plasma free amino acid levels were determined by the ninhydrin reaction (Moore, 1968), with glycine as standard. The metabolite concentration for each adult was measured in $g.l^{-1}$ before and after the reproduction.

7) Differences between A, B1, and B2 experiments.

a) Experiment A: constant environment, single population.

Five communicating sections (total length = 50 m) of the experimental channel were used during the experiment out of the thirteen sections available. Traps were placed as the

downstream limit of the most downstream section to catch drifting individuals, and upstream movement from the most upstream section was prevented with grids. The stream bed was covered with coarse gravel (1-2 cm diameter). Each section provided a spawning ground in its upstream part with a mean depth of 15 cm, as well as a shelter area with visual obstacles (mean depth of 30 cm). Individuals were free to move in all the 50 meter area.

Wild brown trout (29 females and 20 males ranging in size from 18 cm to 38 cm) were sampled by electrofishing in the River Bastan (+43° 16' 2.51", -1° 22' 32.46") in November 2010 and transferred to the experimental channel after having undergone measures, sample collection and pictures as described in § II.II.3. Few fish were found in the traps during the first week of the experiment, and this process ceased afterwards (note that spawners could leave the trap to return into the experimental sections without much difficulty). The last mating episode occurred on the 14th of January 2011, after which adults were collected, underwent treatments described previously before being released in the River Bastan. In March and April 2011, 1088 juveniles were sampled for genetic analyses and the remaining were released in the River Bastan.

b) Experiment B1 and B2: replication of results, population effects and environment control.

A new experimental setup was built from November 2012 to April 2013, with some modifications on the channel habitat arrangement (see below). More importantly, I included two main factors in the experimental design. First, I used different sections of the channel to simulate different hydrological regimes (constant versus stochastic). Second, I mixed individuals from two different populations: this allowed to investigate

inter-populations variations in our results and to study possible reproductive isolation between populations (see § V.III).

Two separated reaches of 30 meters (= 3 channel sections) each were constituted to form two distinct environments controlled by different water flow during the entire experiment: constant water flow (Constant environment: experiment B1) and variable water flow (Variable environment: experiment B2). In the constant environment (experiment B1), water flow was maintained around 210 $m^3.h^{-1}$. In the variable environment (experiment B2), rapid discharge variations were executed and followed three modalities: high discharge (360 $m^3.h^{-1}$) intermediate discharge (210 $m^3.h^{-1}$) and low discharge (180 $m^3.h^{-1}$). The duration (in days) of each modality was drawn randomly in a discrete uniform distribution [1-3], and a natural order was respected: low discharge followed intermediate discharge, and intermediate discharge followed high discharge. The magnitude of discharge variation was relatively low compared to observable variations in natural conditions. However, the speed of water level change was much faster (about 1 to 3 minutes) than in natural environment. Within each environment, as written above, three communicating sections were used, each measuring 10 meters long and 2.80 meters wide. In the middle section, channel bed was set up with specific size of substrate and dedicated to reproduction (diameter =[5-20] mm) (Fig. 7) with water depth between 13 and 15 cm at intermediate discharge, whereas the upstream and downstream sections were arranged with coarser substrate (diameter =[40-80] mm) (Fig. 7), variable depth (from 0 to 60 cm) and visual obstacles, in order to provide hiding and resting areas for fish. Fish were free to move between the three sections in each environment (1 or 2). Traps were placed downstream of the most downstream section of each reach to catch drifting individuals, and grids were placed upstream of the most upstream section of each reach to prevent upstream migration.

As previously mentioned, I also decided to mix fish from two different origins: River Bastan (France, +43° 16' 2.51", -1° 22' 32.46") and River Urumea (Spain, +43° 14' 31.81", -1° 55' 28.98"). These rivers were chosen because they differ in their flow conditions, the River Bastan having more predictable flow conditions than the River Urumea, mainly with less numerous high and low pulse events per year, a lower coefficient of variation of annual discharge, as well as lower coefficients of dispersion for monthly discharge (estimation on daily discharge time series over 31 and 17 years respectively, see Table 4). The rivers presented a comparable annual mean discharge (about 6m3.s-1) in locations where spawners were sampled.

Fish were released in the artificial river from November 21th to December 13th 2012. Fish of both populations were attributed quasi-randomly to an environmental section: we made sure that the distribution of body size between the two different environments were the same. Fish were removed using electrofishing from the artificial river on February 13th 2013, two weeks after the last reproduction observed. Juveniles were captures by checking traps and by electrofishing. Only adults were brought back to their natural river, all juveniles were killed with a lethal dose of 2-phenoxyethanol and placed individually in a tube of absolute ethanol (90°) to later conduct genetic assignment of parentage.

Table 4: *Synthetic indicators of hydrologic alterations as calculated by IHA software. Indicators calculation and interpretation are described in Colwell 1974 and in Poff and Ward 1989. Here, the indicators determine the River Bastan as a snow/rain driven system, whereas the River Urumea is determined as a perennial runoff system. Values in bold style indicate significant differences in the stochasticity level.*

System	Bastan	Urumea
Period of analysis	1981-2011	1995-2011
General parameters		
Mean annual flow ($m^3.s^{-1}$)	6.67	6.08
Annual Coefficient of variation	1.21	**1.56**
Flow predictability	0.53	**0.44**
Constancy/predictability	0.79	**0.72**
Low pulse count	7	**19**
Low pulse duration	6.75	**3**
High pulse count	9	**10**
High pulse duration	4.5	**3**
Monthly discharge coefficients of dispersion		
January	0.9372	**1.191**
February	1.156	**1.868**
March	0.6614	**1.074**
April	0.8299	**1.892**
May	0.535	**1.32**
June	0.5411	**1.072**
July	0.4653	**0.6262**
August	0.3051	**0.3493**
September	0.2559	**0.2633**
October	**1.039**	1.031
November	1.031	**1.792**
December	**1.03**	0.9364

a)  b) 

Figure 7. *Example of a) section dedicated for resting activity with woody debris and b) section dedicated to the reproduction with specific substrate in the Lapitxuri site experiment.*

Table 5. *General informations about the three experiments*

		Experiment number		
		A	**B1: Constant waterflow**	**B2: Variable waterflow**
Reproductive period				
	Period	Nov 2010-Jan 2011	Nov 2012-Jan2013	Nov 2012-Jan2013
	Population	Bastan	Bastan/Urumea	Bastan/Urumea
	Number of females	29	32	31
	Number of males	20	17	19
	Number of copulation observed	11	22	14
Juveniles				
	Subsample of juveniles	1088	555	732

III. Natural environment: experiment C

Females choose habitat of reproduction and dig their nest under a gravel bar to lay their eggs which will be simultaneously fertilized by one or several males. Eggs and later the vesicled alevins, then spend a few months (about 800 degree.days) under the gravel. To survive under gravel, they have to avoid predation, desiccation, hypoxia and scouring, all processes possibly dependent on physical features of the redd, such as depth, water velocity or gravel size. Moreover, the optimal habitat for egg survival may depend on the size of the egg. Field experiment C aimed at testing the effect of features of the spawning habitat chosen by females on the survival of their eggs, controlling for egg size.

1) Sampling sites and reproductive activity

In order to monitor the correlation between female habitat choice, egg size and egg survival, three samplings have been realized during three consecutive seasons of reproduction (from November to January 2011-2012; 2012-2013; 2013-2014) on two rivers: the River Bastan (+43° 16' 2.51", -1° 22' 32.46") and the Lizuniaga brook (43°17'02.9"N 1°37'02.2"W), a tributary of the River Nivelle.

These systems have been selected because of their accessibility, and because the reproduction activity in these rivers have been previously observed by the lab team. The Bastan is more torrential, wider, water level is less variable and fish size is more variable. We combined these two sites in order to maximize the range of variation in fish traits and habitat features. The reader should also be aware that the differences in flow regimes

partially conditioned my decision to sample reproductive activity. For instance, during the second year of sampling (2012-2013), high flow events constrained me to mainly sample the Lizuniaga brook because of its lesser average discharge than the River Bastan, which was not accessible at that time. An attempt on the river Urumea (from where some of the spawners were sampled for experiments B1 and B2) did not provide enough data due to the high difficulty to observe reproductive activity *in situ* for this river (only 1 sampled redd during the whole first winter).

In order to obtain the different samples, the first step consisted in detecting reproductive activity on a spawning site between one female and one or several males (female digging and chases between males) and to measure this female. To determine its size, the female was first photographed. Then, one conspicuous object (stone, stick of wood…) present on the picture was measured with a ruler after the end of reproductive activity on the redd. Female size was then deduced relatively to the object actual size with an image processing software (ImageJ, 1.45s). When the precise moment of fertilization was observed, we waited 30 minutes to let the female cover her eggs before processing to further samples. When we observed reproductive activity without being able to stay until fertilization, we came back the day after to confirm that the nest was finished. Initially, I had hoped to relate reproductive activity before fertilization to female habitat choice and egg survival, by first observing reproductive activity on the redd, and then manipulating eggs and tracking their survival. But the odds of observing a whole reproductive sequence, and then finding eggs were very low, so I decided to limit the protocol to measuring female size, habitat characteristics, and egg size and survival.

2) Habitat variables, egg size and experimental setup

Different variables were measured directly on the redd once the reproduction occurred in order to see their potential effect on offspring survival:

- Particle size of substrate moved by the female for redd construction.
- Depth of burying for eggs.
- Egg volume at the individual scale.

To analyze the potential effect of particle size, pictures were taken directly on the field and were analyzed later on the lab. A reference frame (50 cm x 50cm) was used and disposed around the dome. An umbrella was used to prevent light reflection leading to unusable pictures.

The following step consisted in excavating the eggs from the redd. In order to determine the depth of the nest, the water level above the dome was measured. Then, particles of the dome were removed carefully until the eggs were found. Thirty eggs were pulled out gently with a pipette and placed in a tank. Water level above the place where eggs were found was measured. The depth of the nest dug by the female was thus deduced (*Water level above the dome – Water level above the eggs*). A net was placed downstream the nest in order to intercept eggs drifting because of nest disturbance.

Eggs were aligned in a gutter equipped with a ruler, and a picture was taken to measure individual egg area at the with ImageJ software. From the area measured on each egg, egg volume was calculated (Egg volume = $(4*Area^{1,5})/(3*pi^{0,5})$. To link egg size to survival, eggs had to be "individualized" throughout incubation. For this, eggs aligned in the gutter were numbered from 1 to 30 and distributed systematically in six survival capsules designed to monitor egg survival in the field (Dumas & Marty, 2006, Fig. 8). Five eggs were placed in each capsule (A to F), with 8 glass beads between each

successive eggs to ascertain egg order (so later vesicled alevins could not swap places) and to prevent disease transmissions (Dumas & Marty, 2006). The top stopper of each capsule was marked with different notches to keep track of egg identity.

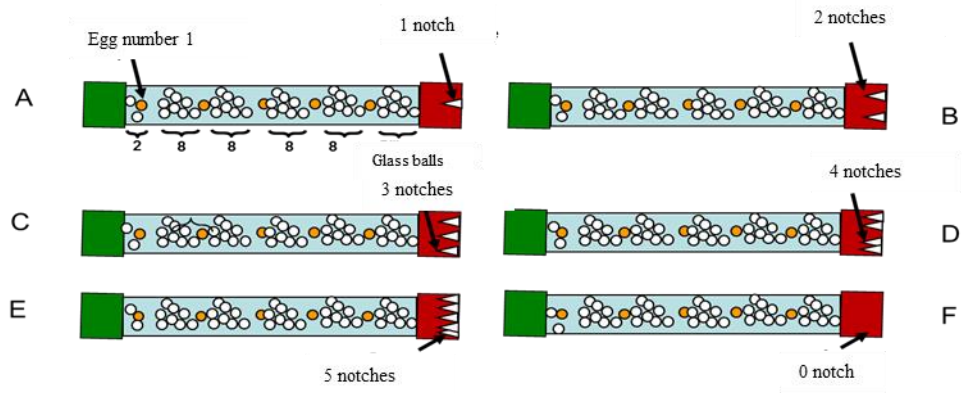


Figure 8. *Capsules (A to F) used to isolate 30 eggs in one redd. Capsules are constituted of stainless steel grids which allow water circulation on it.*

Capsules containing eggs were then horizontally arranged in the dug nest, at the precise place where eggs were previously found in the substrate. They were then covered by the substrate (particles previously removed) until the water level above the dome was similar to the initial measure. Eggs were therefore placed at the same place chosen by the female and the environmental conditions of the redd have been closely respected. Redds were located from the river bank thanks to plastic tape.

3) Survival

At the end of the experiment, each redd was excavated to estimate the survival of eggs just before hatching (approximately 375 degree.days). The mortality of every individual (from 1 to 30) was noted. Also, if one or several capsules were not found, the survival of

every egg in the missing capsules was considered as null. Living individuals were brought to the experimental station of Lapitxuri and placed in a breeding device until 750 degree.days (timing of emergence stage). At this stage, they were released in their river of origin.

4) Particle size analysis

Particle size was measured from pictures taken on the field. The best picture of each redd was selected, according to the quality of the image (image clearness, without any reflection or undulations which would deform the image of the pebbles). Two softwares were used to analyse these pictures: GIMP 2.8.6 and image J version 1.45s. The former software was used to correct the prospect of the photo. To do so, the grading frame served as reference and the objective was to restore a square shape to avoid a bias in the measures due to the picture angle.

The second software was used for particle measurement. To be the most representative of the redd, 100 particles inside the frame were measured. Particles were selected in a systematic way within the redd. To do this, a grid was superposed on the picture of the frame of 50 cm aside. The area of the particles under each intersection of the grid were measured manually. This area is expected to represent at best the actual mass of the particle. When the number of particles (100) was not reached, the grid was again arranged randomly on the image then the new particles indicated by the intersections were measured until to reach 100 particles (Fig. 9). The upper 90 % quantile (Q90) of the particle surface distribution was chosen to describe the particle structure of each redd: the size of the particles is a physical constraint for females to dig their nest, so the Q90 is a synthetic way to represent the biggest particles sizes.

a) b)

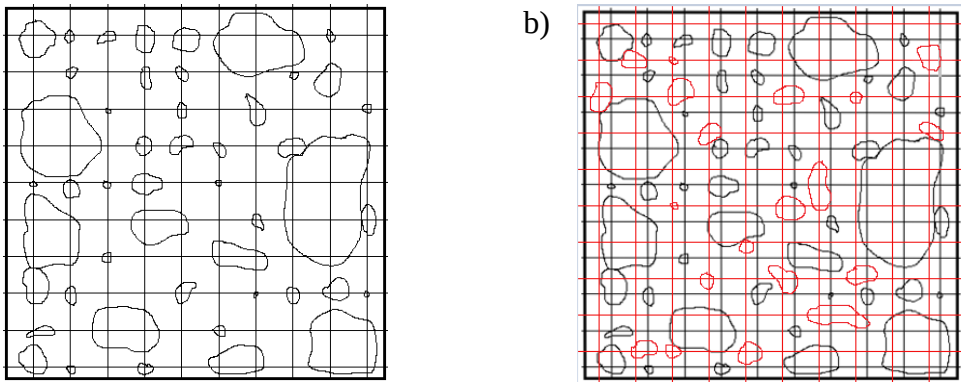


Figure 9. *Systematic sampling of particle size a) first sample b) second sample used when the number of 100 particles was not reach with the first sample.*

IV. A word on statistics

The reader will notice throughout this manuscript that various statistical approaches have been used. They range from simple parametric and non-parametric tests, to linear and generalized linear models, generalized linear mixed models, up to Bayesian hierarchic models. The reasons for that are multiple. First, some but not all questions require complex statistical investigations. Second, I did not always have the time to correctly analyze some of the data with the appropriate method each time (*i.e.* time of modeling is very long).

I will here take the example of reproductive success, which is indeed illuminating: reproductive success estimated from genetic data is not easy to statistically analyze for several reasons. Generally, it results in a matrix containing an important proportion of

zero which is quite frequent in ecological surveys and these dataset are commonly named "zero inflated dataset" (Martin et al., 2005). Two main problems with too many zeros in the data analysis process arise as 1) data do not fit with standard distributions and 2) a zero value may originate from several distinct ecological processes of interest. Additionally, and it is not the least of the problems, data of reproductive success are not independent for males and females, although this problem is generally overlooked in sexual selection.

In Chapters III and V, for the reasons explained above, we will explore results of the effects of several traits on reproductive success using relatively simple and traditional statistical approaches, such as Generalized Linear model (GLM) using Quasi Poisson or negative binomial distribution frequently used for reproductive success analysis. Sometimes, even simple linear regressions will be used, in order to be able to compare our results with already published results using the same approach. In Chapter IV however, I will develop a hierarchical model, representing several behavioural and ecological processes that contribute to sexual selection, and accounting for the various statistical and logical pitfalls of reproductive success data analyses: ecological meaning of zero data and relationship to statistical distribution choices, and non-independence of reproductive success within mating pairs in order. Such development is time costly, and it was therefore not possible to use such framework along all the analyses presented in this manuscript.

CHAPTER III.
Sex alone: individual scale

I. Context

The tradeoff between current reproduction and future reproduction is crucial in iteroparous species since energy invested in current reproduction will not be available for future reproduction (Williams, 1966). Moreover, individuals also face a trade-off within each period of reproduction, since energy invested in reproduction is allocated to different essential functions such as gametes production and behavioural activities (competition for mating access, parental care…). In salmonids, gametogenesis starts a few months before the reproductive season. Reproduction is particularly costly especially because feeding resources are rare at that period and individuals display intense behaviours: females compete for spawning sites and dig nests in gravel bars to protect eggs against predation, whereas males display intense and fierce agonistic behaviour with conspecifics to gain access to sexual partners (Beall & Marty, 1983; Berg, Throanes, & Bremset, 1998; Esteve, 2005; Garcia-Vazquez et al., 2001; Gaudemar & Beall, 1998; Schroder, 1981) or to sometimes defend eggs after fertilization, thereby providing paternal care (Tentelier et al., 2011). All these behavioural activities are highly energy consuming and occur after gametogenesis. Therefore disentangling investment in gametes versus reproductive behaviour seems interesting because individuals may vary in their allocation strategies between gametes and behaviours.

In the present chapter, the energy spent by brown trout in reproductive behaviour will be investigated using two proxies, weight variation and variation in plasma metabolites concentration. In the first and second part of this chapter, I will test the link between reproductive success (estimated from genetic assignment at the end of reproduction) and each proxy separately. In the third part, I will analyse the correlations between both metabolites and weight variations using a principal component analysis, and I will use

these components to study their joint effects on reproductive success. In a fourth part, behaviours involved in reproduction such as occurrence of chases and digging, will also be analysed and correlated to either weight or metabolite variations.

II. How much weight did individual invest in the reproduction? (EXP A)

Variation in individual body mass during the reproductive period is frequently analysed in order to quantify reproductive effort which represent the part of energy invested by each individual in reproductive activities (Anderson and Fedak 1985; McElligott et al. 2003). In salmonids, weight is strongly correlated with body size which is also correlated with age. However, weight corrected for body size or age also depends on the potential of an individual to access and stock energetic reserves during the past year before reproductive season. Moreover, the capacity of individuals to catabolise their reserves may vary regardless of their initial condition. Thus, initial weight at the onset of the reproduction and variation of weight during the reproductive period could reflect different tactics of reproductive effort and their effect on reproductive success will be studied in a first part.

To quantify reproductive effort, we study weight and size, which reflect the condition of an individual at the onset of the reproduction, and the relative variation of weight, which indicates the proportion of its energetic stock an individual invests in reproduction. Additionally, we should also consider the interaction between initial weight and relative variations of weight which corresponds to the absolute variation of weight during reproductive season.

Relative weight variation was therefore calculated as following:

$$Relative\ weight\ variation[i] = \frac{Weight\ br[i] - Weight\ af[i]}{Weight\ br[i]}$$

Where *Weight br* represent the weight before reproduction and *Weight af* for an individual *i*. Hence a positive weight variation indicates a weight loss.

During the reproductive period in experiment A, females lost in average 12% of their initial weight (sd±8.55) whereas males lost around 6% of their initial weight (sd± 4.25). With respect to their initial weight, females generally did lose more weight than males. Moreover, relative variation of weight was higher for bigger males than for smaller ones (linear model: Df =1; F= 8.21, P= 0.01, R^2 adjusted= 0.27). The trend was the same for females (linear model: Df =1; F= 4.11, P= 0.053, R^2 adjusted= 0.1).

Additionally, here weight was measured before and after reproduction and was therefore the outcome of both gamete release during reproduction and potential behavioural activity. Also, even if food is scarce during the reproductive period, individuals may have absorbed some preys such as micro invertebrates, worms and some eggs (oophagy, (Aymes et al., 2010; Tentelier et al., 2011). In comparison, in the nearby Bertiz population (located in the Bidasoa watershed, Navarra) weight variation in females from this experiment (12% of their body weight) seems to correspond closely to loss of weight due to gamete release and to coelomic fluid (N= 55; mean weight variation= 14%, unpublished values calculated from Régnier's data (2011), which leads to the conclusion that body weight variation is probably mainly associated to gamete release, although it can also vary with fat use. Weight is, therefore, an integrative measure, that may not be adequate to isolate components of reproductive investment, such as behavioural investment.

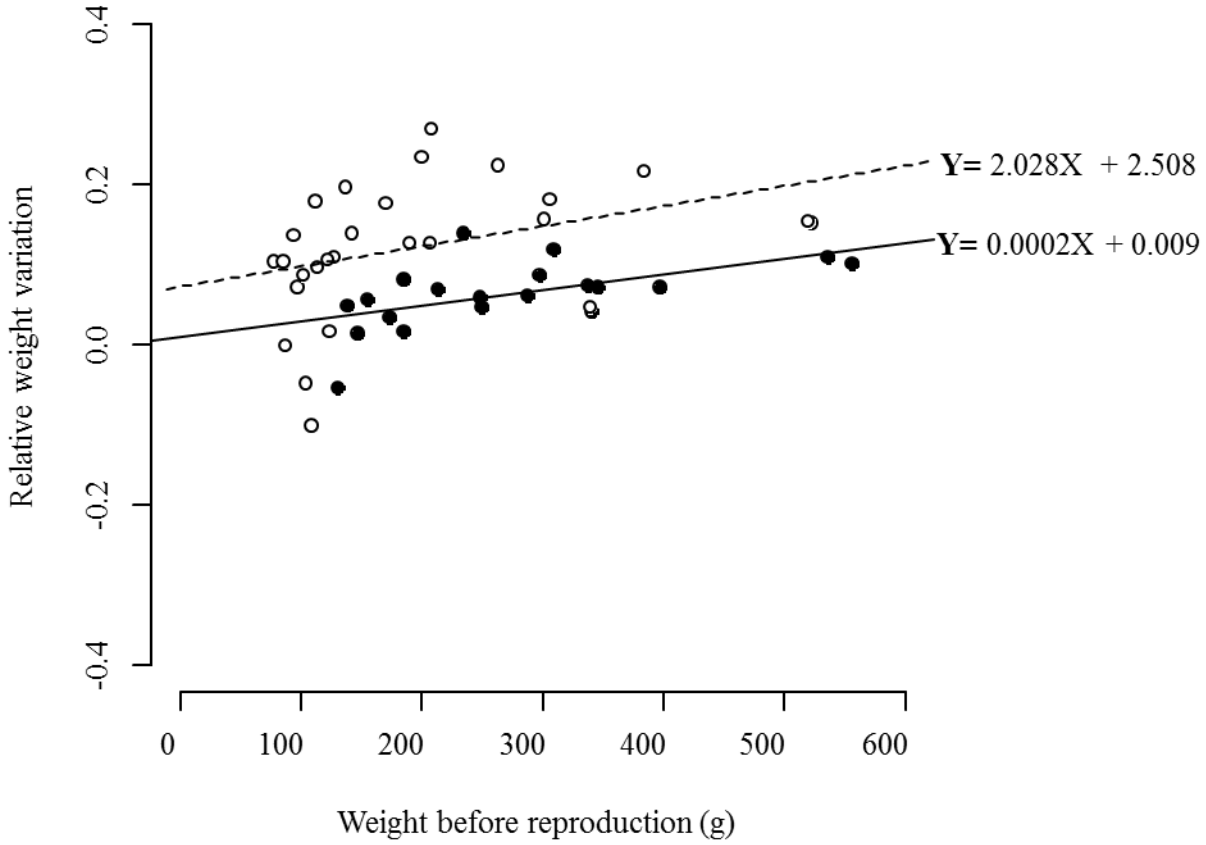


Figure 10. *Relationship between relative weight variation and weight before reproduction in males (black dots) and females (white dots).*

III. Relationship between weight and reproductive success

A GLM (with quasi-Poisson distribution) was used in order to test if initial weight, relative variation of weight and sex had an impact on reproductive success. The results of the model revealed that only initial weight had a strong impact on reproductive success in both males and females (Table 6). On a side note, relative weight variation did not statistically influence offspring number, although a positive trend can be sketched (P=0.08). Finally, the interaction between initial weight and relative weight variation was

not significant: this interaction corresponds to the absolute variation of weight and its non-significant effect therefore highlights the fact that gamete production was not the sole determinant of reproductive success.

Table 6. *Deviance analysis of the GLM results (R software) for the experiment A, testing the effect of initial weight, relative weight variation and sex on reproductive success(offspring number) of individuals. Dispersion parameter value for the quasi-Poisson distribution was 51(*).*

Variables tested	*Df*	*Deviance*	*Residual Df*	*Residual deviance*	*P-value*
NULL Model		3046.8			
Initial weight	1	730.01	45	2316.8	**0.0002**
Relative weight variation	1	154.58	44	2162.2	0.08
Sex	1	114.9	43	2047.3	0.13
Initial weight *Relative weight variation	1	11.88	42	2035.4	0.63
Initial weight* Sex	1	33.84	41	2001.6	0.42
Relative weight variation*Sex	1	63.21	40	1938.4	0.27
Initial weight* Relative weight variation*Sex	1	57.86	39	1880.5	0.29

(*)note: index of dispersion compared to the expectation of the Poisson model is very high and reduce chances to find significant effect (see chapter II, Statistical part). It also indicates that Poisson distribution is not adapted to this type of data.

IV. Metabolites as a proxy of reproductive effort

Using only weight as a proxy of energy expenditure seems insufficient. Because weight variation and relative weight variation do not satisfactorily predict reproductive success, there might be alternative ways to approach other components of reproductive investment, such as behavioural activity. Other methods to measure energy loss during reproduction have used the loss of energy in relation to breeding behaviors (Anderson &

Fedak, 1985; Hendry & Beall, 2004; Murchie, Cooke, & Danylchuk, 2010), but these methods are either lethal or highly invasive and thus cannot be implemented in wild populations, especially in iteroparous ones.

To better estimate reproductive effort, I chose to measure the plasma concentration of metabolites involved in energy production through catabolism (i.e. glucose, triglycerides, free fatty acids and amino acids). This method has already been tested in burrowing parrots *Cyanoliseus patagonus* (Aves, Psittaciformes) and showed that males with decreasing plasma triglycerides improved their number of fledging juveniles (Merilä & Svensson, 1995). A description of the rationale and the methods to estimate metabolites variation in plasma samples is provided in § II.II.6. Here I propose to measure variations in plasma metabolites as a proxy of reproductive effort during a period of reproduction and test its potential relationship with fitness (*i.e.* reproductive success). Using the same approach as for weight, initial level of plasma metabolites and their relative variations were analysed.

1) Initial level of metabolites

The initial observed concentrations (at the onset of the reproduction) of the four metabolites (triglycerides, free fatty acids, amino acids, glucose) were within the range of values shown by several studies in fasted rainbow trout (*Oncorhynchus mykiss*, Panserat et al. 2002; Seiliez et al. 2012; Kamalam et al. 2012; Skiba-Cassy et al. 2013). Initial concentrations of glucose did not differ between males and females (average 1.02 and 0.95 g/L respectively, Table 7, Fig. 11).

On the other hand, there were important differences in initial concentrations in triglycerides, free fatty acids and amino acids between males and females. Females started reproduction with higher concentrations of triglycerides and free fatty acids than males, whereas males had higher concentrations of amino acids (Table 7).

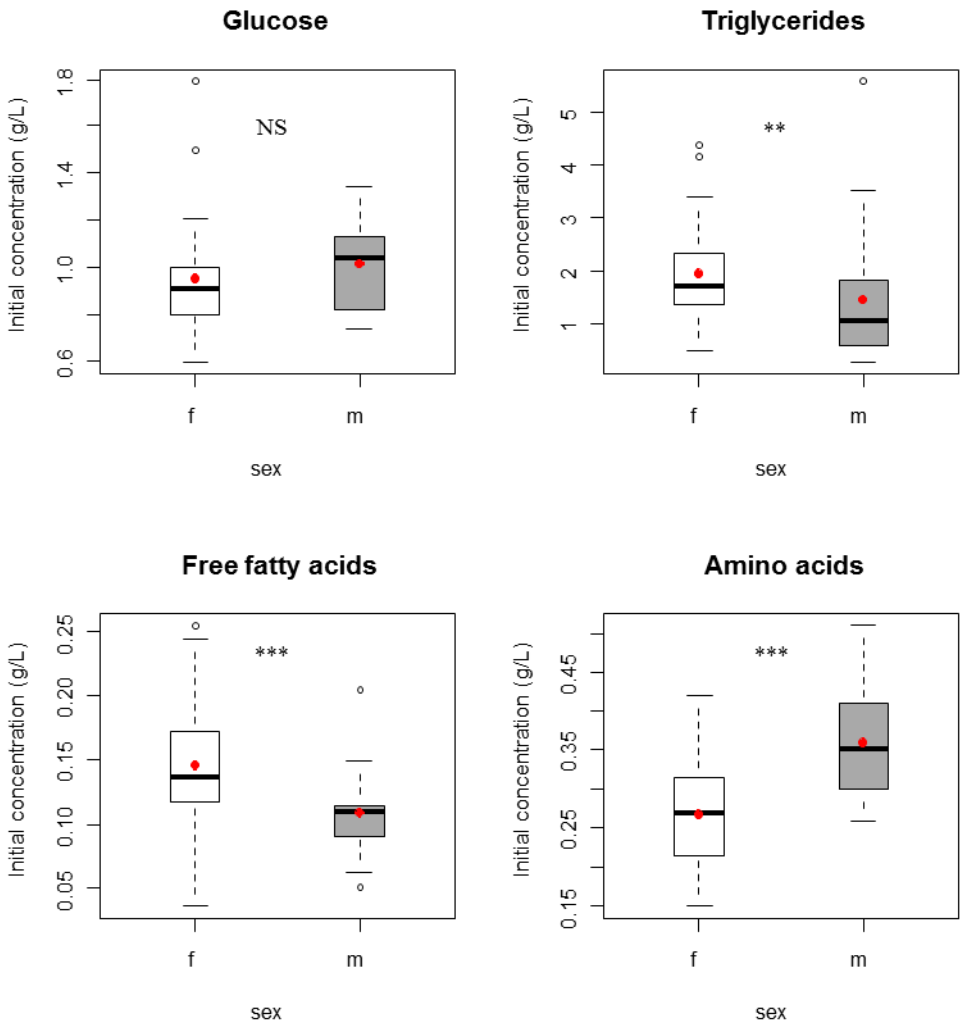


Figure 11. *Box-plots of initial concentrations of glucose, triglycerides, free fatty acids and amino acids in males and females. Red points represent the mean, black bar the median.*

Therefore, females had a higher lipid concentrations for both triglycerides and free fatty acids. In contrast, males showed higher plasma amino acids concentrations. Previous

studies of several salmon species showed that males arrive earlier than females, on average, in the spawning grounds (Gosset, Rives, & Labonne, 2006; Pritchard, 1937). The mate opportunity hypothesis proposes that males who arrive first will increase their mating opportunity (Morbey, 2000). Therefore, males could have already partly used their lipid reserves (i.e. free fatty acids) before their capture for the experiment.

2) Metabolite variations during reproduction

The relative variation of triglycerides, free fatty acids, glucose and amino acids were calculated as the difference of the plasma concentration before the reproduction to plasma concentration after the reproduction, divided by the plasma concentration before the reproduction as following:

$$Relative\ plasma\ metabolite\ variation[i] = \frac{Metabolite\ br[i] - Metabolite\ af[i]}{Metabolite\ br[i]}$$

Where, *Metabolite br* represents the plasma metabolite concentration before reproduction and *Metabolite af* the plasma metabolite concentration after reproduction for an individual i. These variations could genereally range between -1 and 1, hence, when the variation was positive, the level of plasma metabolites decreased during the reproductive season, whereas it increased when the variation was negative.

Because free fatty acids, triglycerides and amino acids are directly used to produce energy, a decrease of this three plasma metabolites is expected during reproduction whereas variation in glucose should be more an indicator of stress during the reproduction. First, there were no significant relative variations in glucose level during the experiment either for males or for females. Additionally, the relative variations in

metabolite concentration during the reproductive season did not differ between males and females for glucose, triglycerides and free fatty acids (Table 7, Fig. 12). Glucose is absent from food intake, but neoglucogenesis is especially used for maintenance activity or during long periods of food deprivation (Enes, Panserat, Kaushik, & Oliva-Teles, 2009; Hemre, Mommsen, & Krogdahl, 2002; Stone, 2003; R. P. Wilson, 1994), and as such, variation in glucose is related to stress in fish (Silbergeld, 1974). Thus, even if some small reductions were detected in the concentrations of glucose (8.7% for males and 0% for females), the steady plasma concentration of glucose in our experiment indicates that individuals did not suffer from stress. Secondly, plasma amino acids did not vary a lot from their initial concentration which indicates that our fish had not engaged intense proteolysis by the end of the reproduction. Because intense proteolysis is a sign of physiological distress in fish, it appears that the reproductive investment of our fish in this experiment did not directly jeopardize their metabolic status, and may therefore not have a direct and rapid impact on their immediate survival. However, the levels of amino acids in plasma decreased more in males than in females. Moreover, a high variability was observed in some females especially in the relative variation of amino-acids. Salem *et al.* (2006) have described that during spawning, some rainbow trout females could even show a high proteolysis. Hence, asynchronous spawning in our experiment could explain this variation in amino acids dynamics between females.

Metabolites, except triglycerides, do not vary monotonously. Therefore, while it is relatively safe to discuss about relative variations of triglycerides, results of both free fatty acids and amino acids are difficult to interpret. For example, we cannot conclude that a decrease in the relative variation of amino acids corresponds to a decrease of the

proteolysis relative to the initial concentration or, on the contrary, an increase of the proteolysis. Thus, two individuals showing a same final concentration may not have the same metabolic status. However, it seems that individuals were not stressed during the experiment (no strong glucose variation) and still have respectively 50% and 40% of their triglycerides and free fatty acids in their plasma. Additionally, weight variation is not surprising and corresponds to usual variation due to gamete release and to coelom fluid. Therefore, it seems probable that individuals are not engaged in intense proteolysis in this experiment.

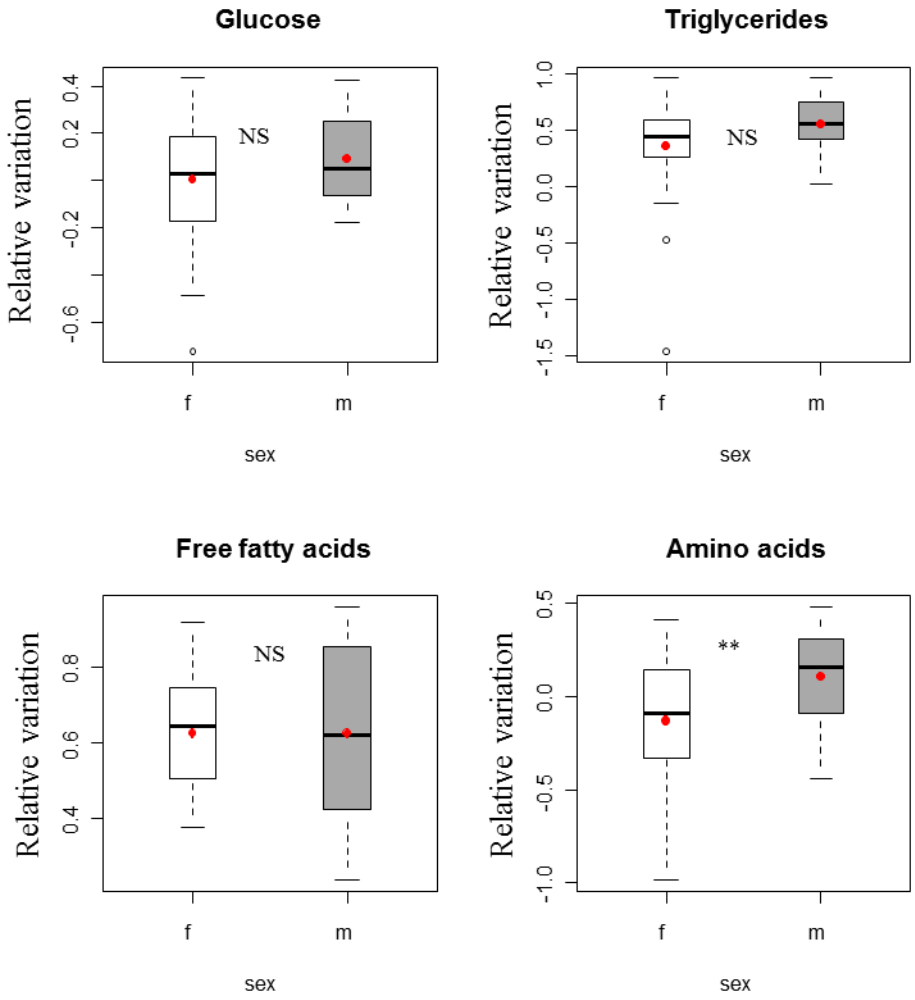


Figure 12. *Box-plots of the relative variations of plasma glucose, triglycerides, free fatty acids and amino acid concentrations in males and females. Red points represent the mean, black bar the median.*

Table 7. *Differences between males and females in initial and relative variation of weight and concentrations of metabolites, and in number of offspring and mates found in the offspring (Kruskal Wallis test).*

	Males (mean ± sd)	Females (mean ± sd)	P-value (Ho: males=females)
Initial level of:			
Weight (g)	273.05 (± 121.49)	194.18 (± 123.91)	**0.005**
Glucose (g/L)	1.02 (± 0.19)	0.96 (±0.25)	0.14
Triglycerides (g/L)	1.41 (± 1.32)	1.89 (± 0.99)	**0.02**
Free fatty acids (g/L)	0.11 (± 0.03)	0.14 (± 0.05)	**0.002**
Amino acids (g/L)	0.36 (± 0.08)	0.27 (±0.07)	**0.0003**
Final level of:			
Weight (g)	252.97 (±106.38)	163.70 (±102.82)	**0.0007**
Glucose (g/L)	0.90 (±0.17)	0.91 (±0.17)	0.81
Triglycerides (g/L)	0.56 (±0.48)	1.08 (±0.77)	**0.06**
Free fatty acids (g/L)	0.04 (±0.02)	0.05 (±0.02)	0.09
Amino acids (g/L)	0.31 (±0.08)	0.28 (±0.04)	0.1
Relative variation of:			
Weight	0.06 (± 0.04)	0.12 (± 0.09)	**0.002**
Glucose	0.15 (± 0.28)	0.06 (±0.27)	0.40
Triglycerides	0.55 (± 0.23)	0.42 (± 0.32)	0.11
Free fatty acids	0.62 (± 0.23)	0.62 (± 0.14)	0.86
Amino acids	0.11 (± 0.24)	-0.13 (±0.37)	**0.02**

Degree of freedom is equal to 1 for each test. Bold values indicate a significant difference between males and females. Number of offspring assigned and number of mates result of the overall matrix of reproductive success.

In contrast, the concentration of triglycerides fell by 50% in both sexes, and by 60% in free fatty acid. Therefore, despite some scarce food availability and potential oophagy (Aymes et al., 2010), lipid reserves were actively utilized during reproduction hinting at high monopolization of fat reserves for reproductive behaviours. It is well known that investment in reproduction can affect lifetime reproductive success in individuals (Stearns, 1992; Williams, 1966). Consequently, an actual measure of the trade-off between current investment and future survival and reproduction opportunities (as both expected by theory and shown for many species) would require monitoring the individuals over a long period with knowledge of their reproductive investment. This could now potentially be undertaken in wild populations since the plasma metabolites are accessible via simple blood samples and fish can be individualized (by tagging or genotyping) and recaptured afterward. Metabolites in variations could then be coupled to weight variation and reproductive success.

V. Link between variation in weight and in metabolites

No significant correlation was found between the relative variation of weight and each of plasma metabolite variations: glucose (males: df = 1; p= 0.28; r = 0.01; females: df = 1; p= 0.59; r = -0.02), amino acids (males: df = 1; p= 0.71; r = -0.05; females: df = 1; p= 0.24; r = 0.01), free fatty acids (males: df = 1; p= 0.15; r = 0.15; females: df = 1; p= 0.68; r = -0.03), and the triglycerides (males: df = 1; p= 0.20; r = 0.03; females: df = 1; p= 0.99; r = -0.04) (Fig. 13). Metabolites variations during spawning period are probably not strongly associated with gamete release and may in consequence describe other parts of reproductive investment, such as behavioural activities.

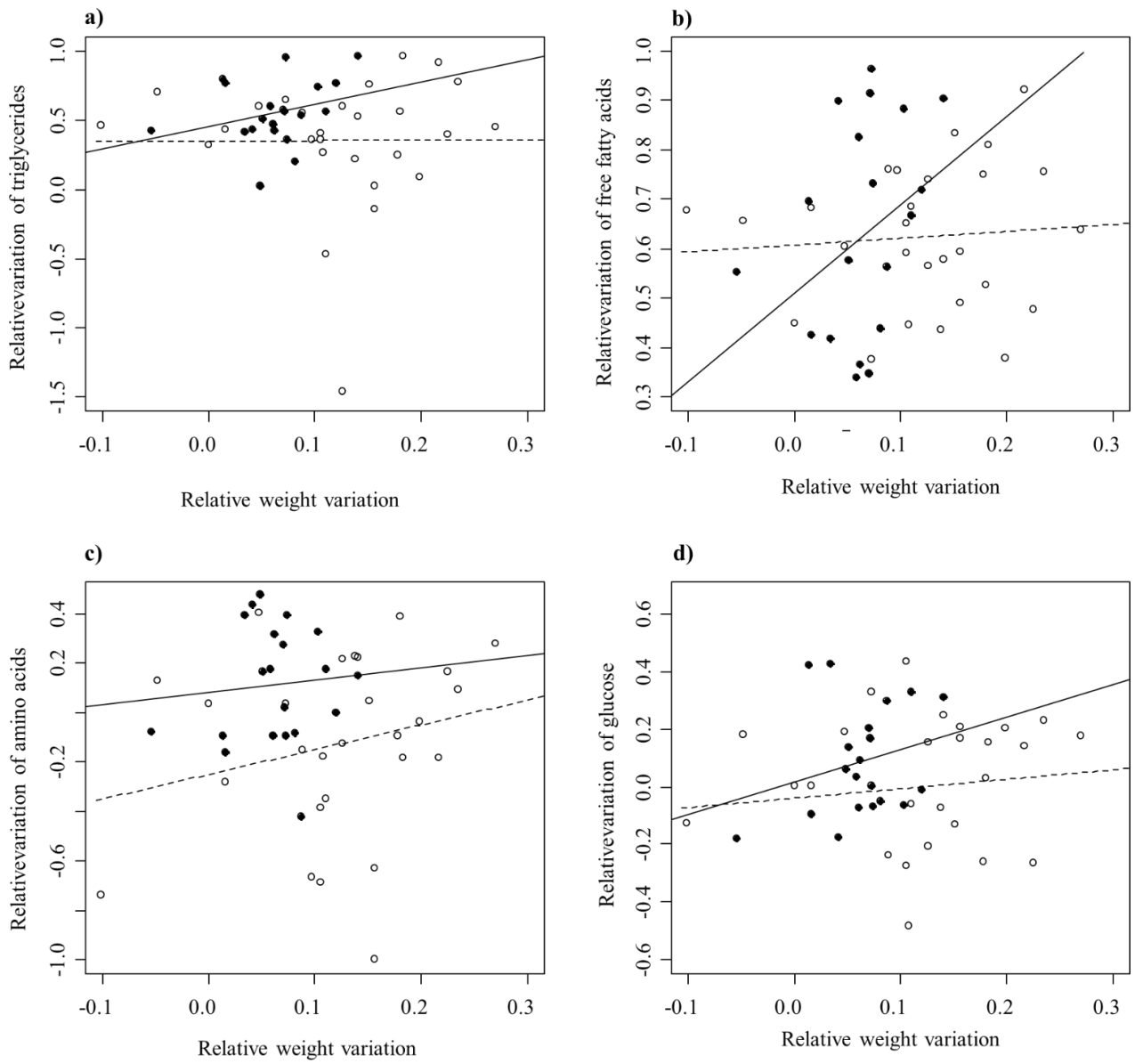


Figure 13. *Relation between relative plasma metabolic variations and weight variations for males (black points and solid lines dots) and females (white points and dotted lines) : respectively a) triglycerides variations; b) Free fatty acids variations, c) Amino acids variations and d) glucose variations.*

VI. Relative contribution of weight and plasma metabolites on reproductive success.

During the study, no significant correlation between the variation of weight and variation of metabolite concentration were found, hinting that metabolite variations during spawning period are probably not associated with gamete release, which is one of the major determinants of weight variation in salmonids during the reproduction. As previously proposed for other biological systems (Kilgas, Mänd, Mägi, & Tilgar, 2006; Masello & Quillfeldt, 2004) this finding supports the general idea that variation in metabolite concentrations is actually a useful and better proxy than weight for measuring energy investment in reproductive behavior. Then, because both weight and plasma metabolites seem to vary throughout the reproductive season, the next question is: do they affect directly reproductive success? To analyze their effect on reproductive success, a general analysis integrating these variables has been conducted. To do so, a scaled principal component analysis (PCA) using the initial concentration and the relative variation of each metabolite (function prcomp, R software, version 2.10.1) on all individuals was performed in order to access to a synthetic index of metabolites data variation.

The scores of individuals on the first two axes of the PCA (A1, A2 representing 37.7% of the total variance) were kept as synthetic indicators of their metabolic profiles during reproductive season. A negative binomial regression model was then fitted with a log link function (package MASS, R software) to infer the effect of A1, A2, initial weight (IW) and relative weight variation (RVW) on offspring number N as follows:

$$log(N_i) = \beta_0 + \beta_1 \times A1_i + \beta_2 \times A2_i + \beta_3 \times IW_i + \beta_4 \times RVW_i + \beta_5 \times A1_i \times IW_i + \beta_6 \times A2_i \times IW_i + \beta_7 \times IW_i \times RVW_i + \beta_8 \times A1_i \times RVW_i + \beta_9 \times A2_i \times RVW_i + \beta_{10} \times A1_i \times IW_i \times RVW_i + \beta_{11} \times A2_i \times IW_i \times RVW_i$$

With (β_0, β_1, …, β_{11}) the parameters to estimate. The negative binomial model was chosen to account for overdispersed [(2)] variance in reproductive success data that prevents to use the Poisson regression model usually adapted to count data. The interaction between A1 and A2 was previously tested and yielded no effect. It is therefore not presented for the sake of simplicity. An analysis of deviance table using a χ^2 test was applied to assess the statistical significance of each parameter. The results of the principal component analysis oppose on the two first axes (37.7% of the total data variance) variation in amino acids on the one hand (axis A1), and triglycerides and free fatty acids on the other hand (axis A2, Fig. 14).

Individuals having a high A1 score entered the reproduction season with high concentrations of amino acids and showed an important relative variation of their initial amino acids level throughout the reproductive period. Individuals having a high A2 score showed an important relative variation of their initial triglycerides and free fatty acids levels.

[2] Here negative binomial distribution was chosen to fit better with data variance rather than the Poisson or Quasi Poisson distribution

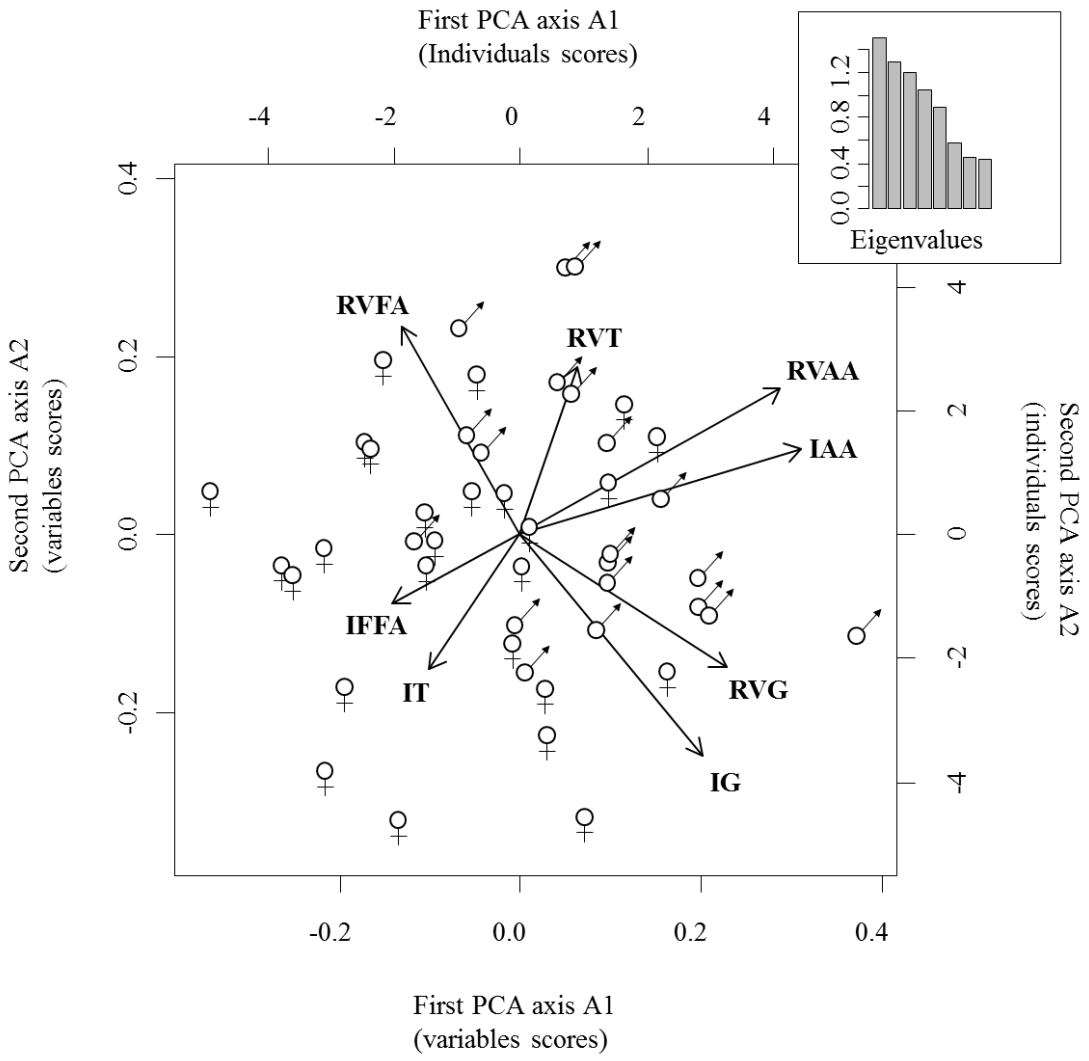


Figure 14. *Principal component analysis biplot of axes A1 and A2. Arrows represent scores of variables on first and second axes (bottom and left scales respectively) and male and female symbols represent individual scores on first and second axes (top and right scales respectively). IG, IT, IFFA, IAA represent respectively the initial glucose, triglycerides, free fatty acids and amino acids concentrations, whereas RVG, RVT, RVFA and RVAA represent the relative variation of these metabolites during the reproductive season.*

The results of the negative binomial regression model indicated that the first axis A1 of the principal component analysis had no significant effect on offspring number, while the second PCA axis A2 had a statistically very significant effect on offspring number ($p < 0.00001$) (Table 8). Relative weight variation also had a significant effect on offspring

number (p=0.03513). Finally, we detected a significant interaction between initial weight and A2 (p=0.00212). The first PCA axis A1, as well as interactions between A1 and initial weight, A2 and relative weight variation, and initial weight and relative weight variation had no significant effect, but all ps were inferior to 0.1. Triple interactions had no significant effect with ps superior to 0.2.

Table 8. *Analysis of deviance table for the negative binomial regression model. A1 and A2 are the scores on the first and second axes of the principal component analysis respectively, IW is the initial weight and RVW is the relative variation of weight.*

Variable	Degrees of freedom	Explained deviance	P-value (α=0.05)
A1	1	3.0379	0.08134
A2	1	21.2474	**4.036e-06 *** **
IW	1	1.0305	0.31004
RVW	1	4.4390	**0.03513 ***
A1× IW	1	3.0533	0.08057
A2× IW	1	9.4411	**0.00212 ** **
A1×RVW	1	0.5168	0.47220
A2×RVW	1	3.0275	0.08186
IW×RVW	1	2.8449	0.09166
A1×IW×RVW	1	1.5815	0.20855
A2×IW×RVW	1	0.7746	0.37880
Residuals	34	51.263	-

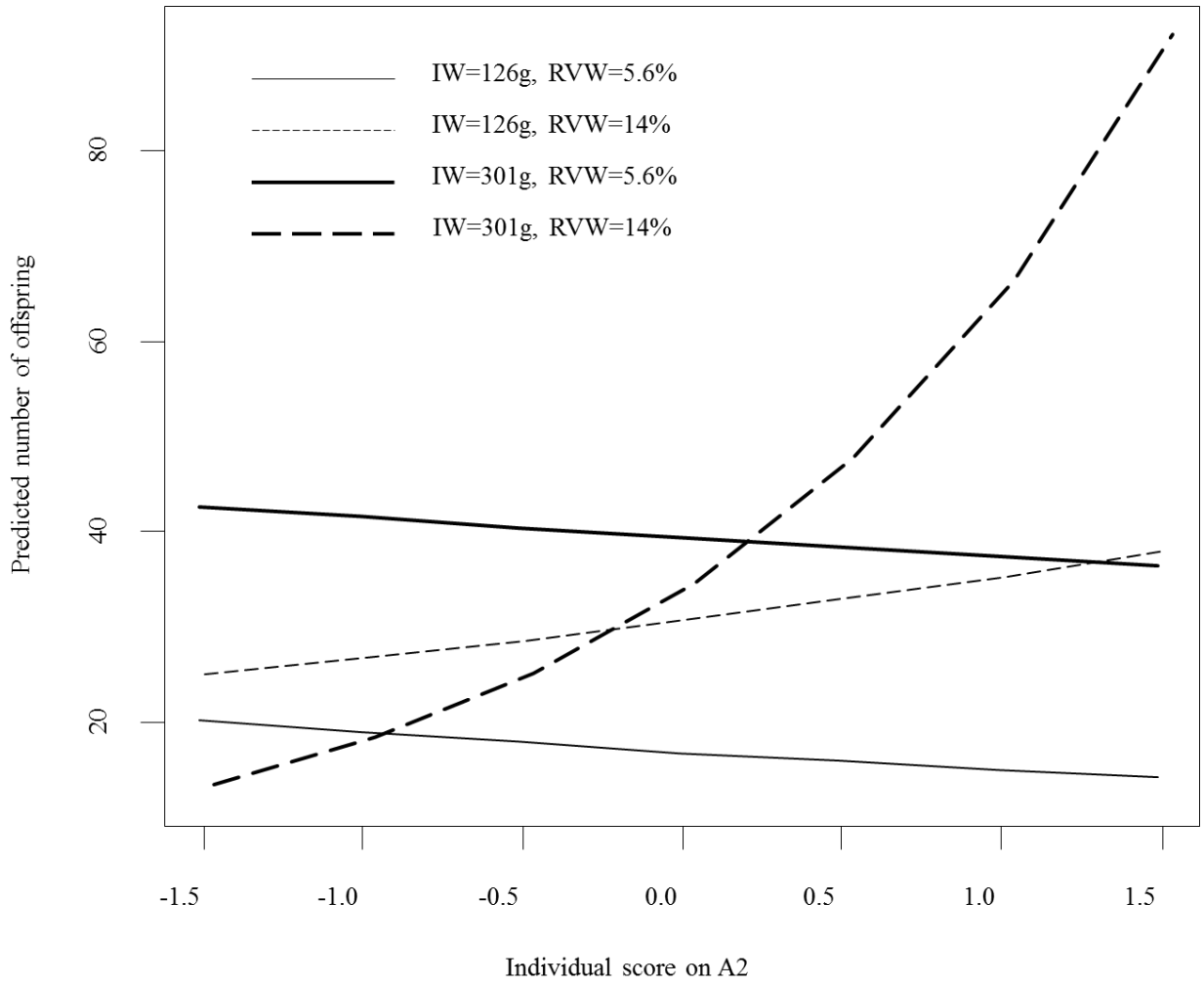


Figure 15. *Predictions of the number of offspring produced made from the negative binomial regression model. Predictions are calculated over a [-1.5;1.5] range on the A2 PCA score, as well as for two initial weight (126g, 301g) and two relative variation of weight (5.6%, 14%), representing each time the 25% and 75% quantiles of their observed distribution in our data.*

Based on this model, an important loss of weight was associated to a high number of offspring (Fig. 15). The effect of high individual scores on A2 was translated also into a higher number of offspring, also depending on initial weight, but it could be overridden when relative variation of weight was too small. In that case only the initial weight positively affected the number of offspring.

Therefore, relative weight variation increased the number of offspring[3]. This result seems logical since weight loss strongly correlates with the number of gametes released (Healey & Prince, 1998), which is in direct link with fertility in females and with the outcome of sperm competition in males (Parker, 1982). While metabolite level variation was uncorrelated to weight variation, metabolite level variation displayed an even greater effect on the measure of the number of offspring. In salmonids, fierce competition between males to get access to females is the norm (Fleming & Gross, 1994; Höjesjö, Johnsson, & Bohlin, 2004). Additionally, paternal care in brown trout occurs when egg cannibalism pressure is high (Aymes et al., 2010; Tentelier et al., 2011). Likewise, an efficient nest digging by females is expected to provide benefits such as protection against predators and environmental stochasticity (Fukushima, Quinn, & Smoker, 1998; Møller & Jennions, 2001; Tappel & Bjornn, 1983). All these behavioural activities can be costly and this cost appears to be well reflected by variations of plasma metabolites such as triglycerides and free fatty acids.

This is exemplified by the fact that metabolic profiles, as synthesized by the A2 individual scores, interact with initial weight. There is therefore a synergistic effect of initial weight and metabolites levels variations, especially when weight loss over the reproduction period is important: initial weight can be for instance a good proxy of intra-sexual competitive ability (Jacob et al., 2007), but it is efficient only if one invests in both gametes production and active behavior such as agonistic interactions or parental care. This finding implies that gametic investment, as approached by weight variation, cannot be used as the sole measure of reproductive investment and that it is possible in wild fish to efficiently complete the picture by a proxy of behavioural investment in reproduction,

[3] With this new model using the negative binomial distribution, we find this time (comparing to the first model using the quasi poisson distribution) an effect of the relative weight variation hinting that the statistical used to analyse reproductive success data including many zeros is probably not the best method. For additional informations, see the chapter II, statistical analysis part.

as approached by metabolites variation. Indeed, when looking at the figure showing relationship between relative weight variations and relative metabolites variations, it seems that all strategies exist including high investment in both gametes (weight) and reproductive behaviour (metabolites).

VII. Behaviours and reproductive investment

The variation of metabolite status and weight has been analyzed previously in order to quantify reproductive investment during a reproductive season. From our conclusions, relative weight variation seems directly linked to gamete investment, whereas variation of both triglycerides and free fatty acids would be a better indicators of parental care and intra-sexual competition. Consequently, one of my objectives was to measure reproductive behaviours directly involved in these two mechanisms (number of chases between males and number of digging provided by females) in order to see their relationship with metabolites variations. In other terms, are behaviours statistically related to plasma metabolites variations? Because variations of plasma triglycerides and free fatty acids were respectively of 50 and 60% in comparison with relative variation of glucose and aminoacids, only relationships between these first two were analyzed as a function of behaviour.

1) Digging behaviour

It was not possible to track all individuals during reproduction, because I had access to a limited number of cameras, and because even in our semi-natural spawning channel, fish could choose locations that were not obvious to spot. Reproductive activity starting after dusk was also sometimes difficult to detect. As a consequence, out of the three experiments combined (A, B1, B2), only 26 females out of 93 females have been

observed digging during reproduction (N_{expA}=4; N_{expB1}=13; N_{expB2}=9) leading to a lack of information about other females.

I will first address relationships between digging activity, reproductive success and metabolite variation for observed females. However, even if not seeing a given female constitutes missing data, it can also be considered a piece of information on the female's behaviour. Hence, in a second step, we will also compare reproductive success and metabolite variation between observed and non-observed females.

Within observed females, the average number of digging (before + after reproduction) per female was 74 with a high inter-individual variance (±147). A significant relationship was found between reproductive success and the total number of digging ($p<0.001$) (Fig. 16). This result is not surprising since digging behavior is directly related to the quality of the nest which provide good protection against egg cannibalism (Aymes et al., 2010) and is essential during the early stage of life of juveniles.

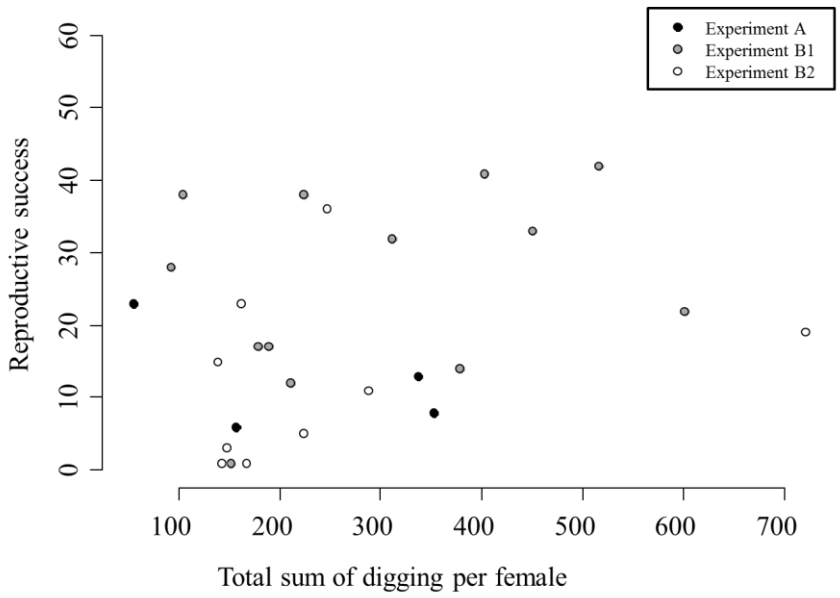


***Figure 16**. Relation between reproductive success (offspring number) and the total number of digging per female observed in experiment A, B1 and B2.*

Additionally, no relationship was found between the number of digging and relative variation of both triglycerides (r=0.13, p=0.19) and free fatty acids (r=0.02, p=0.82).

When we compared metabolite variation between observed and non-observed females, the relative variation of triglycerides was higher in observed females than in non-observed females (χ^2= 4.8984, df = 1, p = 0.027, Fig. 17). For free fatty acids, relative variation was not different between these two groups (χ^2= 0.061, df = 1, p = 0.805, Fig. 17). Hence, despite a limited number of females, we find that digging activity influences reproductive success, but it is not directly related to metabolite variation. Our sample size might be preventing us to find such a relationship. However, the fact that non observed females showed lower relative variation in triglycerides implies that they might have adopted a different behavioural tactic. Our observed samples might not be a good representation of the total behavioural variation present in our experiment. Non-observed females could for instance, pick up different and less conspicuous habitat that would also imply lower energy expenses. A possibility would be therefore that reproductive effort may vary depending on the condition of specific environment for example with low water flow, or higher resource availability.

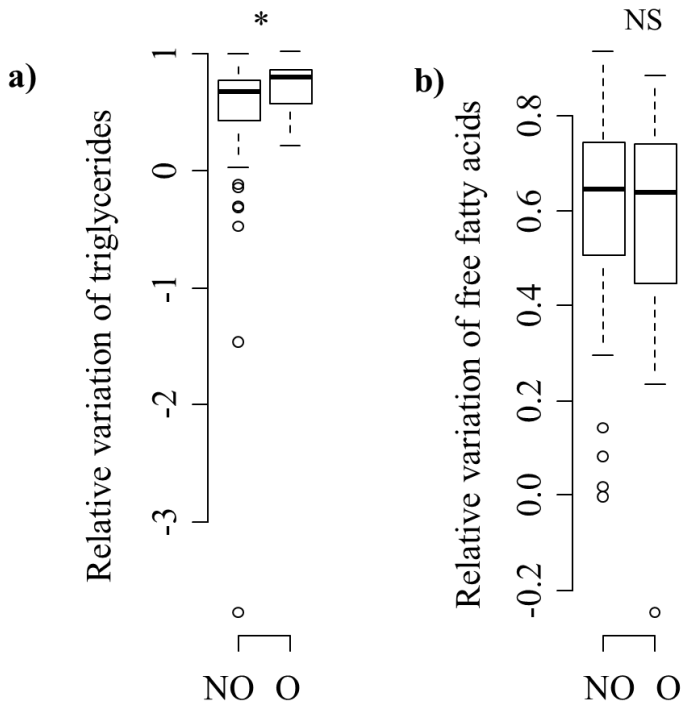


Figure 17. *Boxplot of the a) relative variations of triglycerides and b) relative variation of free fatty acids between non-observed (NO) females on video recording and observed digging (O) females.*

2) Competition between males

Out of 58 males, only 24 males were observed on videos (N_{expA}=7; N_{expB1}=10; N_{expB2}=7). Within these observed males, 16 chased conspecifics, whereas the other 8 did not chase any competitor. In average, the number of emitted chases per individual observed was 188 (±415). Individuals that performed at least one chase had a higher reproductive success than individuals that were observed without performing any chase (GLM: ddl=1, Null deviance=2887.5; Residual deviance=2510.1, $p<0.001$) (Figure 18).

However, the observation time is variable between individuals. It may on the one hand generate some bias in our conclusions, by generating spurious variation in chase numbers, although by comparing chasing versus non-chasing individuals, we reduce this potential bias on chase numbers. But on the other hand, individuals have a higher probability to be

picked up on the cameras when being around the nest, the observation time can therefore be linked to reproductive activity and competition.

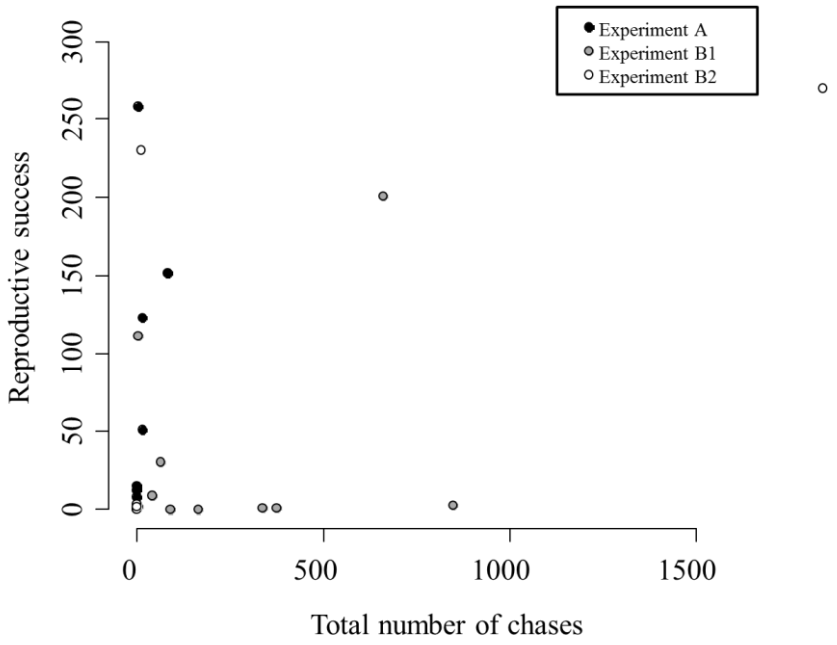


Figure 18. *Relation between reproductive success (number of offspring) and the total number of chases (before and after reproduction) per male observed in experiment A, B1 and B2.*

Additionally, within males observed on spawning sites, no general relationship was found between chases number and either the relative variations in triglycerides ($r = 0.34$, $p = 0.12$) and free fatty acids ($r = 0.08$, $p = 0.728$). However, relative variation of both triglycerides and free fatty acids was higher in males that emitted chases than males that were present but that did not chase, indicating a possible link between metabolites and competitive behaviours (triglycerides: $\chi^2 = 5.70$, $df = 1$, $p = 0.017$; free fatty acids: $\chi^2 = 4.53$, $df = 1$, $p = 0.033$, Fig. 19). Males that emitted chases lost on average 80% of their triglycerides whereas the others only lost about 55%. Additionally, the former lost on average, 70 % of their free fatty acids whereas the latter lost only 50%.

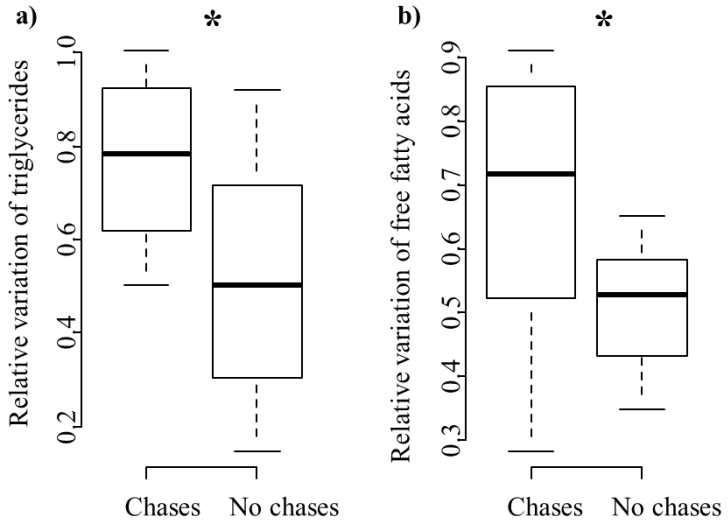


Figure 19. *Boxplot of the a) relative variations of triglycerides and b) relative variation of free fatty acids between males observed on video recording during episodes of reproduction that emitted chases (Chases) and males that dis not emitted chases (No chases). Stars denote a significant difference between the two groups.*

In conclusion, it appears that reproductive behaviours can be somehow related to variation in metabolites variations in plasma, although our sample size is often limited. Chasing males show higher variations of metabolites, and females that are not observed show lower variations of metabolites. It remains difficult to establish a clear relationship between behaviour and metabolites however because 1) our observation abilities are somewhat limited, and 2) because, as previously stated, simple relative variations of metabolites concentrations over the whole reproductive periods may not always translate more complex variations, especially when fish are feeding and when the metabolites dynamics are not monotonous.

CHAPTER IV.

Sex not alone : effect of conspecifics

I. Context

In the previous chapter, I looked at how individual traits may influence reproductive success. However the evolution of costs and benefits of each reproductive strategy is closely related to the *biotic* environment. For example, competition depends on how many competitors are present. Also, the number of sexual partners available affects both mating opportunities, in relationship with competitor density, and choice between phenotypes. Finally, and it is not the least of the factors: one's fitness in sexual selection is influenced by the sexual partner(s).

In the present chapter, I will look first at variations of parameters of interest, such as OSR, phenotypes, relationship between OSR and competition, as well as between OSR and males and females phenotype. This will be done first by simply investigating patterns in our data originating from the various experiments.

Then, by building a unified framework to disentangle different component of fitness in order to measure intensity of sexual selection for each component, I will explore how body size of both sexual partners affects these various components (mate encounter, mating success and reproductive success). This framework is an attempt to use simultaneously behavioural observations, and data obtained from parentage assignments, in order to improve our vision of mating success, and therefore improve our measures of sexual selection

All the data analysed in this chapter originate from experiment B1. The data produced by experiment B2 will be analysed later (chapter V) comparatively, in order to investigate *abiotic* environmental effects.

II. OSR variation

In brown trout, OSR vary considerably. In several rivers of the Pyrénées Mountains, the mean OSR per river varies between 3.67 and 7.25, but punctually, local OSR can reach higher values (up to 13, Labonne et al., 2009). In our experiment (B1, number of reproductive episodes observed= 22), OSR varies from 1 to 7 between our observation units (mean=3.9± 1.28, Fig. 20). Therefore, the OSR value is on the lower range of value known in natural populations from the same region, and each female encounter in average 20 % of the males.

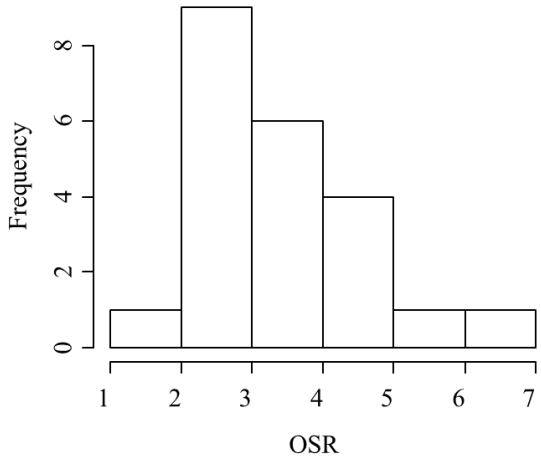


Figure 20. *Distribution of OSR (number of receptive males on number of receptive female) in the different observed units (OU) (N=22).*

III. OSR and competition

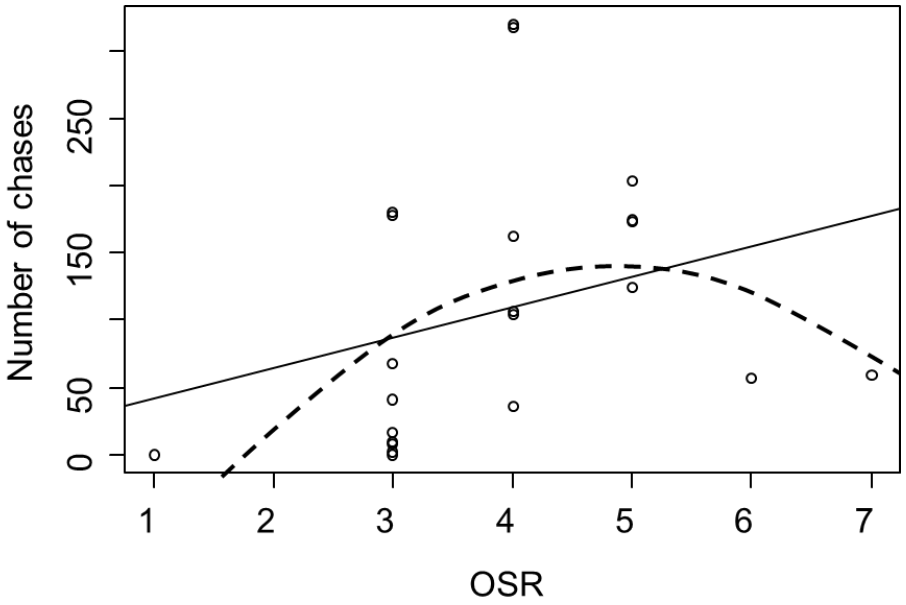


Figure 21. *Number of emitted chases by the dominant male before and after the observed copulation. The circles indicate observations, the full line shows linear adjustment (R^2= 0.0434, p = 0.177) and the interrupted line shows the non-linear adjustment (polynomial fit, R^2= 0.15, p =0.079).*

Variability in mating behavior may be affected by different factors such as OSR which is often used as a proxy of intensity of sexual selection. Emlen & Oring (1977) described the OSR as the ratio of the receptive males to receptive female during reproduction at any time. OSR is known to affect mating system in the sense that it will modify intensity of both intra- and inter-sexual selection. Indeed, with high OSR, competition intensity between males is expected to increase (Kvarnemo & Ahnesjö, 1996; Sutherland, 1985). For example, Weir, Grant, & Hutchings (2011) looked at the effect of OSR across a wide range of organisms on different mechanisms of intra-sexual competition such as contest competition, scramble competition, sperm competition and courtship. They show that male aggressiveness increase with OSR until a certain value, and that mate guarding increases, whereas courtship behaviour decreases. Therefore, OSR can influence mating

behaviours. Additionally, OSR affects the structure of the available phenotypes present for an episode of reproduction (Kokko & Monaghan, 2001).

When looking at the number of chases emitted by the dominant male on each OU (N=22), there was no significant correlation between OSR and number of chases emitted. This is true for a linear relationship, this remains true for a non-linear bell shaped relationship, although in the latter case, the p is not far from the significance threshold (Fig. 21). It is possible that this pattern is similar to the patterns found in Weir, Grant, & Hutchings (2011) and Tentelier et al. (2011), who both found a non-linear relationship. However, our dataset is here probably not sufficient to reach the 0.05 threshold. It is probable then that social environment as approached by OSR has an influence on agonistic behavior occurrence, which are a measure of reproductive investment for males.

Different studies showed a positive link between OSR and cannibalism (Aymes et al., 2010). Cannibalism occurs in 25% of the cases in natural populations of brown trout of the Pyrenean rivers (Aymes et al., 2010). It represents a high selective pressure since it has a negative effect on eggs survival. This behaviour occurs during a short period just after fertilization process, when eggs are not still covered by the female and remain visible against predators. Cannibalism can take two forms: 1) heterocanibalism and 2) filial cannibalism (Manica, 2002). The former corresponds to the egg eaten by one (or several) male(s) peripherals, whereas the latter corresponds to the eggs eaten by the male that fertilized the eggs. These two types of cannibalism are generally observed when intra-sexual competition is high. Moreover filial cannibalism frequently occur with paternity uncertainty (Aymes et al., 2010; Gray, Dill, & McKinnon, 2007), due to multiple mating and egg's survival probability (Lourdais, Brischoux, Shine, & Bonnet, 2005; Thomas & Manica, 2003) which lead the fertilizing male to eat its own offspring in

order to acquire reserves for future reproduction. In the present experiment (B1) cannibalism was observed in 50 % of the OU (N_{tot}= 11; $N_{heterocanibalism}$=5; $N_{filialcanibalism}$=2; $N_{hetero + filialcanibalism}$=4). However, although it is predicted that OSR should enhance cannibalism in natural population, it seems that in the present experiment the average OSR did not vary between OU where cannibalism occurred and that where it did not occur (χ^2= 0.019, df = 1, p = 0.890). In average, the OSR was 3.9 (sd±1.7) in OU without cannibalism and 3.8 (sd±0.75) in OU with cannibalism (including both filial and hetero cannibalisms). Therefore, it appears that in our experiment, egg early mortality – by means of cannibalism - is not directly affected by OSR. Egg cannibalism is also expected to improve male energetic status for further mating episodes, but here again, it does not seem to be controlled by OSR.

Additionally, the evolution of paternal care has been demonstrated in *Salmo trutta*. Parental care is defined as an increase of chases from the fertilized males against peripherals during a short time window (2 minutes after fertilization process: males and females release their gametes) where eggs are particularly threatened by cannibalism (Tentelier et al., 2011). Herein, I did not look precisely at paternal care which can increase with an increase of OSR since I measured the number of chases 1h30 after copulation. In a future study, it will be interesting to look at this behavior.

IV. OSR, attractiveness, and phenotype availability

Another interesting question is whether OSR variations are linked to female phenotype. Indeed, larger females have higher fecundity and could therefore be more coveted by males which could result in a higher competition and higher OSR. However, my results showed that regardless of their size, there was no link between OSR and female size in

the present dataset (Fig. 22) (Df =1, F value= 1.28, P=0.273). Small and high females encountered in average the same number of males.

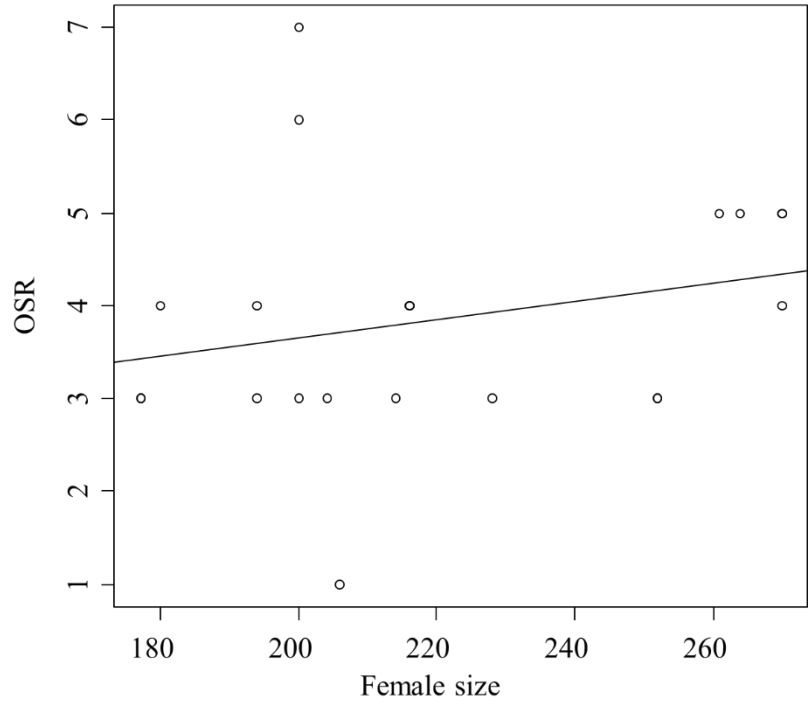


Figure 22. *Relationship between OSR and female size*

Additionally, I looked at how OSR was related to phenotypic availability of males (Fig. 23). There was no relationship at all between these two variables, implying that social environment as seen by OSR did not modify the availability of male phenotypes for females. It is also clear from the range of male phenotypic variation encountered by the females that most of the time, females in this experiment B1 had access to the whole population phenotypic variability.

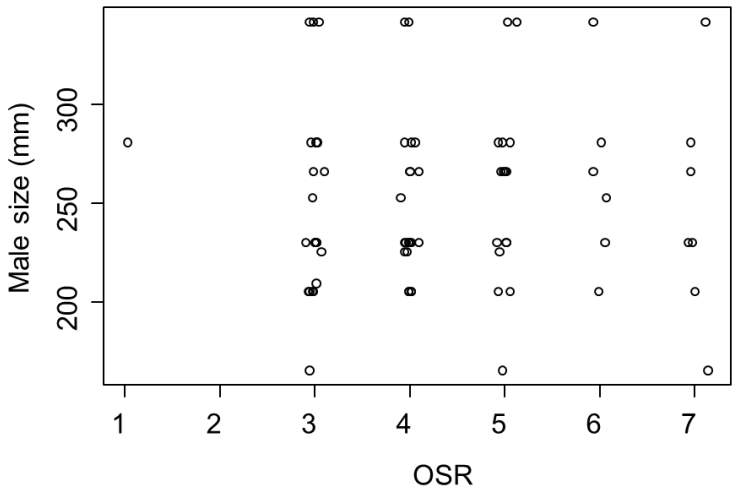


Figure 23. *Relationship between male size and OSR (some random variation was added to the x axis in order to distinguish between superposed points).*

While it is not the focus of this thesis to investigate realized mate choice in terms of trait associations, it is of interest to check whether males and females associated at random with regard to their phenotype. Fig. 24 shows the association of the different male and females phenotypes that encountered through the 22 mating episodes (from observed data). It seems that in experiment B1, males and females mated at random with respect to body size. This is relatively unexpected given previous works on the subject (Labonne et al., 2009; E Petersson et al., 1999). This result could be due to a relatively narrow range of variation in female body size.

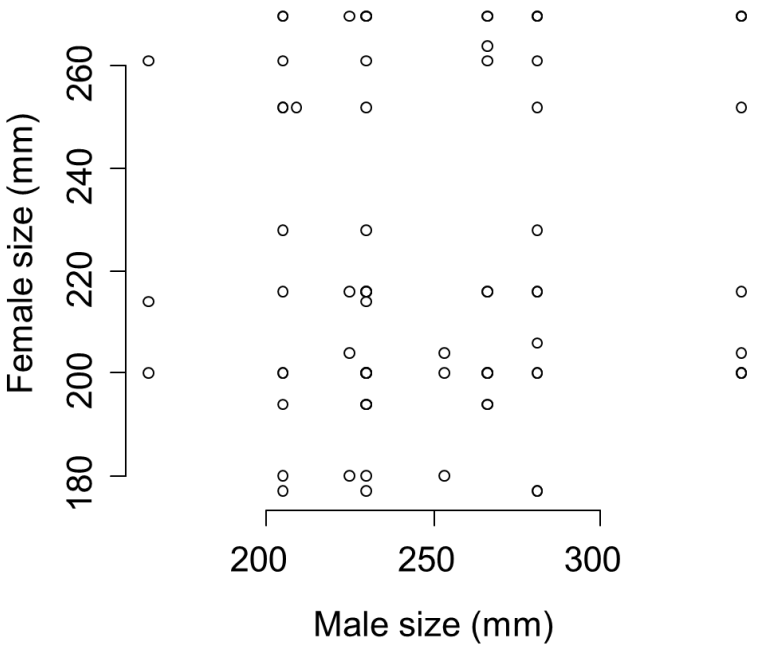


Figure 24. *Observed association between male and female phenotypes for observed encounters.*

V. Fitness model: taking into account conspecific phenotypes in reproductive success

1) Context

Sexual selection is predicted to operate provided there is variance in reproductive success and in mating success, and a strong link between these two.

For iteroparous species, the reproductive success RS_i of an individual *i* over a reproductive season is often used as a proxy of fitness within this reproductive season. The distribution of RS_i in a population for each mating episode[4] is generally summarized

[4] Hereafter, a mating episode will be defined as the situation where at least one female is active and sexually receptive in a given location and time. In my case study, mating episodes are all situations where a female trout dug a nest and was sexually active. This situation may or may not have led to a copulation, and may or may not have produced offspring. A mating episode can therefore include encounter,

by an array of number of offspring produced between all possible pairs of males and females for this episode. Then, the sum over all mating episodes produced a single matrix of offspring produced by each pair, the so-called parental table (Arnold & Duvall, 1994). An estimate of such matrix is typically generated by parentage analysis based on genetic markers (Bateman, 1948; Adam G. Jones & Ardren, 2003; Landry, Garant, Duchesne, & Bernatchez, 2001; Serbezov et al., 2010). possibly complemented by direct observations of mating behaviour (Collet et al., 2014; Coltman et al., 1999; Pemberton, Albon, Guinness, Clutton-Brock, & Dover, 1992).

These parental tables are commonly used to estimate indices of sexual selection and to quantify mating system, such as opportunity for selection (*I*), opportunity for sexual selection (*Is*) using respectively, reproductive success variation and mating success variation in the aim to see if selection can occur. Other indices, such as selection gradient (β) tend to calculate the covariance between absolute traits values relative success such as reproductive success and mating success (Jones, 2009). The Bateman gradient (βss) is a particular type of selection gradient accounting this time for the covariance between reproductive success and mating success as a particular trait. To calculate indices presented here, studies generally reduce the matrix to its margins, individual reproductive success being the sum of offspring on the individual's row or column, and mating success being the number of non-null cells on the individual's row or column, *i.e.* the number of different individuals with which at least one offspring was produced. This approach presents two important caveats: first, the definition and/or the estimation method of mating success, and second, the lack of consideration for the fundamental dependency between the mating and reproductive success of an individual and the mating and reproductive success of its mates. As I explained in the introduction, the definition of

encounter and copulation, or encounter, copulation and offspring production. Additionally, mating episodes are termed “Observation Unit” when they were video recorded during the experiments.

mating success may be influenced by the methods used (for instance, the molecular approach to estimate mating success), and often, authors do not clearly justify why they choose a given definition. The presence of zero values in parental tables obtained with such methods can be attributed to a variety of causes, may they be of biological interest (matings not producing offspring, offspring not surviving until sampling hinting at post-zygotic selection), or just possible bias due to sampling (non-random and non-exhaustive sampling). Because benefits and costs are both essential to understand the evolution of sexual selection, it should be of interest to study both points of view in a single framework to estimate sexual selection indices, especially for iteroparous species that may regulate reproductive investment between reproductive seasons depending on their age or on environmental variation (Jones, 2009; Pélissié et al., 2014). Additionally, matrices of reproductive success are often partially biased because of sampling time or because missing data which can conduce to a spurious measurement of indices of sexual selection since they are calculated from variance in mating and reproductive success (Collet et al., 2014; Snyder & Gowaty, 2007). The second caveat is illustrated by classical methods that only focus on the marginal sums of the parental table, and therefore cannot control for sexual partner trait or mating success variation whereas traits of both partners may influence mating success and reproductive success. Selection indices are estimated by regressing the margins of the parental table against the vector of values of phenotypic traits, independently for males and females. A direct consequence is that we might detect a significant correlation between a trait and mating success or reproductive success for a sex, and interpret it as evidence of direct selection, whereas indirect selection could for instance be at work by mean of non-random association between sexual partners' traits. Moreover, the environment in which individuals encounter each other may also vary and play a role in mating success and reproductive success of a pair at each observed mating

episode. We therefore need an approach in which the mating and reproductive success of a pair of individuals accounts for the phenotype of both individuals and the features of the environment where individuals encounter.

To solve both matters, we propose a model that 1) combines molecular data (parental table) and behavioural data (encounter and copulation matrix) to estimate the different components of reproductive success (here encounter rate, copulation rate, number of offspring produced) for each mating episode within the reproductive season, and 2) infers the joint effects of both male and female phenotypes and characteristics of mating episode on each component of the reproductive success (Fig. 24). The advantage of the model developed is that both behavioural data and parental tables can be incomplete, but each matrix will inform the others.

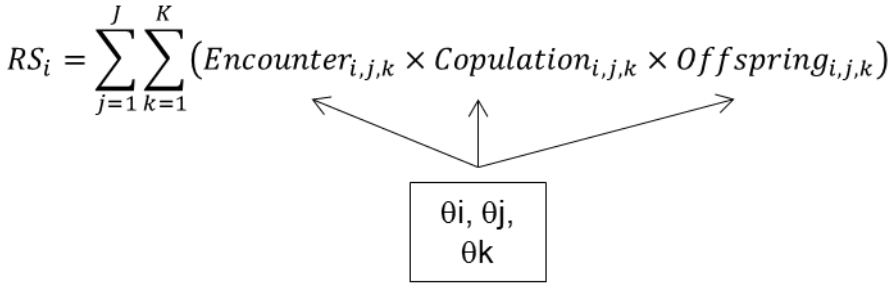


Figure 25. *Decomposition of individual reproductive success (RS_i) as modelled in this study. Individual reproductive success of individual i is the sum across all partners J and all mating episodes K of the product of encounter, copulation and number of offspring produced between partners i and j at the k^{th} occasion. Each component of the reproductive success at a given mating episode may be affected by individual phenotypes (θi, θj = body size, secondary sexual characters...) and environmental features (θk = operational sex ratio, wind speed...).*

I will first look at classical regressions methods using mating success and reproductive success estimated only from behavioural data or from molecular data. Then I will explain in detail the statistical model that allows this fitness decomposition between encounter

rate, copulation rate and number of offspring over pairs of sexual partners and over mating episodes. Then classical indices of selection will be measured from these data but also from the model output and I will discuss how the model can help to define and estimate mating success. Finally, the combined effect of male and female body size will be tested on the different reproductive components, such as the encounter process, the copulation process and the offspring number production.

2) What do we learn from raw behavioural and molecular data?

In total, 22 spawning acts were video recorded (K_{obs} mating episodes) during the reproductive season. Within these K_{obs} mating episodes, 14 females out of 32 and 12 males out of 17 were observed, giving a total of 75 pairwise encounters. 13 females and 7 males were observed releasing their gametes, totalizing 22 pairwise copulations (no multiple mating was observed). Stripping at recapture showed that almost all individuals (especially females) had released their gametes by the end of the experiment (only two females did not lay their eggs). Redds were detected in places where we did not place our cameras so we must have missed a proportion of mating episodes.

A total of 555 juveniles and 49 parents were genotyped. Among those individuals, 551 juveniles were assigned to 41 pairs of parents (10 males and 22 females) at a confidence level of 95%, corresponding 41 pair of copulations. Number of offspring varied from 0 to 201 for males (mean ± sd= 32 ± 64) and between 0 and 86 for females (mean ± sd= 17 ± 24). Only 12 pairs were both seen copulating and assigned offspring.

a) Bateman gradient

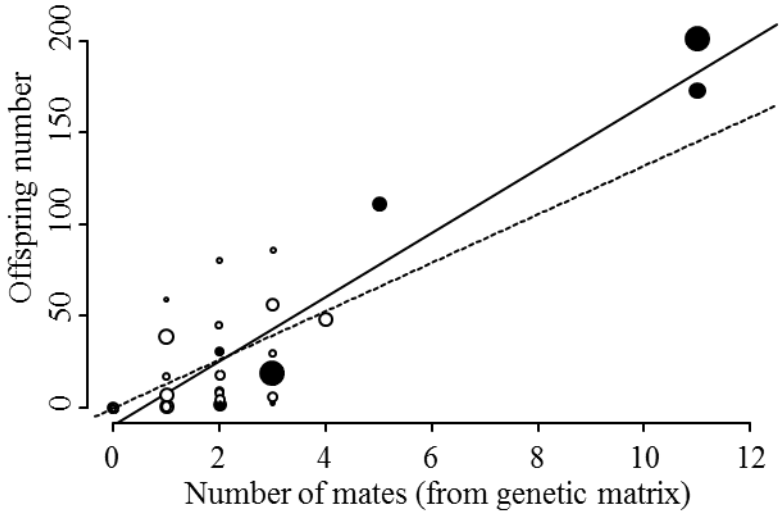


Figure 26: *Bateman gradient estimated from a linear regression between offspring number and number of mates (individuals that produced at least one offspring with a sexual partner to the next generation) in males (black points) and white (female points). The size of the points indicates individual body size.*

The Bateman gradient shows the relationship between offspring number and number of mates in males and females (Fig 29). In our example, from molecular assignation, the relationship between number of mates and number of offspring was very strong in males (linear regression: estimate ± SE= 17.62 ± 1.33, F=175.13, df=1, $p<0.001$) but it is also significant in females (linear regression: estimate ± SE= 13.19 ± 3.14; F=17.603, df=1, $p<0.001$) (Fig. 26). Then, if a phenotypic trait is correlated with the number of mates, it will enhance reproductive success and will be selected to the next generation. Classical theory suggests that in typical "sex roles" species, females should not increase their number of offspring by multiplying their number of partners. Two hypotheses have been proposed by Collet and collaborators (2014) stipulating that positive Bateman gradient in females may arise if more fecund females 1) mate with more males and 2) produce a bigger number of offspring for an equal number of mates. In their paper, Serbezov et al (2010) also found a positive Bateman gradient in brown trout even if its value was very inferior that the one found here (Serbezov et al., 2010): 1.43 against 13.19, see Table 9

below). These differences in the Bateman gradient may be due to the time of juvenile sampling, as in the present experiment, they are sampled at emergence stage whereas in Serbezov et al (2010), sampling was done at different and later dates which could affect variance in reproductive success. As a consequence, post-zygotic selection could be higher in their data than in ours, thus dampening the slope of the Bateman gradient.

b) Body size as a trait of interest under selection

Males did not increase the number of females encountered with increasing body size (GLM, quasi-Poisson distribution: estimate ± SE= 0.006± 0.005, dispersion parameter= 5.25, df=1, p=0.256) (Fig. 27). On average, regardless of their body size, males encountered 4±4 females. However, in males, body size increases significantly the opportunity to release gametes (copulation process) (GLM, quasi-Poisson distribution: estimate ± SE=0.017 ± 0.01, dispersion parameter= 2.27, df=1, p<0.005). Larger males produce more offspring than smaller males (GLM, quasi-Poisson distribution: estimate ± SE= 0.02 ± 0.005, dispersion parameter= 55.83, df= 1, p <0.001).

According to the raw data, female body size is not related to any variables related with sexual selection. Whatever female body size, they did not increase the number of males encountered (GLM, quasi-Poisson distribution: estimate ± SE= 0.001 ± 0.01, dispersion parameter= 6.64, df=1, p=0.942). Females did not spawn more when they are bigger (GLM, quasi-Poisson distribution: estimate ± SE= -0.003 ± 0.01, dispersion parameter= 1.41, df=1, p =0.762). Moreover, number of offspring did not increase with an increase in female body size (GLM, quasi-Poisson distribution: estimate ± SE= -0.001 ± 0.01, dispersion parameter= 36.48, df=1, P=0.865), a contradictory result with Serbezov et al., (2010) that found a weak positive relationship in females. Therefore it seems that body

size seems not to be under selection in females although larger females are known to have larger and more eggs (Blair, Rogers, & Quinn, 1993) which could lead them to be more attractive for males. Schroder (1981) referred that female readiness to spawn is a more important criterion for males than female size which could explain why all females regardless of their body size access to mates. Also, the ability to dig the nest and depth of the nest which provide good protection against scouring or predation, could be traits that inform males about the quality of the female but I was not able to look at it in this experiment because it requires to "destroy" the nest to access to this information. However, Freychet (2011) showed that the more the females dig before spawning, the less the fertilizing males cannibalise the offspring. Freychet also proposed that digging behaviour could be an indicator for males to increase their reproductive effort in paternal care.

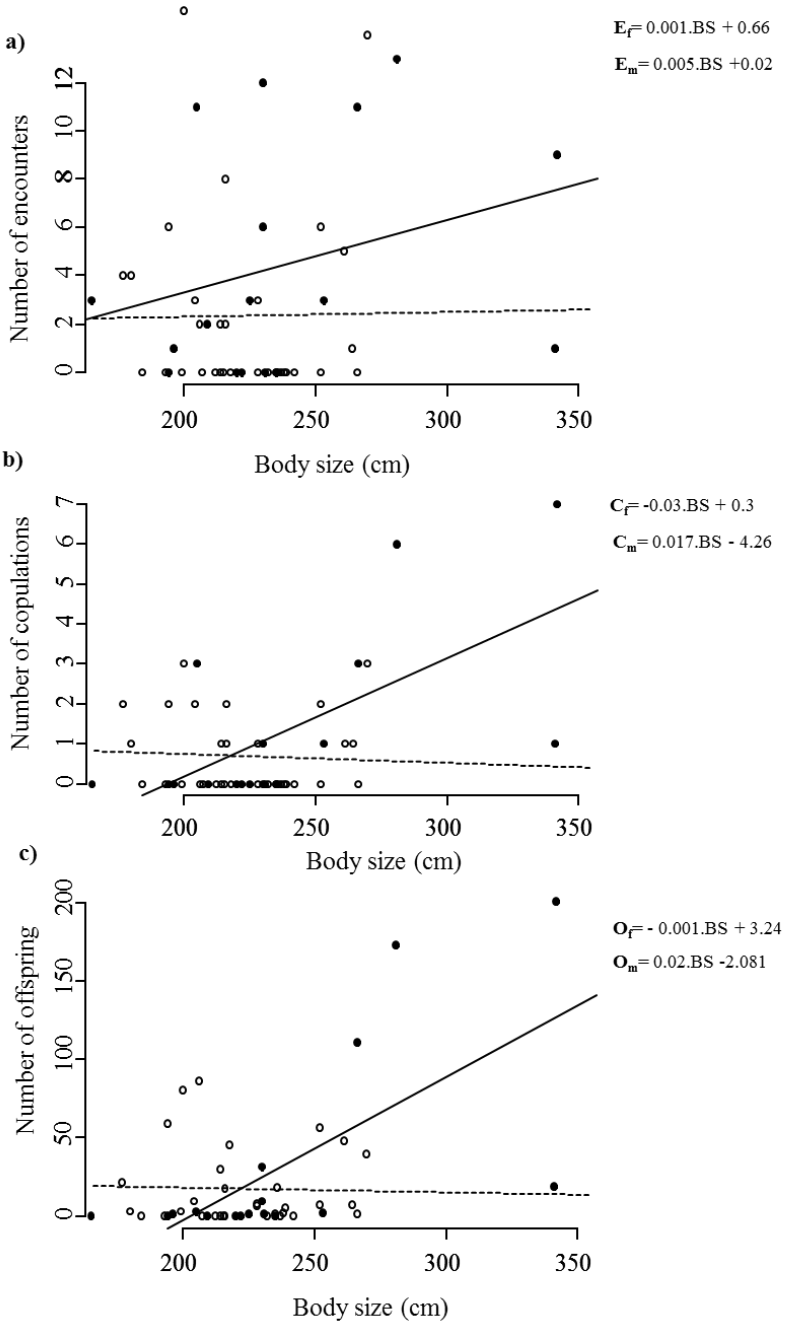


Figure 27. *Effect of body size on male (black points, solid line) and female (white points, dotted lines) number of encounters (from video observations) (a), number of copulations (from video observations) (b) and reproductive success (c) (computed as the number offspring of each individual, according to molecular analysis). The slope of the regressions are significantly positive for males but not for females.*

These results obtained from classical methods allow concluding that body size should be under directional selection in males through copulation access and production of offspring.

3) Statistical model

The model uses both behavioural data and molecular data. The philosophy is to make use of any data structure, provided the functional connection between data is explained. Here, my approach is inspired by our own data, which come in three different arrays confronting pairs of individual of each sex:

(1) a three dimensional array of observed pairwise encounters for each of the K_{obs} observed mating episodes that were video recorded (Fig. 28): $OE_{i,j,kobs}$,

(2) a three dimensional array of pairwise copulations observed for each of the K_{obs} observed mating episodes that were video recorded (Fig. 28): $OC_{i,j,kobs}$ and

(3) a two dimensional array of the total number of offspring produced by each pair (i.e. the parental table), over the whole reproductive season (i.e. summed over the K mating episodes), estimated from genetic assignment (Fig. 28) $N_{i,j}$.

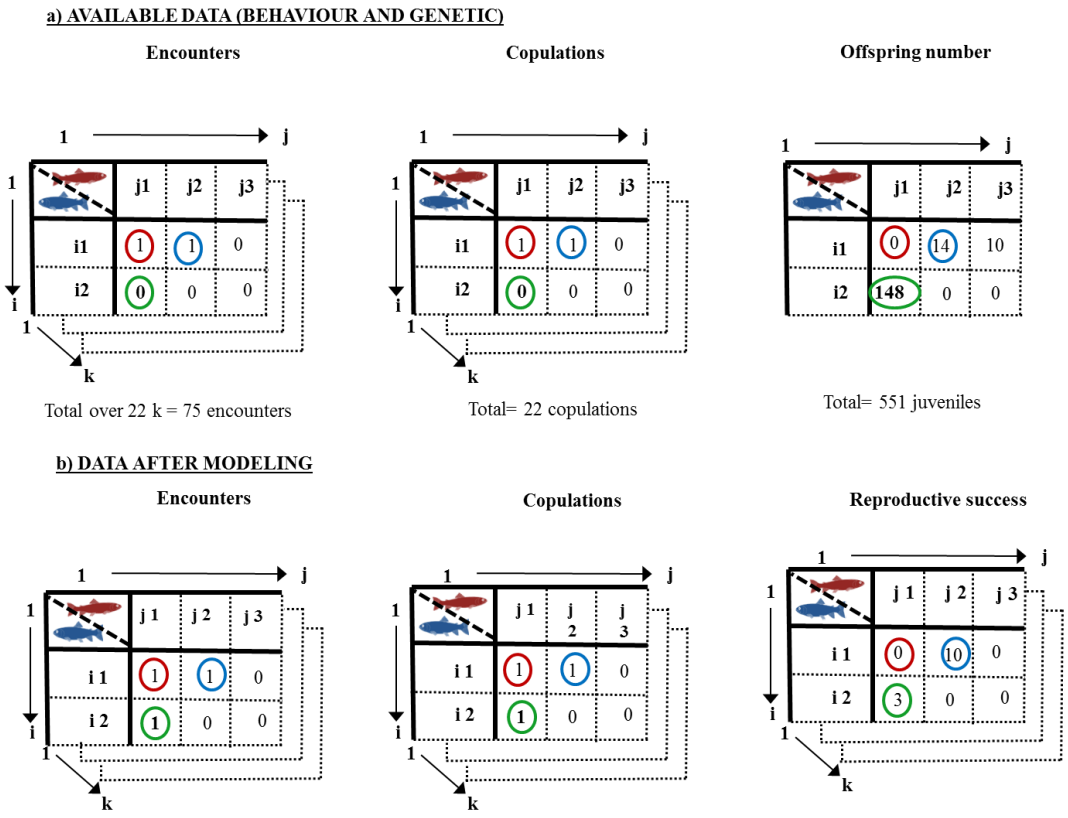


Figure 28. *Matrices describing encounters, copulations and number of offspring as obtained from a) observed data and b) after modeling process. In the present example, encounters and copulations could be observed between a pair of individuals during the behavior analysis for the first mating episode (k=1), leading to a positive or null reproductive success estimated from parentage assignment. In the blue example, female j_2 and male i_1 encountered, copulated and had a positive reproductive success (N=14) for the total reproductive season. The model decompose reproductive success for each mating episode, reproductive success between these two individuals falls to 10 for the first mating episode (k=1). In the green example, male i_2 and female j_1 produced 148 offspring during the whole reproductive period but were not observed on video cameras. The model aims at unfolding the reproductive success matrix into the k mating episodes. The consequence is that we might be able to attribute a copulation between these two individuals for the first mating episode (k=1) despite not having observed it, which also attributes a positive score for the encounter process. In the red example, encounter and copulation were observed between j_1 and i_1 but no offspring were produced.*

The goal of the model is to estimate the real data variation between all male-female pairs at each *k* mating episode: the encounter (a binomial variable indicating if male *i* met female *j* at mating episode *k*), the copulation (a binomial variable indicating if male *i* mated with female *j* at mating episode *k*), and the number of offspring produced (a

discrete quantitative non negative variable describing the number of offspring produced by male *i* and female *j* at mating episode *k*).

A first problem is the unfolding of the reproductive success matrix $N_{i,j}$ in *K* sub matrices, with *K* the total number of mating episodes that occurred in the reproductive season. This problem arises because usually offspring are sampled at the end of the reproductive season and all clutches are therefore pooled. We here assume that:

$$N_{i,j} = \sum_{k} N_{i,j,k}$$

Because behavioural data are generally incomplete within a mating episode (for instance, some individuals participated but were no identified), we assume that:

$$OE_{i,j,k} = E_{i,j,k} \times O_{i,j,k}$$

Where $E_{i,j,k}$ and $O_{i,j,k}$ are both binomial variables sampled in Bernoulli distributions of mean *pe* and *po*, respectively the probability that the encounter for the pair *i* and *j* at mating episode *k* happened and the probability that it was observed (both individuals I and j were correctly identified). When $O_{i,j,k}$ is zero, we have no observed behavioural data for the pair *i* and *j* at mating episode *k*, so encounter rate and copulation rate cannot be directly estimated.

A second problem lies in the probability to actually observe all *K* mating episodes. In general, this is not so, and we observe K_{obs} mating episodes. If $K_{obs} < K$, then no behavioural data are available for some mating episodes. Additionally, the actual number of mating episode *K* may not be known. In such case, I propose to simply simulate expected behavioural data using the posterior densities from estimated parameters for the K_{obs} mating episodes where behavioural data are known.

The value of *K* itself depends on the reproductive system studied as well as the population characteristics, but its value can hopefully be estimated directly in the model because the posterior distribution should reveal the best combination of behavioural and molecular data conditional on the value of *K*.

In short, encounter and copulation processes are mainly informed by behavioural data on observed mating episodes (*Kob*s). Relationships between these two processes and parameters of interests (traits, for instance) are then used to re-simulate what is the most probable distribution of encounters and copulations at the scale of the whole reproductive season for each of the K observed or non-observed mating episodes. Moreover, the posterior distribution of the parameters and variables of interest should indicate what the most probable value of K is.

If we now leave aside these problems of sampling bias, we can define the relationships between processes and parameters of interest. For instance here the effect of male and female body size (BSM_i and BSF_j) on encounter rate ($E_{i,jk}$), copulation rate ($C_{i,j,k}$) and offspring number ($N_{i,j,k}$) were modeled as following:

Encounter rate

$$logit(E_{i,j,k}) = a_1 + b_1 \times BSM_i + c_1 \times BSF_j$$

Copulation rate

$$logit(C_{i,j,k}) = a_2 + b_2 \times BSM_i + c_2 \times BSF_j$$

Offspring number

$$log(N_{i,j,k}) = a_3 + b_3 \times BSM_i + c_3 \times BSF_j$$

Statistical inference was conducted in the Bayesian framework. The joint posterior distribution of all unknown quantities of the model was approximated by MCMC sampling as implemented by the OpenBUGS (version 3.21) software. A MCMC sample of 11320 draws with a thinning of 100 was used, after checking its convergence by applying the Gelman-Rubin test (Gelman & Rubin, 1992). We used non informative Gaussian and independent priors distributions (mean= 0, precision= 0.001) for hyper-parameters a_1, a_2, a_3, b_1, b_2, b_3, c_1, c_2, c_3, a Beta prior distribution $\beta(1,1)$ for po, and a uniform distribution [0,100] for K. The full code in OpenBugs language and the posterior density of parameter value are provided in Supplementary information 5 andSupplementary Information 6.

a) Selection indices from raw data and from the model output

In order to estimate different measures of sexual selection between the raw data and data simulated from the model combining behavioural and molecular data, we computed different quantitative measurements of sexual selection for each sex. Opportunity for selection (I) and sexual selection (Is) were computed as the ratio of variance on squared mean of reproductive success and mating success, respectively (Wade & Arnold, 1980). Bateman gradient (β_{ss}) was measured using a simple linear regression between reproductive success and number of mates. To compute these indices from the raw molecular data, we conformed to the classical view: individual reproductive success was considered as the number of offspring produced (sum of the individual's line in the parental table) and individual mating success was considered as the number of different individuals with which the focal individual produced offspring (number of non-null cells

in the individual's line in the parental table). From the raw behavioural data, we computed opportunity for selection on the number of partners encountered and opportunity for selection on number of mates with which copulation occurred. For the latter, we only considered individuals for which at least one encounter was recorded. To combine behavioural and molecular data, we ran the model, then simulated behavioural and molecular data using parameter values drawn from the joint posterior distributions, and finally computed the indices of selection on these simulated data. Here, individual reproductive success was again computed as the number of offspring produced, but mating success was decomposed in encounter success, *i.e.* number of individuals of the other sex encountered by the focal individual, copulation success, *i.e.* number of individuals of the other sex with which the focal individual emitted its gametes, and mating success *sensu* Bateman, *i.e.* number of individuals with which the focal individual produced offspring. Opportunity for sexual selection and Bateman gradient were computed using each definition of mating success.

Although only 22 pairwise copulations were recorded on video and 41 families were detected by behavioural and molecular analysis, the results from the model tell a different story: accounting for the possibility that 1) a brood was the result of several copulations, 2) some copulations led to no offspring production, and 3) some copulations could not be observed, the model estimated that 41 (sd = 9) mating episodes occurred involving 56 (sd= 14) pairwise copulations.

Additionally, the reconstruction of the mating process from both behavioural and molecular data allowed to reduce variance and increase the mean of mating success processes and reproductive success by correcting “zeros value” previously accounted as an absence of copulation whereas it could be due to a copulation that failed to achieve

fertilization. The reduction of variance of mating success and reproductive success thus allowed calculating more precisely indices of sexual selection.

The opportunity for selection and sexual selection, Bateman gradient and the maximum standardized sexual selection gradient, computed from both raw molecular data and output of the model, are given in Table 9. This table shows the ability of this statistical method to provide access to many different definitions of mating. For example, from the output of the model, I was able to calculate new indices of opportunity for selection for each of the components and also new selection gradients (derived from Bateman gradient) for different definitions of mating success: number of genetic mates $(I_{genetic\ mates}, \beta_{genetic\ mates})$, number of other-sex individuals encountered $(I_{encounters}, \beta_{encounters})$ and number of other-sex individuals with which copulation occurred $(I_{copulations}, \beta_{copulations})$. However, the reader should be informed that the different indices from molecular and behavioural data cannot be compared to indices calculates from the model for the single reason that the model (in the presented form) takes into account the effect of both sexes phenotypes.

Table 9. *Opportunity for selection ($I_{offspring}$), opportunity for sexual selection and Bateman gradient computed for males and females, on raw molecular data and on data simulated from the output of the model. Opportunity for sexual selection and Bateman gradient was computed for different definitions of mating success: number of genetic mates ($I_{genetic\ mates}$, $\beta_{genetic\ mates}$), number of other-sex individuals encountered ($I_{encounters}$, $\beta_{encounters}$) and number of other-sex individuals with which copulation occurred ($I_{copulations}$, $\beta_{copulations}$).*

	$I_{encounters}$	$I_{copulations}$	$I_{genetic\ mates}$	$I_{offspring}$	$\beta_{encounters}$	$\beta_{copulations}$	$\beta_{genetic\ mates}$
From molecular data							
Males	-	-	2.15	3.93	-	-	17.45
Females	-	-	0.80	2.07	-	-	13.19
From behavioural data							
Males	1.05	1.36	-	-	-	-	-
Females	1.82	0.34	-	-	-	-	-
From model output							
Males	0.05	1.46	1.48	3.38	7.78	8.84	8.84
Females	0.06	0.40	0.40	0.61	-0.01	-0.08	-0.09

Opportunity for selection on number of partners encountered was much lower when computed from the model output than from raw behavioural data, whereas opportunity for selection on number of sexual partners was the same for both methods. Opportunity for selection on number of mates (obtained from genetic analysis) and number of offspring was the same from both methods for males, but was much lower for females when estimated from the model output.

The data simulated from model output allowed computing sexual selection indices on behavioural dimensions of mating success, which are also given in Table 9. Selection gradient on number of individuals encountered and number of copulation partners were significantly positive for males but did not differ from zero for females. Finally, the

selection gradient on number of mates (obtained from molecular analysis), Bateman gradient, were also positive for males and null for females.

b) Combined effect of male and female phenotype on the components of reproductive success

Sexual selection on phenotypic traits is classically quantified for each sex separately, by regressing the number of mates against phenotypic trait in a separate model for each sex. However, one offspring has two parents and traits of each sex may influence mating its fitness. Therefore, mating success and reproductive success should be analysed taking into account traits of a pair of individual and not testing separately the trait effect of each sex independently on mating and reproductive success.

Here, the unified framework consider the mating episode as the statistical unit, and infer the effect of traits borne by individuals (and environment) involved in that event on its outcome. This approach departs from selection theory, to which regression models fit well (Lande & Arnold, 1983), but allows insights on the mechanisms by which traits affect reproductive success.

Thus, as described in the previous paragraph, the model includes the effect of male and female body size on the probability of encounter, the probability of copulation and the average number of offspring. Result (Fig. 29) show that body size had a positive effect on all components of male mating and reproductive success. Comparing to the results above accounting only for behavioural and molecular data, body size increased each component of reproductive success. Larger males had a greater probability to encounter females, had a greater probability to copulate with the females they encountered, and produced a larger number of offspring once mated. The effect of body size was quite different for females and, as it did affect neither the probability of encounter nor the probability of copulation

upon encounter. Regardless of their size, a weak positive effect in encounter probability was found for larger females. Larger females are generally the most attractive females because have the same probability to encounter males and copulate because the readiness to spawn is probably at least as much attractive for a male than a big female with potentially more eggs (Shroder, 1981). Additionally, if smaller males have a lower probability to encounter and copulate, they could be less selective which let the possibility to all females to encounter and mate. However, and quite counter-intuitively, larger females produced less offspring once mated, a surprising result that was hidden when taking into account only molecular data. Several possibilities could explain these results. First bigger females could attract a bigger proportion of males (high OSR male biased) that could increase the probability of filial and hetero cannibalism (Aymes et al., 2010). In the experiment no multiple mating was observed, which reduce the probability of the filial cannibalism hypothesis since it is related with parentage uncertainty in males. Another possibility is that if bigger females mated with bigger males, the positive effect of male size could balance the negative effect of female size. This discrepancy between results of the two methods points at the benefits of our methods when data are not independent.

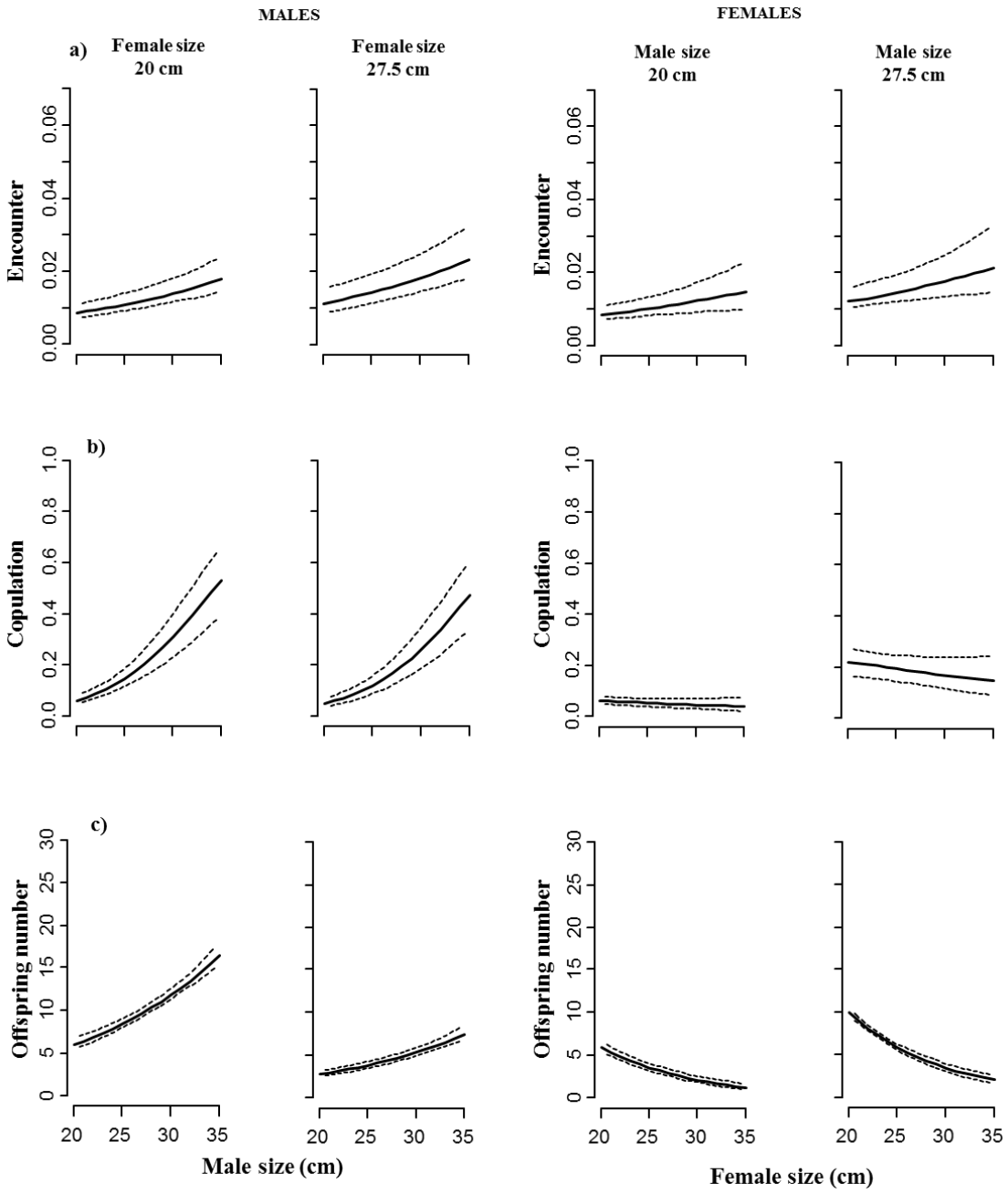


Figure 29. *Model predictions on the effect of male (on the left) and female (on the right) body size on the probability of encounter (a), the probability of copulation upon encounter (b) and the average number of offspring produced upon copulation (c). Predictions are computed after the joint posterior distributions of hyper-parameters of the model including effects of body size of both males and females for each iteration once the MCMC chains have converged (=11320 iterations). Solid lines represent the median, and dashed lines represent 5% and 95% quantiles. When predicting the effect of one sex's body size, body size of the other sex is set at two different conditions: 20 and 27.5 cm*

While presenting the model, I described how variation at the level of the mating episode could also be accounted for. In the presented case study, this advantage allowed us to first draw some information from observed mating episodes, and then expand this information at the scale of the whole reproductive season. However, much more information could be drawn from inference at this scale: for instance, any variable that could vary between mating episode could improve our estimates of sexual selection. For instance, it could be possible to include the effect of observed values of OSR on copulation rates and number of offspring produced. Such models are currently processed, but I did not have sufficient time to obtain clean estimates for their hyper-parameters. Additionally, random effects could also be integrated, to better account for uncontrolled sources of variation. Here again, I am still at the preliminary stage to obtain stable results. Such estimates can sometimes be very long to produce using currently available softwares and algorithms (weeks of simulation).

CHAPTER V.

Sex in habitats: population scale

I. Context

Environment may vary consistently between and within rivers which will have direct consequence on population structure. When faced with new environmental pressures (natural or human induced, biotic or abiotic environment), populations can respond differently (Davis, Shaw, & Etterson, 2005; Gienapp, Leimu, & Merilä, 2007; Holt, 1990): 1) populations can escape by dispersing to others and more favorable habitats, 2) they can display plastic strategies by modifying their phenotypic responses without altering their genetic construction or 3) they can adapt to the new conditions through evolutionary processes. Theoretical climate models predict an increase of the magnitude and frequency of extreme precipitation events and consequently an increase of droughts and floods in Europe (Dankers & Feyen, 2008).

The results in the previous different chapters showed that 1) individual reproductive investment (plasma metabolites and weight) is closely related to reproductive success 2) reproductive success depends on traits of both sexual partners. To a larger scale, we can now wonder how physical environment is going to affect fitness and therefore how it influences natural and sexual selection. With global change, an increase of temperatures with higher precipitation level and higher atmospheric moisture has been observed and the trend will continue (Milly, Wetherald, Dunne, & Delworth, 2002). We will here explore two possible paths by which environmental change can affect reproductive output.

The first one will look at the post-zygotic stage: because eggs spend a few months under gravel, quality of the nest dug by the female should provide good protection against biotic and abiotic pressures (predation, scouring, oxygen level…). We will therefore address how female may adjust their reproductive habitat choice, with respect to 1) the effects of environmental stochasticity within rivers, and 2) their own reproductive investment. To

do so, we will use the Experiment C, and assume that females strategies related to habitat choice may evolve in order to maximize their own fitness.

The second path will focus on the differences of environmental stochasticity between rivers and their respective effects on social environment, reproductive investment and reproductive isolation mainly at pre-zygotic stage. To that end, we will use two populations with different gene pools and we will investigate how reproductive investment is affected in each population as a function of the ecological contrast used in the experiment B1 (constant discharge) and B2 (variable discharge). By placing these two populations in sympatry under the same ecological contrast, we will also monitor what could be the consequence on gene flow between the two populations.

II. Within river contrast in environment

Environment may vary locally and change the direction of natural and sexual selection thereby opening new pathways for the evolution of reproductive behaviours. Brown trout display an impressive range of behavioural variation related to environmental cues. Within a river, spawning site is apparently selected by females and is expected to have a strong impact on offspring fitness. Indeed, in salmonids, females cover their eggs (previously fertilized by one or several males) under gravel bars. Eggs will hatch about at 420 degree.days^{-1} after fertilization and will stay under gravel up to 800 degree.days^{-1} (emergence time). Until this stage, juveniles continue their development using their vitellin reserves as the only energetic resource. Thus, offspring survival mainly depends on the spawning site where they will be buried, and on the reserves provided by their mother (egg size). The physical characteristics of the redd is expected to impact their own survival since it affects protection against predators (Dumas, Olaïzola, & Barriere, 2007). Additionally, the physical environment of the redd, such the depth of the nest or the water

flow conditions and their potential effect on the oxygen level can also have an impact on offspring survival. Moreover, water flow variations may modify habitat structure and particularly reproductive habitat, large floods leading to gravel bars scouring, whereas drought could leave redds dry, two phenomena known to affect offspring mortality. In a global context, environmental stochasticity, such as the frequency and magnitude of floods and droughts, should dramatically increase in the future and should be therefore considered as important selective pressure (Milly et al., 2005).

Based on these observations, we can predict that to maximize both their own fitness, and possibly their offspring fitness, a possible evolutionary path is that females should be able to discriminate their environment. Thus, females should do an optimal choice of spawning site in order to realize the best trade-off between reproductive investment in offspring and their own fitness (Trivers, 1972).

In the present experiment (Experiment C), I could only related female phenotype, redd environment and habitat selection by females (§ II.II). I initially attempted to relate these data with observations of reproductive behaviour directly in the wild, knowing that effect on variation of habitat choice would potentially act as a selective pressure leading to variation in offspring mortality which would in turn affect the social environment and males reproductive behaviour (e.g. increase of OSR, cannibalism, paternal care). However, during the first year of sampling, I could only get a complete sequence of data (reproductive behaviour, habitat selection and survival) for 7 redds: the odds of being both able to capture a useful behavioural sequence, the spawning, and monitoring egg survival were too low to produce sufficient data during the span of this study. Therefore, the experiment C only allows to look at the impact of certain biotic and abotic characteristics of spawning site on natural selection (effect on offspring survival).

1) Effect on offspring survival: redd scouring as the biggest environmental pressure?

During experiment C, eggs survival was estimated at hatching developmental stage. Over the three reproductive seasons, a total of 56 redds were equipped with a total of 324 capsules. Only 101 capsules out of 324 were found at hatching time. In other terms, 70 % of the capsules were not found in the substrate due to redd scouring in both rivers sampled (Fig. 30).

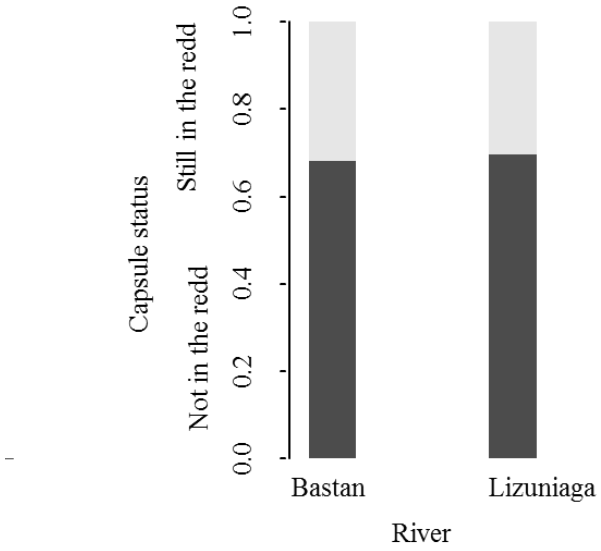


Figure 30. *Proportion of capsules still in the redd (light gray) and outside of the redd (dark gray) in the two sampled populations over the three reproductive seasons (Bastan and Lizuniaga). Total number of capsules is equal to 324 (Bastan=135; Lizuniaga= 189).*

According to this result it seems pretty clear that water flow discharge leading to scouring appears to be an important mortality driver that could potentially influence female strategies of reproductive habitat choice. If 70 % of offspring mortality is due to scouring, then females could be therefore more selective in their choice of spawning site to avoid scouring, and different strategies should evolve across time.

In order to test different characteristics of spawning habitat on resistance to scouring probability, a generalized linear mixed model was run using Open bugs software (version 3.2.2). Scouring probability (R) was measured as following:

$$R = 1 - (Probability\ of\ a\ capsule\ to\ be\ scoured)$$

This model accounts for partial scouring of the redd: within a redd, some capsules might be scoured, whereas some others may remain. The model looked at the effect of female size (FS), depth of the nest (D), the 90% quantile of substratum particle size (Q90) and the double and triple interactions involving these three variables, on R. Sampling year, river, and redds were considered as independent random effects:

$$\begin{aligned} Logit(Ri) = {} & ai * FS + bi * D + ci * Q90 \\ & + di * FS * D \\ & + ei * FS * Q90 \\ & + fi * D * Q90 \\ & + gi * FS * D * Q90 \\ & + ki * random\ effect\ (redd) \\ & + li * random\ effect\ (river) \\ & + mi * random\ effect\ (year) \end{aligned}$$

Depth of the nest, which could increase resistance to scouring because it may provide a better protection of the nest, had no effect. Also Q90 and interaction with the different variables had no effect on resistance to scouring. Surprisingly, results of the analysis showed that only female size has an effect on resistance to scouring (Table 10). Redds built by smaller females had a higher probability to resist to scouring (Fig. 31). Female effect on resistance to scouring could be explained by the fact that the quantity of gravel displaced is different between large and small females. Gravel displacement also modify

impoundment and silting, making gravels easier to get carried away by shear stress forces in case of flood: river flow shapes river bed by following least cost path for erosion and sediment transport. If large females displace more gravel due to their physical force, their redds represent an important surface of clean gravels, and they could then be scoured with a higher probability than small redds.

Moreover, in the present experiment, I assessed that eggs present in capsules removed from the substrate where considered as dead. However, capsules are heavier than eggs and could be taken away differently. Also, the future of the eggs removed from their nest remains unknown. Therefore a specific experiment in the fluvarium – a closed experimental looping channel available at the Saint-Pée lab - could be done in order to simulate the effect of scouring on the fate of these eggs. It is not excluded that eggs could also roll between substrate and find other places to develop themselves.

Table 10. *Parametes estimates for the model of resistance to scouring.*

Factors	**Offspring survival**	
	Mean of the parameters	sd
Female Size	**a= -15.53**	13.09
Depth	b= 1.35	24.82
Q90	c= -5.55	21.86
*FS*D*	d= 7.37	13.18
*FS*Q90*	e= -0.91	11.94
*D*Q90*	f=2.21	20.87
*FS*D*Q90*	g=4.21	10.15

Numbers in bold indicate parameters for posterior distribution is at least 90% above or below zero. D= Depth; Q90= 90 quantile for particle size; FS= Female size.

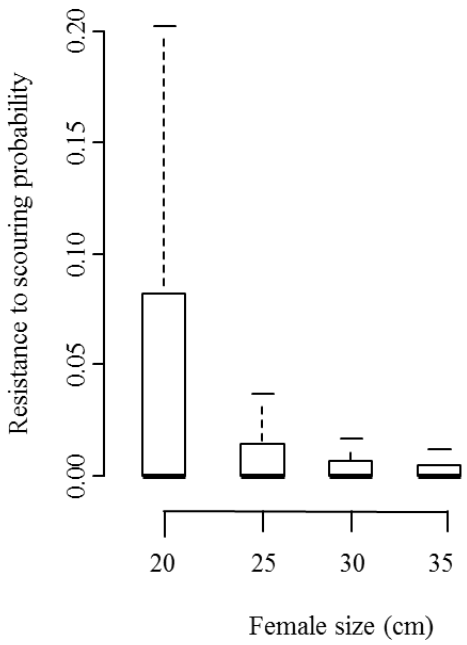


Figure 31. *Predictions of the resistance to scouring in function of female size. Depth of the nest was fixed at 10 cm and X90 was fixed at 15 cm².*

Hence, environmental stochasticity within the river strongly affects egg survival, and habitat choice by female does not seem to counteract it effect. Only female size appears to influence the outcome.

2) How to explain egg size variability in *Salmo trutta*?

Egg size reflects a part of maternal investment in their offspring, the quantity of their resources directly allocated to their offspring. Indeed, during ovogenesis, the vitellus (which is used as a nutritive resource for embryo and juvenile development) is accumulated in female's oocytes. Quantity of lipids invested may be influenced by age of females (Kamler, 1992). Therefore energetic reserves are directly linked to maternal investment and may vary from one female to another one. Egg size is therefore an

important fitness component in salmonids and it has been shown that offspring fitness tend to correlate with egg size after hatching. However, different studies show opposite effect of egg size on offspring survival. For example, Einum & Fleming (2000) predicted that bigger eggs have a better chance to survive, whereas Régnier, Bolliet, Gaudin, & Labonne (2013) showed the contrary. For example, juveniles that emerge from larger eggs benefits of bigger size after hatching which confer them an advantage to move, disperse and then to food competition (Einum & Fleming, 1999). More generally, in fish bigger individuals have better locomotion capacity which helps them to faster escape predators and therefore increase their survival (Ojanguren, Reyes-Gavilán, & Braña, 1996). In that case, evolution should favor larger eggs in natural populations. However, egg size could also influence offspring fitness before hatching since pre-hatching environment is essential to confer a good protection against biotic and abiotic environmental pressures (predation, oxygen and temperature variability, see Régnier, Bolliet, Gaudin, & Labonne 2013).

Theoretical studies predict that after natural selection episodes, there should exist an optimum of egg size in a single environment, although the relationship between egg size and offspring fitness may vary from one environment to another one (Smith & Fretwell, 1974). However, egg size variation has been observed both *between* and *within* different populations (Bagenal, 1969; Régnier, 2011), which is also the case in our study (Fig. 32). It is noteworthy that in our two populations, there was no strong link between female size and egg size (Fig. 32).

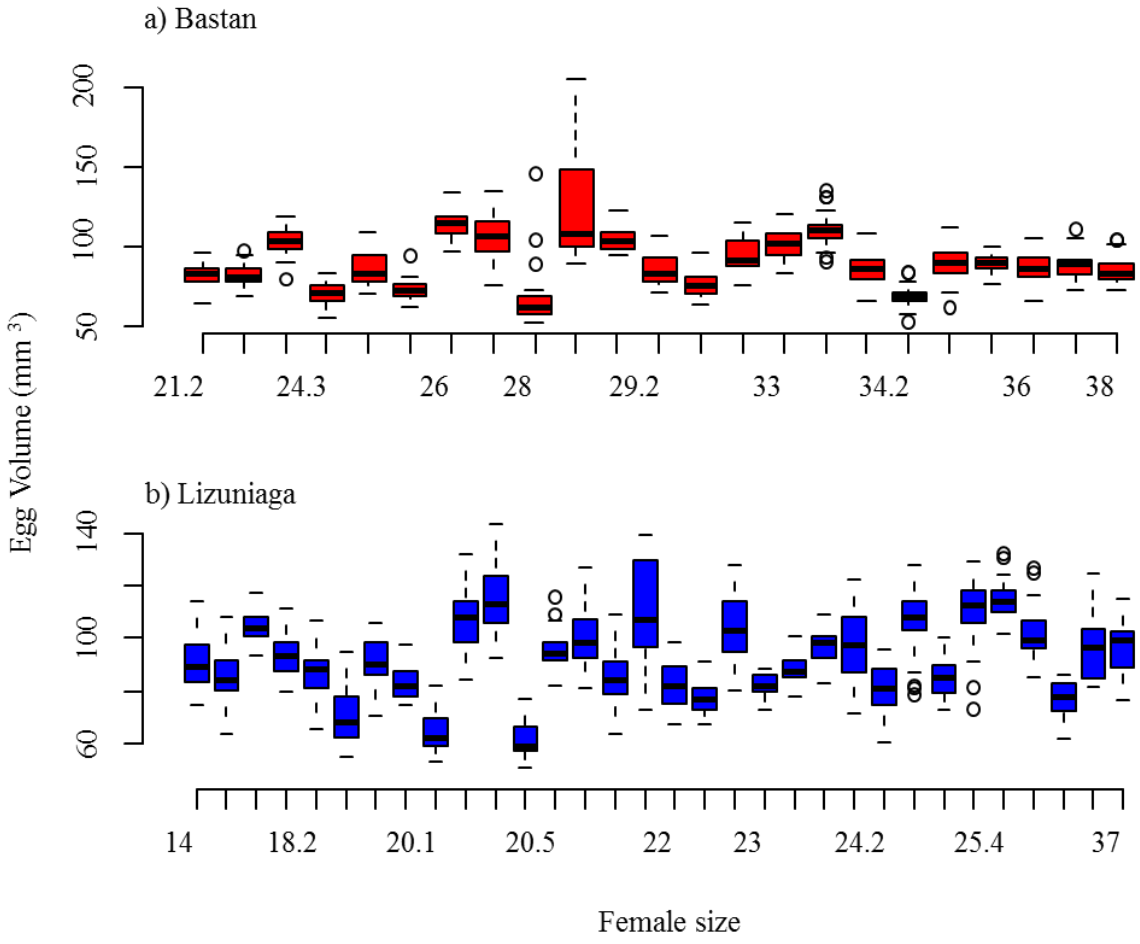


Figure 32. *Egg volume variability ranked as a function of female size in two different rivers a) Bastan and b) Lizuniaga over the three reproductive seasons. Some random variation was added to the x axis in order to distinguish between superposed female sizes.*

Few hypotheses have been proposed to explain variability in egg size. The first one and the more common one is the fact that small females should produce smaller eggs than larger females, maternal investment could therefore be dependent on individual age (Kamler, 1992). Second, under the parental-conflict hypothesis (Trivers, 1972), females could adjust their investment in offspring by doing the best trade-off between its fecundity and fitness of its offspring which will in turn affect egg size. Additionally, kin

competition is predicted to be a strong selective pressure leading females to choose between number and egg size. This tradeoff implies that more fecund females should produce fewer but bigger eggs in order to avoid increased kin competition for resources at the critical stage of emergence (Einum, Nislow, Mckelvey, & Armstrong, 2008; Einum & Nislow, 2005). Egg size variability within and between populations is often related to female size (Kamler, 1992), but other factors such as environmental quality (Gregersen, Haugen, & Vøllestad, 2008) or density (Fox & Czesak, 2000) of individuals have been shown to influence maternal investment. Régnier and collaborators (2013) showed that small eggs tend to have better survival than large eggs when incubation temperature derive from their optima and also that larger eggs have lower tolerance to high temperature. Consequently, if a relationship exists between egg size and environmental conditions, females should adapt their egg investment depending on environment quality. This is the theory of phenotype habitat matching (Hendry & Day, 2003; Hendry, Day, & Cooper, 2001). In other words, there could exist different optima for egg size within population if there is a correlation between maternal investment (egg size) and environment that increase egg survival. In sedentary brown trout, females start vitellogenesis in the summer, whereas the reproductive season occurs in December-January (Estay, Neira, Diaz, Valladares, & Torres, 1998; Tyler, 1990). Then, a prediction is that females should adapt their egg investment depending on the environmental conditions. For example, Hendry and collaborators (2001) predict better survival for small eggs because the oxygen demand is higher for large eggs. However, Einum, Hendry, & Fleming, (2002) predict the contrary, assuming that larger eggs survived better than small eggs when challenged with low oxygen.

In the current dataset, egg size varied from 46 to 205 mm^3 (mean= 89.34; sd±17.20) indicating that females invest differently in their eggs. Female size varied from 14 to 38

cm (mean=25; sd±6.05). The depth of the nest was also variable since it ranged from 2 to 17 cm between the different females (mean=9; sd±3.13). Small particles are the most represented on each redd. The Q90 of the particule surface distribution varies from 3.92 cm^2 to 31 cm^2 in area (mean=13.1; sd±6.15). Therefore, redds presented variation in depth of the nest, in Q90, associated to variation in egg volume. All these variables provide many options for females to invest in reproduction in order to maximize egg survival.

3) Does phenotype habitat matching exist in our populations?

Spatial and temporal variations in reproductive success have been widely described in the salmonids literature (Serbezov et al., 2010) but no experiment in the field has been undertaken in order to study how selective pressure can lead to the evolution of reproductive strategies. The experiment C (§ II.II) was designed to answer this question in natural environments, with the aim to study female spawning choice. To test the hypothesis of phenotype habitat matching in *Salmo trutta* implies to investigate potential interactions between characteristics of the spawning habitat chosen by the female, egg volume, and offspring survival. The effect of interactions between egg volume and different environmental characteristics, such as the nest depth and particle size (Q90), have therefore been tested on offspring survival.

Over the three reproductive seasons, a total of 56 redds were dug up and 1624 eggs were excavated and placed in 324 capsules from them in two different rivers (Bastan and Lizuniaga) (Table 11). However, as previously mentioned, only 101 capsules containing 503 eggs resisted scouring. The current test relies on the eggs contained in these 101 capsules, and does not account for scoured capsules.

Table 11. *Number of redds dug up and number of eggs excavated from the two different rivers per sampling season.*

	River			
	Bastan		Lizuniaga	
Year	Number of redds	Number of eggs	Number of redds	Number of eggs
2011-2012	3	75	3	75
2012-2013	4	120	20	584
2013-2014	16	480	10	290

In order to investigate the effect of different characteristics of the spawning site on offspring survival (S_i) and to see if phenotype habitat matching can be detected in our experiment, a generalized linear mixed model was built. The effect of the depth of the nest (D), maximal quantile of particle size (Q90) and individual egg volume (V) were tested. Also, random effects of the capsule, river and year were added to the model to avoid pseudo-replication due to these various factors.

$$\begin{aligned} Logit(Si) = {} & ai * D + bi * Q90 + ci * V \\ & + di * D * Q90 \\ & + ei * (D * V) \\ & + fi * (Q90) * (V) \\ & + gi * D * Q90 * V \\ & + li * random\ effect\ (capsules) \\ & + mi * random\ effect\ (redd) \\ & + ni * random\ effect\ (river) \\ & + oi * random\ effect\ (year) \end{aligned}$$

The results showed no effect of depth, Q90 or egg volume on offspring survival. Also the two way interaction involving D and Q90, or D and V and the three wayinteractions between the three variables had no effect on offspring survival (Table 12). Only the interaction between Q90 and egg volume (V) had an effect on offspring survival. Then it

seems that small eggs survival (75 mm^3) did not vary whatever the Q90 was, whereas big eggs showed higher survival rate at higher values of Q90 (Fig. 33). In that case, it seems that the hypothesis of phenotype habitat matching is weakly supported, leading to the conclusion that females should select habitat with large substratum particle size when egg volume are large. Therefore, large eggs should be favoured and evolve under directional selection and phenotype habitat matching hypothesis does not allow here to explain the maintenance of egg variability within populations.

In this experiment, only few variables have been tested, while others may also affect for offspring survival depending on egg size. For example, temperature may vary in the nest over the reproductive season, and have an impact on egg survival (Régnier, 2011). Moreover, oxygen availability can vary with egg surface. It would be then interesting to add in the experiment a thermometer in each redd and be able to measure oxygen level through time, but this remains a costly process. But as a matter of fact, if some footprints of phenotype-habitat matching concept could be found here, it does not seem to control for a large part of survival variation, and it has much less impact on mortality than stochasticity in water flow.

Table 12. *Parameters estimates for the phenotype-habitat matching model.*

Factors	**Offspring survival**	
	Mean of the parameters	sd
Depth	a= 2.21	5.12
Q90	b= -1.84	3.49
Volume	c= -0.14	0.51
*D*Q90*	d= -0.52	3.38
*D*V*	e= -0.23	0.52
*Q90*V*	**f=0.43**	0.47
*D*Q90*V*	g=-0.04	0.37

Numbers in bold indicate parameters for posterior distribution is at least 85% above or below zero. D= Depth; Q90= 90 quantile for substratum particle size; V= Egg volume.

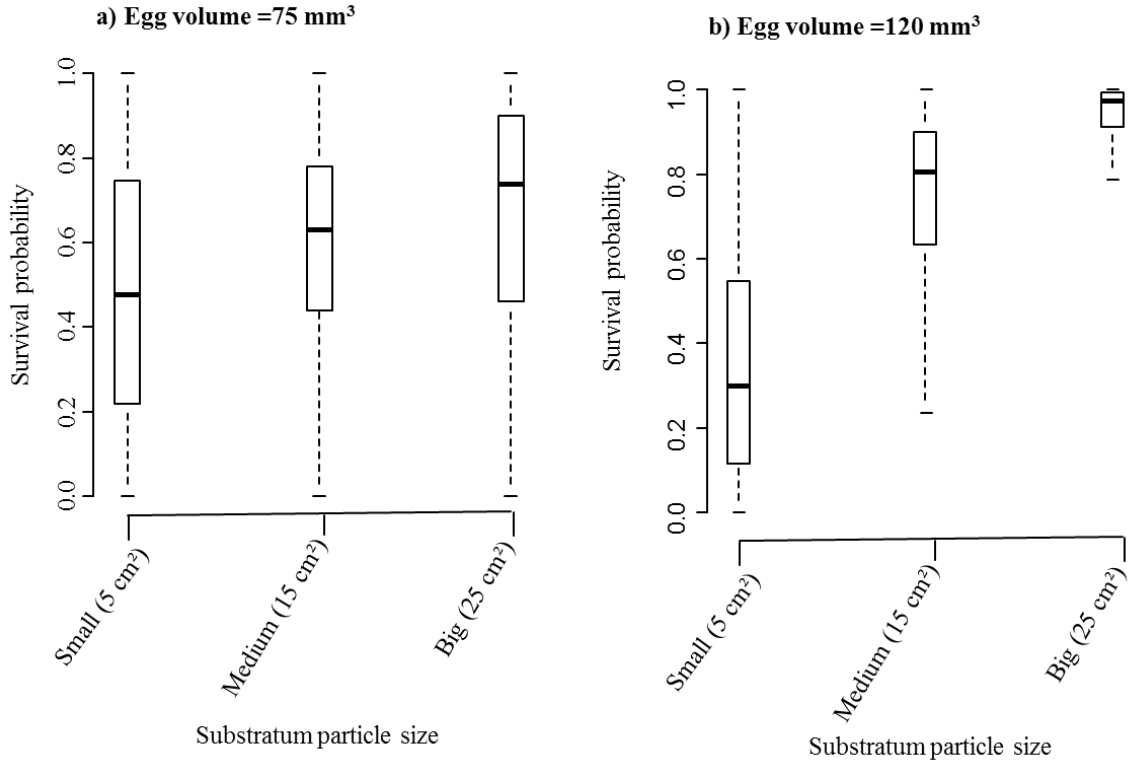


Figure 33. *Predictions of offspring survival at hatching from the model as a function of substratum particle size (X90= {5cm²; 15cm² and 25 cm²}) for a) an egg volume fixed at 75 mm^3 and b) an egg volume fixed at 120 mm^3. Depth of the nest was fixed at 10 cm.*

4) What solution to face unpredictable variation?

According to the previous results, it seems that females do not have optimal strategies to face unpredictable environment leading to scouring (at least on the habitat variables measured here). Only female size seems to play a role but this result is hard to interpret and measuring more environmental variables is necessary. Moreover, it seems that only large egg volume and egg with large particle size have a positive effect in offspring survival, leading larger eggs to have better survival in larger particle size. In that case, if females are not able to perceive environmental risk (such as scouring risk), evolution of bet hedging (Meyers & Bull, 2002) could occur through generations. In other words, females could make multiple nests with different environmental characteristics that may 1) decrease the average impact of environmental stochasticity and 2) favor both small and big eggs. However, based on the present results, small eggs should be never favored and should be counter-selected by natural selection in nearly any case, predicting therefore that bigger eggs should be selected under directional selection. It is obviously not what we observe currently in our populations: either there are other factors that maintain egg size variation (as previously mentioned, kin competition, survival differentials between small and large eggs conditional on temperature or oxygen), or that directional selection has not yet produced its effects.

It is interesting to envision a scenario where females would multiply their nests: it obviously would raise the cost of reproduction for females, and it would also increase the mating opportunities for males while decreasing the number of offspring that can be produced per mating success. In fact, the whole cost and benefit balance would be affected by such evolution in female nesting strategy, and the outcome remains difficult to forecast: decrease of intrasexual competition in males? Increase of intrasexual competition in females for nesting sites? Evolution of sex roles regarding parental care?

III. Between river contrast in environment

The ecological contrast used in this study opposed a constant discharge (Experiment B1) versus a randomly timed stochastic discharge (Experiment B2, see § II.II.7). The magnitude of discharge variation was not very large in the stochastic environment compared to natural environments, and water levels were not low or high enough to either leave the redd dry or scour the redd substratum – whereas it is often the case in natural rivers. In the present part, we will investigate if contrast in discharge stochasticity affects 1) social environment through OSR, 2) reproductive investment of individuals of both sexes and both population origins and 3) reproductive isolation between population of different origins.

1) Does contrast in discharge stochasticity affect OSR?

Operational sex ratio was significantly higher in the constant environment than in the stochastic environment (χ^2= 7.9652, df = 1, p = 0.005) (Fig. 34). Indeed, in the constant environment, females encountered in average 4 males (sd ± 1.28), whereas in the stochastic environment they encountered only 2 males (sd ± 1.48). Several explanations may explain this variation in the sex ratio between the two environments. First, it is possible that water discharge variation leads to a modification of the female's reproductive strategies: females could lay their eggs in several batches (bet-hedging), a strategy known to evolve in stochastic environments. Unfortunately, our experiment did not allow recording all copulations to acquire these data. However, the matrix of

reproductive success (supplementary information 7) gave the information on the number of females with who each female mate and no significant difference was observed between the two environments (χ^2= 1.946, df = 1, p = 0.163) indicating that individuals are potentially able to adjust their reproductive effort in facing environmental changes. It appears that females did not change their reproductive strategy in terms of batch number between the environments.

Regarding OSR variation, more episodes of reproduction were observed in the constant environment (constant environment: N=22; stochastic environment: N=14), which could influence our results. Indeed, due to water discharge, it was sometimes difficult to observe reproduction which can influence the number of spawning events observed: it is possible that the non-observed mating episodes had a higher OSR, especially in the stochastic environment. Moreover, it is possible that sometimes females selected nesting places outside the most favorable sites (in resting sections in fact), and in that cases, when we could observe the mating episode, OSR could be relatively high (up to 5).

If OSR actually changed between the different environments, it could modify the costs of the competition, and consequently influence the allocation of energy in the reproductive season.

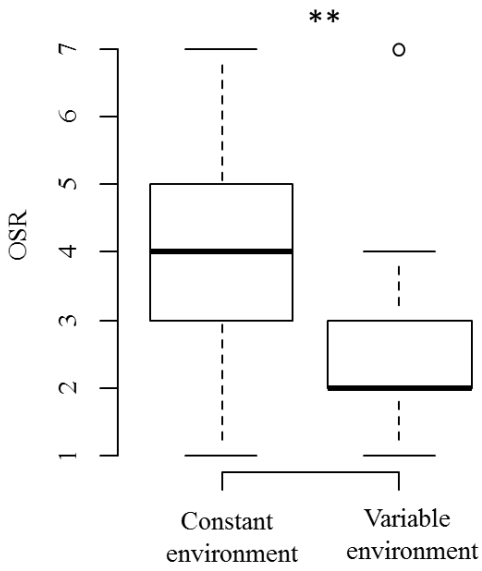


Figure 34. *Operational sex ratio (Number of active males for a given number of active females during an episode of reproduction) between constant environment (experiment B1) and stochastic environment (experiment B2). Stars denote a significant difference between the two groups.*

2) Does contrast in discharge affect reproductive investment?

Environmental variations may directly affect the allocation to different biological functions (McNamara & Houston, 1996). Therefore, individuals able to discriminate environment fluctuations could display plastic strategies by investing or reducing their reproductive investment in the current season that will affect their future opportunities of reproduction (Bardsen, Næss, Tveraa, Langeland, & Fauchald, 2014; Bardsen, Tveraa, Fauchald, & Langeland, 2010; Williams, 1966). Additionally, if different populations are adapted to their own environment, changes in reproductive effort may occur between when placed in a manipulated environment. Herein, the manipulated environment is water discharge (Experiment B1 & B2), the variable environment presenting a very fast change in water level. Such extreme changes in water levels are not common in natural

environments, and can only be expected in regulated rivers due to intense hydropower plant activity. Also, theoretical model of global change predict, with the increase of temperatures, an increase of climatic stochasticity leading to more extreme events of rainfall and intermittent droughts. These sudden changes in water level might be picked up by some individual as a signal of strong environmental unpredictability, and used in the decision to postpone reproductive effort.

In the experiments B1 and B2, two populations from two different rivers were mixed. Using relative weight variations and relative metabolites variations (triglycerides and free fatty acids) as proxies of reproductive investment, I looked at differences in reproductive effort between the two different environments: constant *vs* stochastic. I also used Experiment A as a pseudo-control of the experiment B1 (constant environment) for relative weight and metabolites variations, since experiment A also had a constant discharge and was using fish from Bastan population – although some parameters differed. Kruskal-Wallis rank sum test were used to compare relative variations of weight and metabolites between environment and sex for each population. Tests were corrected with the Sydak adjustment method to adjust the classical alpha threshold (alpha = 0.02532 instead of 0.05 in that case) because of multiple comparisons.

a) Experiment A vs experiment B1

For a sake of clarity I present here the differences in the relative weight, triglycerides and free fatty acids variations between experiment A and B1 as the experiment A can be seen as a control.

- *Relative weight variation* (Fig. 35)

For Bastan males, there was no difference in weight variation between experiments A and B1 (Kruskall Wallis test: Df=1, P = 0.242) and therefore results were replicable. However, when comparing Bastan females of experiment A with experiment B1, relative

weight variation was higher in females of experiment B1 (Kruskall Wallis test: , df=1, P = 0.012).

- *Relative triglycerides variations* (Fig. 36)

Triglycerides variations for Bastan females were more important in experiment B1 than in previous experiment A (Kruskall Wallis test: Df=1, P = 0.001). For Bastan males, there was no difference in triglycerides variations between experiments A and B1 (Kruskall Wallis test: Df=1, P = 0.127).

- *Relative free fatty acids variations* (Fig. 37)

Free fatty acids variations for Bastan females were not different between experiments A and B1 (Kruskall Wallis test: Df=1, P = 0.366). For Bastan males, there was no difference in free fatty acids variations between experiments A and B1 (Kruskall Wallis test: Df=1, P = 0.525).

To summarize, here I note only the significant differences between the experiments A and B1. Results shows that 1) relative weight variations was larger for females in experiment B than in the experiment A and 2) relative triglycerides variations were higher for females in experiment B than in the experiment A.

Such differences may be explained by the variation of the experimental set up: experiments A and B1 differ in their spatial organization of favorable particle size for reproduction, as well as for the length of channel used. In experiment A, favorable sites were distributed among five connected sections, whereas in B1 experiment, all favorable sites were located on the same section, and the total length of river was shorter (30 m versus 50m). Additionally, in experiment B1, although density and sex ratio were in the same range than for experiment B1, fish were placed in sympatry with the Urumea population. All these factors may have modified the decision of females from Bastan to invest relatively more energy in reproduction. For the Bastan males, these differences did

not seem to have modified their reproductive investment at all. Overall, the range of variation observed here in experiment B1 confirm the previous results from Gauthey et al. (in review): both weight and metabolites concentration decline over reproductive season, in relatively similar proportions, respectively.

b) Experiment B1 vs experiment B2

- *Relative variations of weight between the two environments*

Between the stochastic (experiment B1) and the constant environment (experiment B2), only Urumea females showed a significant difference in their relative weight variation. Females in the constant environment B1 lost in average more weight than in the stochastic environment B2 (df=1, p = 0.029). Males and females from Bastan and males from Urumea did not show differences in their relative weight variations between environments B1 and B2 (respective P for df=1: P = 0.698; p= 0.869; p = 0.573).

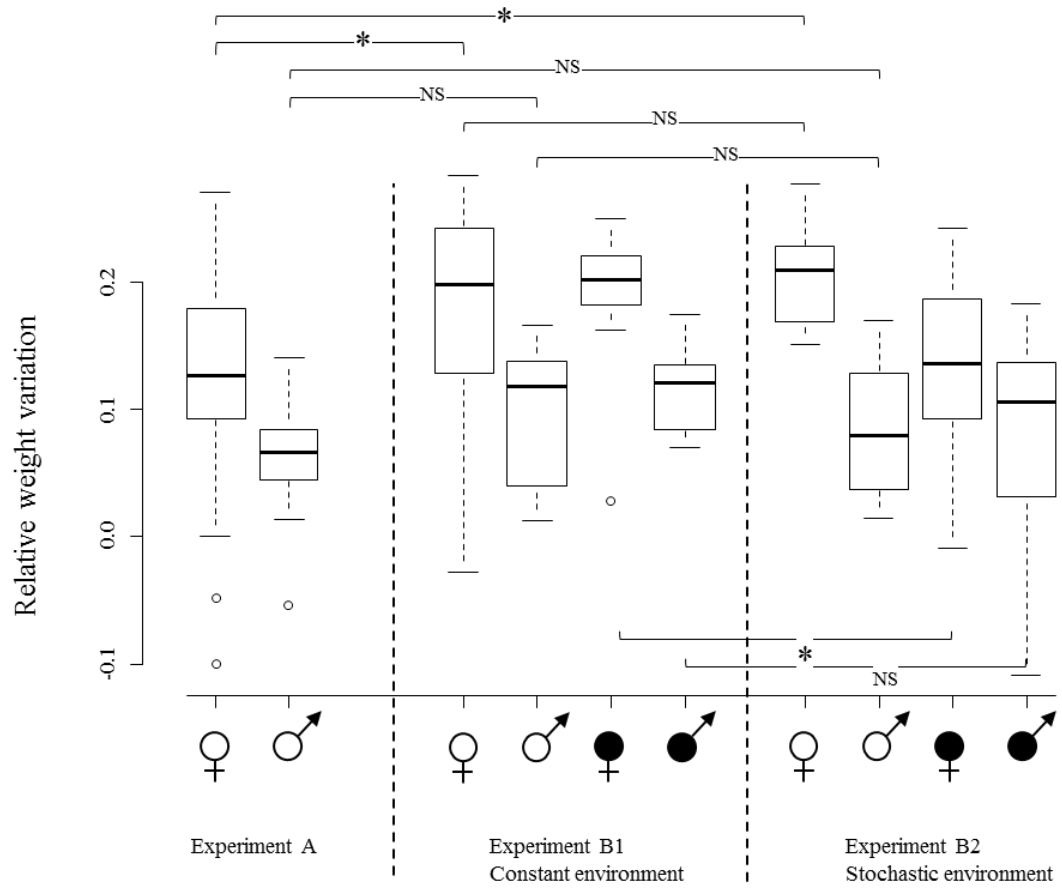


Figure 35. *Relative weight variations in males and females of Urumea (black male and female symbols) and Bastan (white male and female symbols) in the different experiments A, B1 and B2.*

- *Relative variations of triglycerides between the two environments*

Between the stochastic and the constant environment, once again only Urumea females showed a significant difference in their relative triglycerides variation. Females in the constant environment B1 lost, on average, more triglycerides than in the stochastic environment B2 (Kruskall Wallis test: Df=1, $p = 0.001$). No difference was observed in males between experiments B1 and B2 (Kruskall Wallis test: Df=1, $p = 0.21$). For Bastan individuals, no difference in triglycerides variations were found between B1 and B2 environment in either males (Kruskall Wallis test: Df=1, $p = 0.897$) or females (Kruskall Wallis test: Df=1, $p = 0.796$).

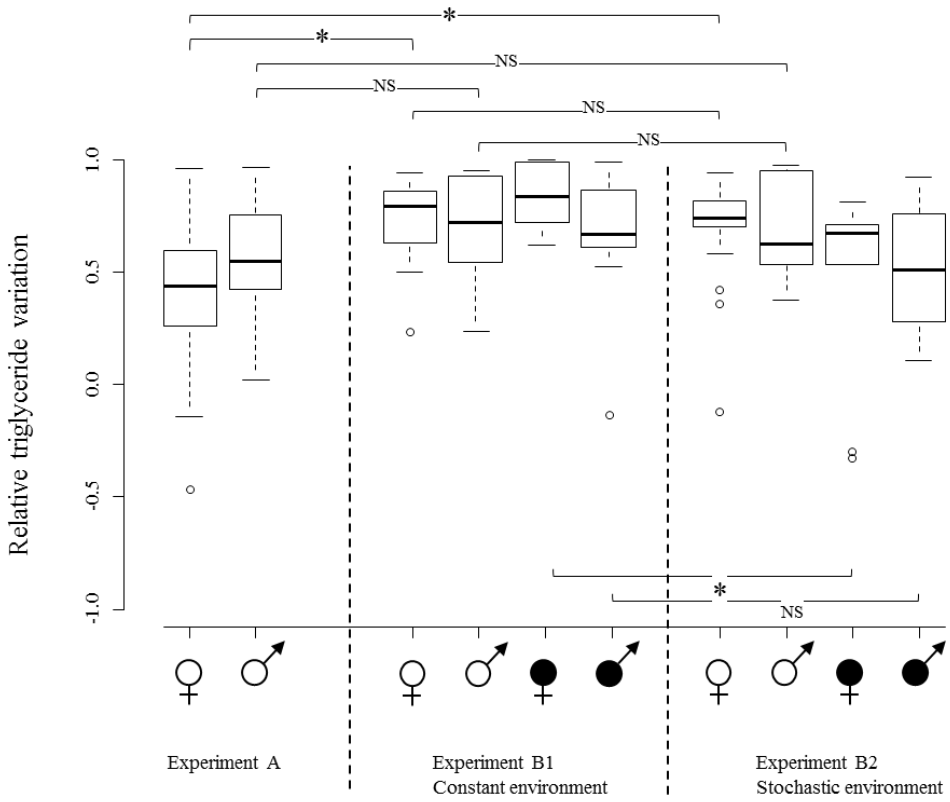


Figure 36. *Relative triglycerides variations in males and females of Urumea (black male and female symbols) and Bastan (white male and female symbols) in the different experiments A, B1 and B2.*

- *Relative variations of free fatty acids between the two environments*

For Urumea females, relative variations of free fatty acids variation was higher in experiment B1 compared to experiment B2 (Df=1, p = 0.02029), whereas no differences were found for the males (Kruskall Wallis test: Df=1, p = 0.622).

For Bastan individuals no differences of the relative free fatty acids variations were found between the two environments B1 and B2 in males (Kruskall Wallis test: Df=1, p = 0.214) and in females (Kruskall Wallis test: Df=1, p = 0.987).

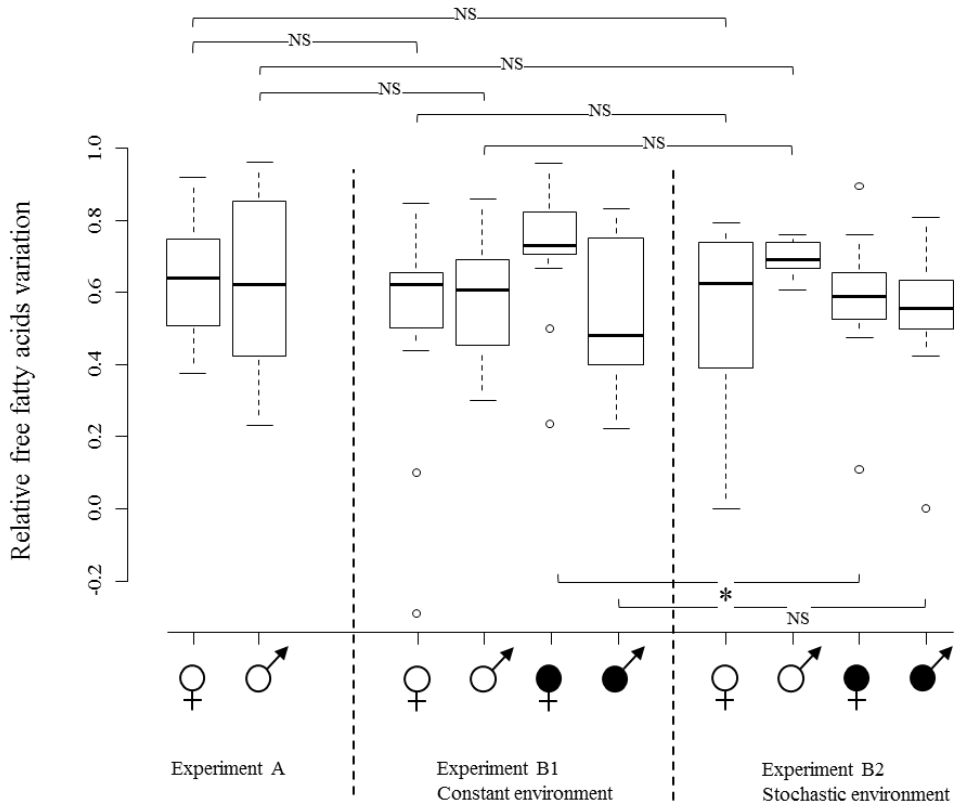


Figure 37. *Relative free fatty acids variations in males and females of Urumea (black male and female symbols) and Bastan (white male and female symbols) in the different experiments A, B1 and B2.*

To summarize, only females from Urumea tend to lose more weight, more triglycerides and more free fatty acids in the constant environment B1 compared to the stochastic environment B2. These differences were found neither for Urumea males nor for males and females from Bastan. It seems therefore that Urumea females decrease their investment in reproduction when environment is stochastic and that they could adjust their reproductive behaviours that are costly depending on the environmental conditions. It would be interesting to be able to compare if differences in digging behaviours between these females occur, but the number of females observed was too small ($N_{UrumeaB1}$=7; $N_{UrumeaB2}$=2). A possible cause of this result would be that Urumea females could be adapted to stochastic environment leading them to allocate less in the present reproduction in order to keep energy for future reproductive seasons. Females seemed to

be able to perceive environmental variations and adjust their reserves, which will in turn have direct consequences on their own fitness. In a more global context, if stochasticity of water flow increase in rivers and had an effect on offspring survival, then populations not able to adjust their investment could invest for nothing during a given reproductive season and the loss of energy would affect their future reproduction potentially leading to a fitness decrease, and possibly ultimately the population extinction.

3) Variability in reproductive effort may lead to reproductive isolation?

Differences in energy allocation between populations may be the result of divergent selection between environments. Indeed, populations may evolve differently facing different environmental pressures, leading to ecological speciation (Coyne & Orr, 2004; Howard D. Rundle & Nosil, 2005; Schluter, 2000). When populations evolved in allopatry, pre- and post-zygotic barriers can appear leading genetically individuals incompatible to mate and reducing, consequently, gene flow between population. Ecological processes are therefore central to the formation of new species as a result of ecologically-based divergent selection. Dobzhansky (1937) classed barriers that do not allow gene flow in two distinct parts: 1) barriers acting before mating and fertilization (pre-zygotic barriers) and barriers acting after reproduction and the production of zygote (post-zygotic barriers). Different forms of pre-zygotic isolation exist, such as habitat and temporal barriers reducing the probability for two different populations to encounter (Rice & Salt, 1990) and therefore reducing gene flow. Also pre-zygotic isolation may arise when migrants are counter selected before reproduction due to poor adaptation in a new environment leading to a low survival rate (Funk, 1998). Another form of pre-

zygotic isolation is due to sexual selection in which individuals from different populations might not mate randomly, due to divergent evolution of signaling traits or sensorial abilities involved in intersexual preference (Boughman, 2002; Rundle & Nosil, 2005).

Most studies exploring the process of ecological speciation focus on the very early stages of the above process: i.e., adaptive divergence and partial barriers between conspecific populations (Rundle & Nosil, 2005; Schluter, 2000). One set of these studies focuses on tests of genetic differences between populations inhabiting different environments (Orsini, Vanoverbeke, Swillen, Mergeay, & Meester, 2013; Sexton, Hangartner, & Hoffmann, 2014; Shafer & Wolf, 2013; Thibert-Plante & Hendry, 2010). Another set of these studies focuses on quantifying specific reproductive barriers between populations (review: Nosil, Vines, & Funk, 2005) and thus focus on assortative mating between two populations.

Studies of assortative mating between conspecific populations have often been successful at inferring ecological speciation, but they frequently suffer from several limitations. First, many studies are conducted in the laboratory with controlled pairings, and so the estimated assortative mating is of questionable relevance to more natural contexts and reproductive isolation failed in many experiments (Rice & Hostert, 1993; Rundle, 2003). Second, most studies do not assess assortative mating in multiple environmental contexts, and so the context-dependency of the barrier is not known. In the context of climate change, increased stochasticity in discharge regime is an expected outcome (Hartmann et al. 2013): extreme events such as droughts and floods are predicted to occur more frequently in currently temperate areas from where brown trout originates.

As already exposed in § II.II.7, experiments B1 and B2 consisted in placing the populations of Urumea and Bastan in sympatry in two contrasted environments: B1,

where water flow was maintained constant, and B2, where water flow follows stochastic conditions. Populations from Bastan and Urumea have evolved in different drainages and are genetically different (Weir & Cockerham 1984) genetic distance calculated on parents genotypes, θ=0.147) and could therefore be locally adapted to their own environment. The two rivers have (as previously mentioned in the chapter II) comparable annual mean discharge (about 6m3.s-1), but the River Urumea presents less predictable flow condition than River Bastan (§ II.II.7). Therefore, here assortative mating was tested in order see if reproduction isolation occurred in these populations. Using parentage analysis of offspring (§ II.II.5), reproductive isolation will be quantified based on mating success (between- vs. within-population matings that produced offspring) and total reproductive success conditional on mate number (between- vs. within-population production of offspring). I inform the reader that although we manipulated the discharge, variation of discharge in the stochastic environment (Experiment B2) was not strong enough to lead to scouring episodes or air exposure for redds in the artificial channel and should therefore have no strong influence on offspring mortality.

a) Assessment of mating success and reproductive success

After the reproductive season, a total of 1305 juveniles were sampled in both environments and 1287 were used for parental assignment (the 18 others were not used because of poor DNA quality). In the constant environment, a total 555 juveniles, in addition to the 52 parents, were successfully genotyped. 551 juveniles were safely assigned to two parents with a confidence level of 95%. In the stochastic environment, 732 juveniles, in addition to the 50 parents, were successfully genotyped. 731 juveniles were safely assigned to two parents with a 95% confidence level.

Parentage analysis revealed that at least 40 successful matings occurred in the constant environment, whereas 55 successful matings occurred in the stochastic environment (Table 13). Out of 63 females, only four (constant environment: one from population of Bastan; stochastic environment: two from population of Urumea and one from population of Bastan) were still ovigerous at the end of the reproduction (they did not lay their eggs during the experiment). Within these females, only one had a reproductive success superior to 0 (7 juveniles assigned). Regarding reproductive success, parentage analysis showed that the total number of offspring varied between 0 and 201 in the constant environment and between 0 and 270 in the stochastic environment for males. For females, it varied between 0 and 86 in constant environment and between 0 and 112 for stochastic environment.

Table 13. *General informations of experiment B1 (constant environment) and B2 (Stochastic environment).*

	Constant environment	***Stochastic environment***
Number of females	33 (19 Bastan/ 14Urumea)	31 (17 Bastan/ 14Urumea)
Number of males	19 (8 Bastan/ 11Urumea)	19 (8 Bastan/ 11Urumea)
Offspring number sampled	555	732
Successful offspring assignations	551	731
Successful matings	40	55
Number of copulations observed	22	14

b) Reproductive isolation calculation

The reproductive success data calculated from the parentage assignment resulted in a matrix of non-negative integers quantifying the number of offspring obtained between all possible pairs of males and females over the course of reproductive season for

constant environment and stochastic environment (Supporting Information 7). We considered a successful mating when one pair of male and female had at least one offspring. We therefore kept a record of number of intra-population and inter-populations matings. We also counted the total reproductive success of each individuals descended from both intra-population and inter-population crossings in each environment.

From these values, two reproductive isolation indexes were calculated in each environment, as followed below from the method described by Sobel & Chen (2014): the first one represented the reproductive isolation based on mating success (RI_{ms}) and the second was based on the total reproductive success conditional on having mated (RI_{rs}).

$$\mathrm{RI_{ms}} = 1 - 2 \times \frac{(M_{between})}{(M_{between} + M_{within})}$$

Where $M_{between}$represents the number of matings between population A and population B; and M_{within} the number of matings within populations.

$$\mathrm{RI_{rs}} = 1 - 2 \times \frac{(O_{between})}{(O_{between} + O_{withn})}$$

Where $O_{between}$ represents the total number of offspring produced by pairs from different populations; and O_{withn} total of juveniles produced within populations.

To check if RI_{ms} and RI_{rs}observed in each environment were larger or smaller than expected, we calculated expected values under a panmictic scenario bootstrap. To do so, we generated 10000 new matrices of mating success from the observed matrix: a zero represents a pair of individual that did not mate, whereas a 1 indicates a successful mating. For each new matrix, a new RI_{ms} was calculated. At the end of the simulations we tested how many times our observed RI_{ms} was larger than expected.

To compare observed RI_{rs} to expected RI_{rs}, we bootstrapped 10000 times on reproductive success within individuals that have already mated. At each simulation, a RI_{rs} was calculated as the equation above and we tested the number of times observed RI_{rs} was blarger than expected.

c) Results

In environment B1 (constant water flow), 17 successful matings occurred between individuals from Urumea and individuals from Bastan and 23 matings within individuals from the same population (Table 14). In the constant environment, reproductive isolation calculated from the number of matings ($RI_{ms,}$) was not significantly different from zero (RI_{ms}=0.15, p=0.87) (Fig. 38), implying a random mating pattern between individuals from the two populations (Fig. 40).

Table 14. *Number of matings between ($M_{between}$) and within (M_{within}) populations, number of offspring produced between ($O_{between}$) and within (O_{within}) populations, and associated reproductive isolation indexes ($RI_{ms\ and}$ RI_{rs} respectively) for each environment.*

	$M_{between}$	M_{within}	RI_{ms}	$O_{between}$	O_{within}	RI_{rs}
Constant environment	17	23	0.15	246	302	0.11
Variable environment	17	38	0.38	210	521	0.43

Additionally, RI_{rs} calculated from reproductive success did not differed significantly from expected values conditional on mating success (RI_{rs}=0.11) (Fig. 39, Table 14). In the constant environment, it therefore appears that agents of sexual selection – intra-sexual competition and inter-sexual preference - did not lead to any reproductive isolation due to mating success. In other terms, each sex from each population had access to sexual

partners irrespective of their origin. As no post-zygotic reproductive isolation was detected, it would appear that gene flow between these two populations in the constant environment would be total.

In environment B2 (stochastic water flow), 17 successful matings occurred between individual from Urumea and individuals from Bastan and 38 matings within individuals from the same population (Table 14). In this environment, reproductive isolation calculated from the number of matings ($RI_{ms)}$ was significantly different from zero (RI_{ms}=0.38, p=0.002) (Fig. 38), implying that individuals did not mate randomly (Fig. 40).

Indeed, when looking at matings more in details, the number of matings between males and females of Bastan was higher than expected (Fig. 40). At the opposite, it appeared that males from Urumea population achieved a lower than expected mating success with females from Urumea population as well as with females from Bastan (Fig. 40). However, the observed number of matings between males from Bastan and females from Urumea did not differ from the expected value.

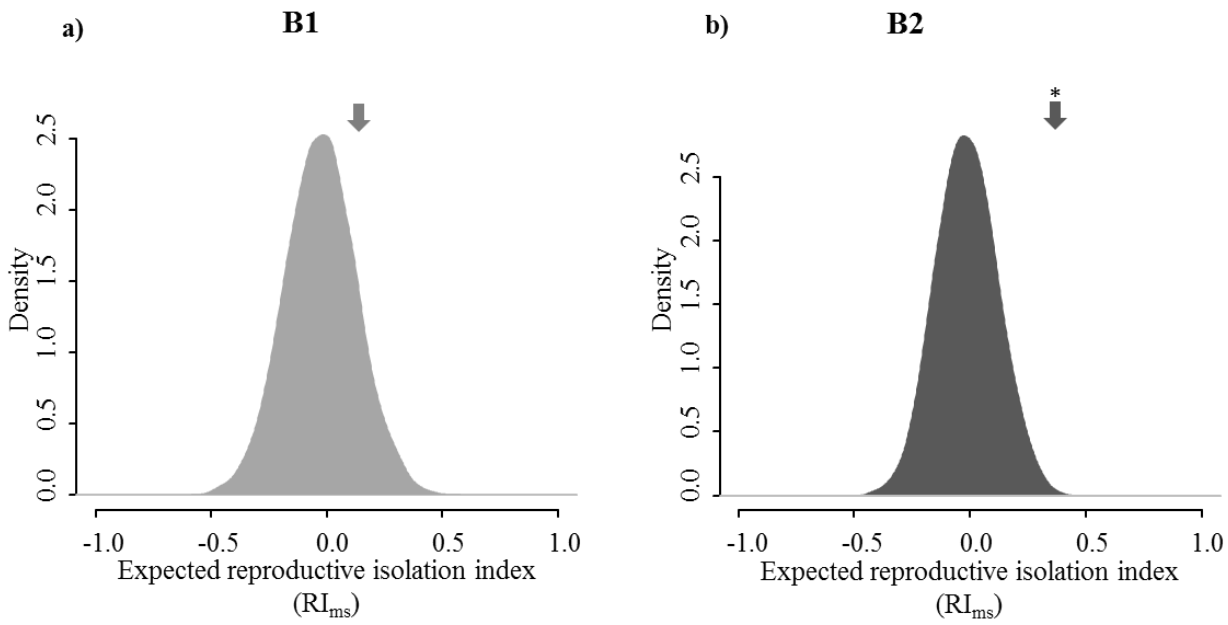


Figure 38. *Density of expected reproductive isolation index calculated under a pancmictic scenario and based on mating success in a) Environment B1 (constant water flow) and b) Environment B2 (stochastic water flow). Arrows show the observed* Ri_{ms} *and stars denote significant differences between expected and observed distribution.*

Reproductive isolation calculated using reproductive success was stronger in the stochastic environment (RI_{rs}=0.43) than in the constant environment (RI_{rs}=0.11, Fig. 38). The stochastic environment was significantly departing from a panmictic scenario (0.046) when not considering realized matings. However, when bootstrap method accounted for realized matings, RI_{rs} for both environments was not significantly different from zero (p=0.3189 for the constant environment B1, p=0.168 for the stochastic environment B2, Fig. 39), indicating that variation in RI_{rs} was fully driven by variation in RI_{ms}. Consequently, no post-zygotic isolation *per se* was detected neither in the constant nor in the stochastic environment.

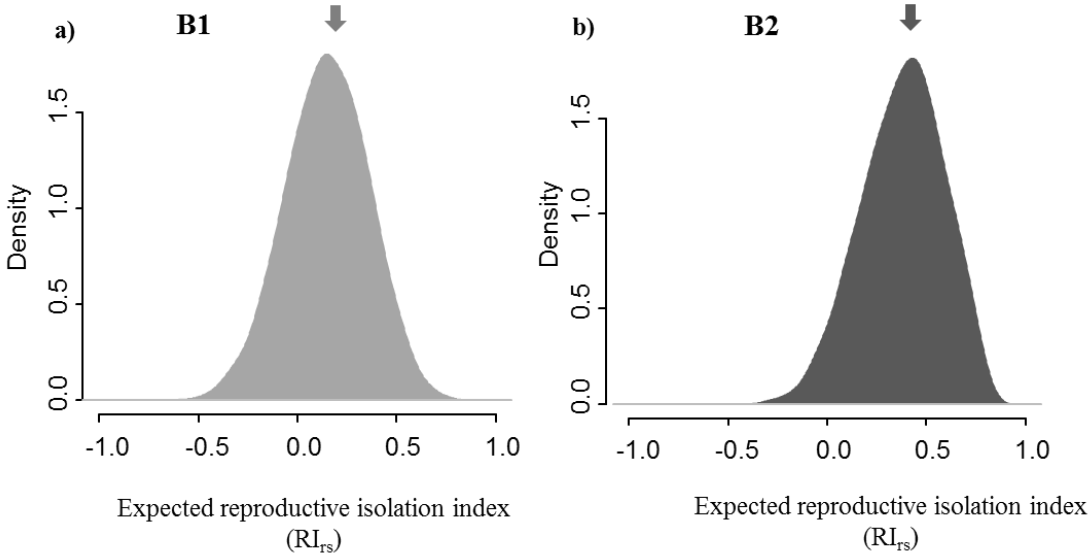


***Figure 39**. Density of expected reproductive isolation index calculated under a panmictic scenario and based on reproductive success conditioned on mating success in a) Environment B1 (constant water flow) and b) environment 2 (stochastic water flow). Arrows show the observed Ri_{rs}.*

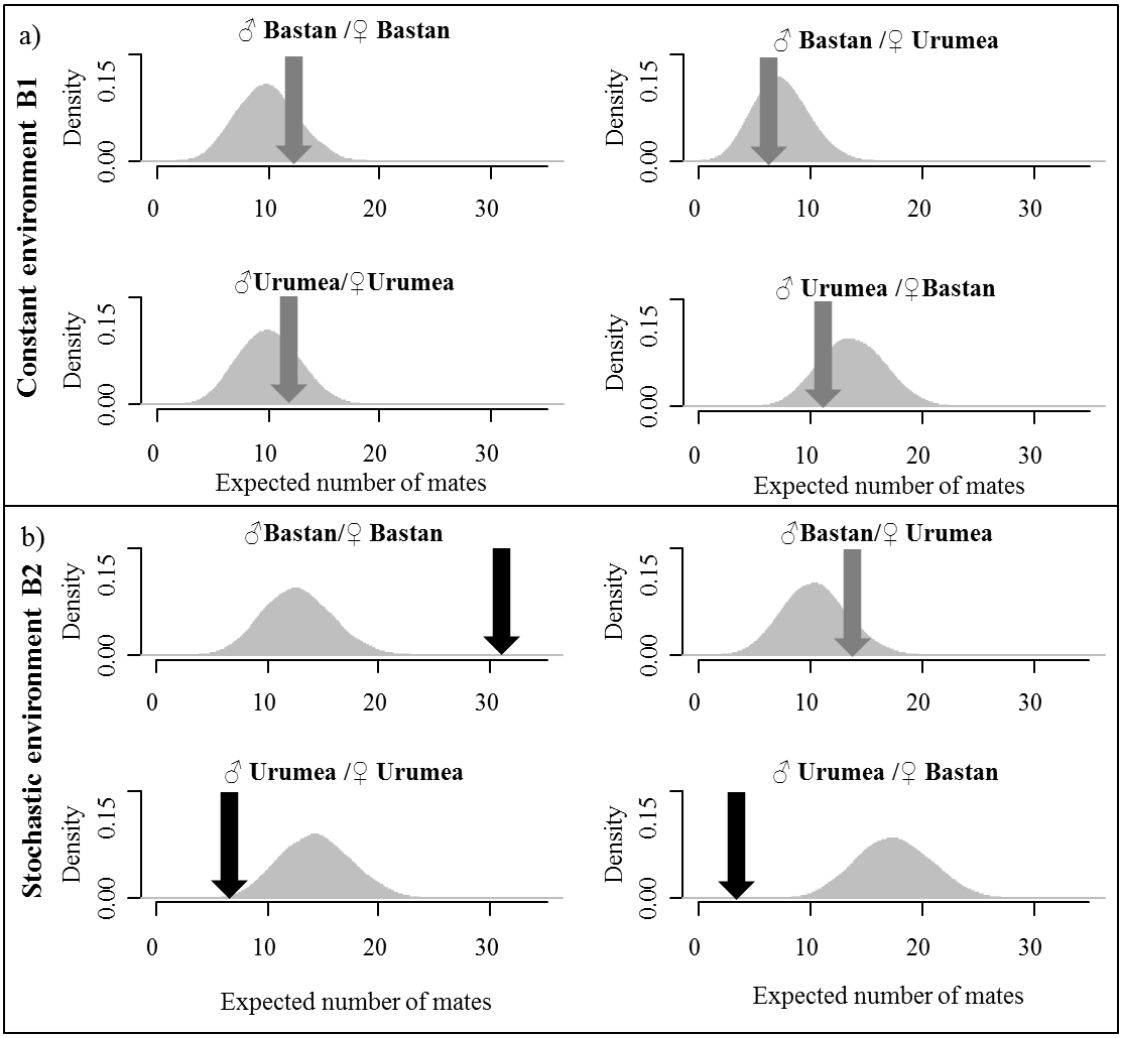


Figure 40*. Density of expected number of mates calculated under a panmictic scenario in a) Constant environment B1 and b) Stochastic environment B2. Arrows show the observed number of successful matings from genetic matrix. Black arrows represent a significant difference between the expected distribution and the observed number of matings.*

4) Conclusions relative to environmental stochasticity effects on reproduction in Experiments B1 and B2.

To conclude, it seems that in the stochastic environment of experiment B2, individuals did not mate randomly and that there is a reproductive isolation due to mating success. This result seems to be due to males from Urumea population and should be therefore sex dependent. We have seen in the previous section (§ V.II.2) that females from Urumea

diminished their investment in the reproduction in the stochastic environment with a decrease of variation in weight, plasma triglycerides and free fatty acids relative to their initial quantity. Despite this reduction in reproductive investment, females from Urumea mated normally with males from Bastan. On the contrary, males from Urumea did not show a reduction in variation of weight and metabolites in the stochastic environment and showed here a decrease in matting success with females of both populations. It appears that males from both populations are unable – or unwilling – to adapt their reproductive investment conditional on environmental stochasticity, but their reproductive output differ in the present experiment B2. A first hypothesis is that despite being on par in the constant environment, with an approximately equal trade-off between reproductive investment and reproductive success, the Bastan males could outcompete the Urumea males in the stochastic environment. This is surprising if one assumes that adaptation may have shaped the trade-off between investment and reproductive success in wild populations: it could be an evidence against adaptation. Another potential explanation is that post-zygotic isolation triggered by stochastic variation of discharge is a no go in the experiment, whereas it can easily occur in natural environments: thereby the reproductive success of Bastan males in a stochastic environment could be significantly lowered.

Alternatively, the reproductive success of males could be a consequence of female behavior: females from both rivers may have preferred males from Bastan in the stochastic environment which could explain why that Bastan males mated more than Urumea males. However, this hypothesis is not easy to document since generally brown trout show preference for particular sexual traits, such as body size (Labonne et al., 2009) and adipose fin size (Petersson et al., 1999), but to my knowledge, no evidence of population assortative mating has been published.

Finally, skipped mating is frequent in fish species (review in (Rideout & Tomkiewicz, 2011), but even if Urumuea males were to skip this single reproductive season, they invested as much weight and metabolites than Bastan males, making this strategy more costly than the Bastan strategy.

No post-zygotic isolation was detected, as expected, since the experimental design did not lead to drying or scouring episode that may affect offspring viability (previous section, Goode et al.2013). Therefore, this result was not surprising. Hybridization in brown trout populations between different lineages occurs generally without proble. However, the present protocol did not allow following hybrid viability and fertility and I cannot conclude on this point here. In natural populations, if discharge stochasticity lead to 70% of the mortality at the egg and vesicled alevins stages (§ V.II.1), then individuals could postpone their reproduction for more favorable conditions.

Even if the present experiment does not allow precisely to point out the mechanisms generating reproductive isolation with changing water discharge regimes (intra-sexual competition, intersexual preference or reproductive investment), it shows how reproductive barriers can arise with contrasting environments, a result that had not been obtained before to my knowledge. The magnitude of this result implies that it should be investigated further in relationship with expected increase of stochastic events in river regimes as predicted by the coupling of climatic and hydraulic models (Milly et al., 2002; IPCC 2013). Genetically distinct populations react differently to this precise environmental factor. Depending on the mechanism at work (genetic selection, phenotypic plasticity, or both), it may on the long term affect locally both the demography and the genetics of the populations, and it can also affect future gene flow between populations. This study also illustrates that the general background of ecological speciation can provide operational insights on biodiversity dynamics once placed in an

experimental context such as in the present study. Here we demonstrate striking potential effects on a single reproductive period that may ultimately affect gene flow and therefore adaptation of populations to environmental variation, which will likely be increased by climate change.

Chapter VI.
Discussion

As explained in introduction, I discussed chapter specific aspects previously. In this general discussion, I will first focus on the most innovative findings provided by the overall work done in this thesis. Then, I will synthesize the potential effects of environmental change on sexual selection in brown trout, using the various findings of this thesis: although this exercise will be speculative, it may enlighten future research paths. I will finish this discussion by a brief evocation of several limitations that the reader may have noticed throughout this manuscript, or that I may have stumbled upon, and for which I would like to propose some potential solutions.

I. Remarkable results

1) Trade-off between investment and reproductive success: a new avenue

In comparison to semelparous species, for brown trout, reproductive investment in current reproduction is predicted to affect future reproduction and, therefore, lifetime reproductive success of individuals since energy invested is no longer available for future reproduction. Accordingly, individuals must select tactics to balance the trade-off between investment in reproduction and energetic status and survival (Abrahams, 1993), a trade-off often assumed but rarely proven in fish. My results allow documenting further these predictions. First, I was able to decompose reproductive investment within a reproductive season in sub-components, mainly gametic versus non-gametic investment. I also showed that non-gametic investment, indicated by metabolites concentrations variations, could potentially be good indicators of behavioural investment (competition, preference and parental care), although more investigation would be needed in that department to better relate behaviour and plasma metabolites dynamics. Second, I showed that both weight and metabolites variations contributed to fitness, and I provided an actual measure of this trade-off, thanks to a good aestimate of reproductive success in the semi-

natural experimental channel. It is also noteworthy that I was able to record ample variation between individuals in their ressource: some lose a lot of weight whereas some show important decrease of plasma metabolites concentrations, without correlation between both traits. This leads to a wide range of tactics to maximize their fitness within a reproductive season. Third, methods allowing estimating behavioural reproductive investment through measurement of metabolites variations measures could be easily extended to other experiments or natural environments, the present data allowing a comparative approach.

2) The benefits of handling various data sources to test a single hypothesis.

In Chapter IV, I developed a new statistical approach in order to correct some biases related to the partial analysis of the data available in parental tables and behavioural interactions matrices, in order to measure indices of sexual selection. To do so, I mixed both behavioural data and molecular data to disentangle fitness in different components, as Wade and Arnold (1984 a,b) already suggested. Reproductive success within a reproductive season was therefore used as a proxy of fitness of a pair of individuals depends on encounter rate, copulation rate and number of offspring produced. Additionally, the model proposed to measure the effects of traits on each of these components improving the estimation of mating success compared to classical methods. Using the example of the brown trout, I showed that it was possible to use different type of data sources in a single unified model. I also took care to model possible bias, such as observation probability for behavioural data. Other improvements could now be made to include additional components, such as the fertilization process and direct or indirect

benefits, and one could also improve our grasp of sexual selection by directly integrating fecundity as a part of the fitness function. This model will also allow testing mating patterns or ecological variation at the scale of each mating episode. I look forward to applying this approach to other species with different reproductive systems and see how it can improve our understanding of sexual selection.

In the present case, I also showed that it was possible to improve the mating success estimation (and get access to various definitions) using different data sources from my experiments. Such insight readily provides access to new measures of sexual selection indices that can be tuned to each process of interest: here, encounter, copulation, production of offspring. I also demonstrated how the dependency between both sexual partners phenotypes was shaping reproductive success.

3) Take-home results for effects of environment on sexual selection

I explained in the introduction why I chose environmental stochasticity as the main ecological driver to study evolution of sexual selection. The reader will have noticed that a part of my work was initially devoted to develop methods and models, and I may have not been able to investigate in depth all aspects of this question. And yet, several very clear results should be put forward.

Environmental stochasticity of discharge is the main driver of egg survival: 70 % of monitored eggs were scoured by river floods. Habitat selection by females does not seem to influence that fact. All eggs appeared to face the same odds in front of this random

risk. For the remaining eggs, there was no relationship between egg size, female traits or habitat selection, and egg mortality.

Environmental contrast between constant water flow and stochastic water flow appears to drive reproductive investment in some populations and for a given sex: females from Urumea definitely modified their reproductive investment (weight and metabolites concentrations variations) depending on environmental contrast. I have no knowledge of such results, demonstrating that reproductive investment could be tuned to environmental variation for some populations only. This pattern could have direct impacts on the evolution of the trade-off between reproductive investment, reproductive success, and survival. The fact that populations diverge regarding that relationship between reproductive investment and environmental variation implies that in face of the expectations of future climate change, not all populations should be considered as replicates simply because they belong to the same species: they was react differently, using different evolutionary mechanisms.

A second result related to environmental contrast between constant water flow and stochastic water flow is as much surprising: such contrast completely governs assortative mating between genetically distinct populations. In a constant environment, we found no reproductive isolation (as measured by mating success), whereas in a stochastic environment, strong assortative mating was detected, in which one sex from one population had very low mating success, and therefore did not sire offspring. While it is not simple to clarify the links between the environmental driver (here discharge stochasticity level) and the reproductive barrier (assortative mating), it provides new insights on possible consequences of climate change on the evolution of biodiversity, that is, gene flow.

II. Effect of stochastic environment on reproductive investment and fitness

In a larger context, theoretical model predict an increase of the intensity of hydrological events, such as floods and dry as a possible consequence of global change. Then, I tested how physical environment such as discharge stochasticity may affect different components of sexual selection (such as OSR) which will in turn affect reproductive investment in the current reproduction, habitat selection and competition or preference (not directly measured here). Although my work is limited to within reproductive season investigation, it is interesting to draw some potential evolutionary perspectives, by synthesizing our main results.

First, results in natural conditions showed that 70% of the total offspring mortality was affected by scouring. Additionally, reproductive investment could be modified between populations originated from different rivers suggesting that populations can adjust their investment when changing environment pattern. Consequences of variations in reproductive investment are diverse but they are predicted to play an important role on the trade-off between the current *versus* future reproductions and therefore affect the lifetime reproductive success.

Based on these two considerations, I propose here a speculative scenario regarding the evolution of life time reproductive success in both populations under stochastic variation. Assuming momentarily that sympatry did not influence either mating success or reproductive success depending on origin (i.e., all individuals react similarly to all other individuals whatever their genetic background, but differ in their response to physical environment), I envision how reproductive investment may influence survival, mating

access, and reproductive success. I also consider the uniform effect of environmental stochasticity on egg survival, which will in turn affect the lifetime reproductive success (Fig. 41). Relative to the constant environment, males and females from River Bastan do not change their allocation in the reproduction, neither in gamete investment nor in behavioural investment. Accordingly, I presume that their potential survival will not be different from the constant environment. However, it seems that males from this same population increase their mating success whereas females do not. Under the hypothesis that egg mortality is highly and uniformly affected by stochastic event (scouring), overall reproductive success in males and females should be reduced. However, by increasing their mating success, males from Bastan also increase their chance to produce more offspring *in fine* and will, therefore, potentially maintain their reproductive success in comparison with the constant environment. On the contrary, Bastan females reproductive success will be reduced through redd scouring (except if they adopt a bet-hedging strategy, which we do not consider here), while they will maintain their reproductive investment. In the present case of individuals from Bastan, males would therefore maintain their lifetime reproductive success by increasing their number of matings whereas stochasticity should have a negative effect on female lifetime reproductive success.

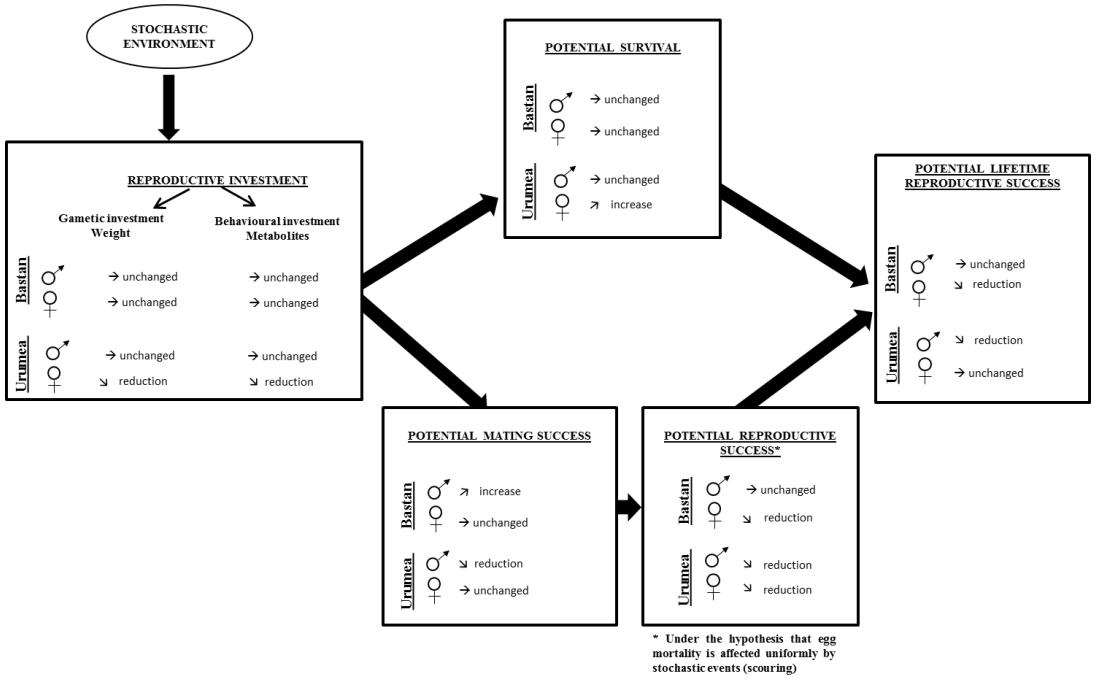


Figure 41. *Figure showing the effect of stochastic environment on reproductive investment of two populations (Bastan & Urumea) relative to the constant environment (exp B1), and its potential consequences on survival, mating success, reproductive success and lifetime reproductive success. Potential Reproductive success is predicted under the assumption that stochasticity increase eggs mortality uniformly (supported by results from experiment C). Additionally, herein, possible interferences due to artificial sympatry are not taken into account for the two populations.*

Now, looking at individuals originating from a more stochastic river (River Urumea), results indicated that, in comparison with the constant environment, females dramatically reduce their reproductive investment in current reproduction (Fig. 41). Indeed, their relative weight variation was reduced, as well as reduction in metabolites. Consequently, if they allocate less in the reproduction, energy can be allocated to other available functions and will potentially increase their survival for future reproductions. However, their reproductive success within a season will be lowered by increased effect of environmental stochasticity (higher scouring probability). Urumea females will thus maintain their lifetime reproductive success. In Urumea males, reproductive investment remained unchanged, thereby leading to an unchanged potential survival in comparison to the

constant environment. However, these males had a strong reduction in mating success leading to a mechanical decrease in reproductive success, that will be additionally also decreased by redd scouring. Consequently, their lifetime reproductive success should decrease.

The outcome of this speculative scenario is a bit counter-intuitive: males from Bastan, assumed to be potentially less adapted to environmental stochasticity, will maintain their lifetime reproductive success (LRS). Males from the Urumea, on the opposite, will decrease their LRS. Females from the Bastan will decrease their LRS, while females from Urumea will maintain their LRS, this result regarding females seeming more expected.

The results concerning males would be contradictory to the hypothesis that these two populations are differentially adapted to their environment (unlike what would be generally expected). Another interesting path of reflection is that while Bastan males and Urumea females would manage to maintain their fitness (LRS), they do not do so using the same mechanisms: males increase their mating success, while females reduce their investment.

Finally, in this little exercise, we assumed that individuals reacted similarly to all other individuals alike, whatever their genetic background. This is probably not so: it is possible that, for instance, intra-sexual competition in males was influenced by origin in this stochastic environment, thereby providing a better access to females for Bastan males. Additionally females from Urumea could express preference for males from Bastan. In short, putting these two populations in sympatry in this precise environment shuffled the trade-off of costs and benefits depending on sex and origin.

In any case, if LRS of any sex in any of the two populations was affected in such a situation, we should keep in mind that in sexual selection, the costs and benefits of each mating tactic for each sex depends on the evolution of other conspecifics costs and benefits. Therefore, a decrease of LRS in one sex should potentially affect variation in mating tactics in the other sex. In this particular context of sympatry, in this stochastic environment, our experiment, if it were to be prolonged in time, would favor the male genes from Bastan via their increased mating success, and the female genes from Urumea via a plastic reproductive investment. In the constant environment, sexual selection would not affect the cocktails of genes transmitted to the next generation.

III. Limitations of the present approach

During this thesis, I focused much of my attention on reproductive investment, fitness decomposition and, effects of trait but limited to body size. I also explored some relationships between behaviours, social environments, and reproductive investment. And, yet, many other areas remain to be investigated. The reader may have wished to get more information regarding agents of sexual selection, the partition between direct and indirect benefits, or parental care. I will here browse rapidly these various mechanisms that also control the evolution of sexual selection, and give some bridges between my own research and potential research paths for these mechanisms. But also, as the reader will notice, these mechanisms are numerous, and it therefore constitutes a huge challenge to combine them all in a single approach. This question is critical in sexual selection. I will therefore discuss it in a second paragraph, to outline related obstacles and possible improvements.

1) Other traits involved in sexual selection

As stated above, many other traits involved in sexual selection in brown trout could be tested. For example, coloration has been demonstrated as a honest signal that can inform about individual condition, immunity, social status of the individual or the quality of a potential mate ("good genes hypothesis"). Brown trout present wide variations in their body colorations (red and black points) and Wedekind and collaborators (2008) have shown that melanin coloration of fathers (black coloration) was positively related with offspring viability, whereas red coloration (carotenoid based colours) decreased their viability. In Atlantic salmon, a previous study also hinted that emergence timing of offspring and their viability increased with an increase of melanin-based coloration of sneaker males. In parallel to my thesis project, one master student investigated the effect of inter-individual variation in melanin-based coloration on reproductive investment, by measuring the relationship between coloration, weight variation and metabolites variation (Roussille, 2014). The results showed that the darker the robe, the stronger relative weight variation during the reproduction in males. However, the robe coloration was not significantly related to metabolites variations. Additionally, males were on average, darker than females. Therefore these results indicate that melanin based coloration could be related with energy allocation in gamete production and could be potentially involved in sexual selection. Indeed, black coloration could signal male quality and be preferred by females. However, in contradiction with the literature (Marie-Orleach et al., 2014; Wedekind et al., 2008), adult variation in melanin based coloration did not have consequences on individuals reproductive success in our study. However, herein, reproductive success was analyzed with a simple linear regression and did not take into account fitness decomposition. In future work, looking at the variation on male coloration

on each components of fitness (*i.e.* encounter rate, copulation rate or offspring number production) would be therefore interesting in order to see if one of these components is positively or negatively influence by this traits. A prediction could be that darkness could influence positively copulation rate, simply because females would thereby maximize their fertilization probability.

Moreover theory of mate choice predicts that individuals should avoid mating with genetically too closely related sexual partners (inbreeding avoidance). Indeed, mating with an individual sharing the same alleles can affect offspring survival through deleterious mutations. Therefore, higher fitness of offspring is predicted between unrelated parents. Major histocompatibility complex (MHC) regroups genes coding for protein involved in antigen recognition (Wedekind et al., 1995). Relatedness between a pair of individuals could be therefore regarded using (MHC) and microsatellite complex predicting to play a role in mating system (Forsberg et al., 2007; Jacob, Evanno, Von Siebenthal, Grossen, & Wedekind, 2010). In wild Atlantic salmon (*Salmo salar*), it has been shown that MHC genes were involved in mate choice (Landry et al., 2001). It is somehow telling that in my own experiment of reproductive isolation (B1 and B2), females either mated randomly (in constant environment) or mated more frequently with non-related males (Urumea females).

Even if initial weight, initial level of plasma metabolites at the onset of the reproduction and their relative variation were not tested in the fitness model, I initially spent some time developing a simplified decomposition fitness model, which was in fact an embryo of fitness decomposition model presented in chapter IV. To do so, I used this time only the matrix of reproductive success estimated by molecular data containing a large proportion of zero which is quite frequent in ecology surveys and these dataset are commonly

named “zero inflated dataset” (Martin et al., 2005). The two main problems with too many zeros in the data analysis process were once again that 1) data do not fit with standard distribution and 2) a zero value may originate from several distinct ecological processes of interest. In the data set, a zero value in the matrix could reflect the outcome of two distinct processes between a male and a female, 1) either fertilization did not occur because individuals never mated, or the eggs were fertilized but did not survive until sampling. The first process provided therefore information about the ability of individuals to access fertilization with a given partner, and therefore deals with intra-sexual competition for mates and mate choice. This “access to fertilization” is like a black box regrouping all pre-copulatory processes, such as encounter rate and copulation rate, over the whole reproductive season; 2) the second informed about their ability to actually obtain living offspring conditional on having mated, which relies on the capacity of individual to do parental care or to provide good genes to their progeny (direct or indirect benefits). To solve the problem, the “zero inflated” data set was analysed with a mixture model which used a combination of probability distributions to estimate parameters and disentangle the different ecological processes (Martin et al., 2005). Results showed that relative variations of both plasma triglycerides and weight increased the number of offspring per fertilization for males and females, and conditioned access to fertilization only for males. Fierce competition between males to get access to females (Fleming & Gross, 1994; Höjesjö et al., 2004) necessitates a lot of energy to win the competition toward conspecifics and to be present at the moment when the females lay the eggs which could explain the positive effect of relative variation of plasma triglycerides. This first result corresponds in part, to the results that I found when testing the joint effects of metabolites variations and weight variations on reproductive success in chapter III. It is therefore clear enough that variables such as, metabolite variation and weight variation,

could easily improve our understanding of sexual selection once accounted for in the model presented in Chapter IV.

In the same line of thinking, I did not investigate directly how environment was shaping reproductive success in the experiments B1 and B2. These contrasts between environments could have been used to make inference on the different components of sexual selection :does environment influence mate sampling, copulation rate, or offspring production? Although I did approach the answer to this question in my study of environment dependent reproductive isolation, it would be better handled to actually integrate environment in the model presented in chapter IV.

2) Modeling limitation

In the previous, I brushed the surface of a complex world, where many factors may contribute to sexual selection evolution. It is mirrored indeed by a wealth of scientific publications, and a relative difficulty to produce a synthetic approach (Kokko & Jennions, 2008, McNamara & Houston 2014).

In the present study, the effects of many traits have been tested on reproductive success but most of the time they have been tested separately using different statistical approaches: classical linear regression (effect of traits on reproductive success), GLMs with either Quasi-Poisson or negative binomial distributions (effects of weight and PCA axes built on metabolites variations), or the unified framework described in the chapter IV (but here, only body size effect of both sexes was inferred). It would be theoretically possible to include more factors in these various models, such as the ones evoked in the

previous paragraph, or such as behavioural investment (number of chases, number of digging). However, the comprehension of each traits and interactions between them in a single model is not intuitive as soon as numerous variables are taken into account. Additionally, the required size of data sets to estimate correctly all these parameters would be frightening. Another side effect is that, at least for some statistical approaches, the convergence time will increase exponentially with the model complexity. Such technological path leaves us with huge complexity in results to struggle with, and potential overuse of computing power: as an example, for the model developed in chapter IV, estimation process took approximately one month to estimate only parameters of males and female body size, with seven parallel instances of calculus. Because I did many different tests before that, one can easily evaluate that at least 6 months of calculus on one workstation were realized. I did only use a third of my available data regarding reproductive success and behavioural informations (experiment B1), and I only tested the effect of body size. A key reason for that problem is that I needed to unfold data matrices between unknown numbers of sub-matrices, because the question is biologically relevant and this process was really time and calculus consuming.

The positive side of my model is that I was able to make use of different datasets, and to somehow remove various kind of bias simultaneously: this was possible because I decided that there were some mechanical conditioning between processes of interest (encounter conditions copulation, and copulation conditions offspring production). But the other way around is also true: non-null offspring production, because of this conditioning, will improve estimation of the above processes (copulation, then encounter). I believe this approach should be further explored, by better stating the mechanical relationships between all processes and factors of interest. For instance: does available energy constrain behavioural investment? Or does behavioural investment (in

competition for instance) condition used energy? The answer is *qualitatively* easy: both paths are real. However, estimating the *quantitative* relationships between available energy, behaviour, and used energy will require new experimental protocols. However, it would improve our view on mechanical aspects that could be then be directly integrated into the model as new processes, instead of simply piling factors and testing naively for their interactions. In short, it implies that mechanical relationships have to be formulated when testing a hypothesis, whereas we usually use standard statistical tools not tailored to our scientific question. The benefits would be multiple: it would increase the synergy between various data sources, it would also probably decrease the quantity of data to sample, and it would indeed improve our scientific approach.

Discussion

Les résultats indiquent tout d'abord que la variation de certains métabolites énergétiques dans le plasma sanguin (triglycérides, acides gras insaturés) pendant la période de reproduction n'est pas liée à la production des gamètes, et pourrait donc être un indicateur de l'investissement comportemental (recherche de partenaire, interactions agonistiques, soins parentaux). Cette hypothèse est fortement étayée par le fait que cette variation est étroitement liée au succès reproducteur des mâles comme des femelles, confirmant au passage un compromis (ou trade-off) entre énergie investie et succès reproducteur souvent supposé mais très rarement démontré chez les poissons. En revanche, lorsque l'on fait varier l'environnement, les géniteurs issus de populations différentes ne réagissent pas de façon similaire : en environnement constant (débit stable), les géniteurs semblent tous montrer la même variation de concentration de métabolites pendant la reproduction. Mais en environnement variable (débit aléatoire), les géniteurs issus de la population la plus soumise à des débits stochastiques montrent une variation des métabolites moindres que les autres. Une interaction population/environnement qui gouverne une partie de l'investissement reproducteur est donc mise en évidence. Par ailleurs, à l'aide d'indices d'isolement reproducteur, il a été montré lors de cette thèse que cette interaction génère un isolement reproducteur dépendant du sexe dans l'environnement stochastique : les mâles issus d'un environnement stochastique ne participent plus du tout à la reproduction. Cet effet pourrait être dû à une adaptation permettant de percevoir les signaux environnementaux comme la stochasticité, et à réguler son investissement reproducteur en fonction.

A l'aide de notre modèle de décomposition de la sélection sexuelle, il a été montré que la taille corporelle affecte positivement chaque étape de la sélection sexuelle chez les mâles (recherche de partenaire, succès d'appariement et succès reproducteur). En revanche chez les femelles, la taille corporelle affecte très peu les deux premières étapes, et a un effet négatif sur le succès reproducteur. Ce résultat semble contradictoire avec d'autres résultats publiés, mais il est la conséquence directe du modèle développé qui permet d'évaluer l'effet des traits des deux partenaires simultanément sur le succès d'appariement ou le succès reproducteur : ainsi le succès reproducteur des femelles dans d'autres études pourrait être affecté (positivement) par le phénotype des mâles avec lesquels elles s'apparient.

Enfin, outre les effets de la variabilité des débits sur l'investissement reproducteur et l'isolement reproducteur entre les populations, l'étude en milieu naturel concernant comment les femelles choisissent leur habitat de reproduction en milieu naturel, en fonction de leur investissement gamétique (taille de l'œuf). Nos résultats indiquent que la survie des descendants est en grande partie gouvernée par la variation du débit et l'arasement de tout ou partie de la frayère. Il ne semble pas que les choix d'habitats, mesurés par la granulométrie ou la profondeur d'enfouissement, influence la probabilité d'arasement, mais les frayères creusées par les petites femelles sont moins soumises à l'arasement. Ainsi, la taille de la femelle pourrait devenir une cible de la sélection naturelle mais aussi sexuelle (préférence des mâles) en cas d'augmentation de la stochasticité des débits. Une solution alternative serait une augmentation du bet-hedging, à savoir, multiplier le nombre de frayère par femelle pour moyenner les risques. Lorsque les œufs ne sont pas arasés, nous montrons que leur survie est en partie affectée par la granulométrie, les plus gros œufs survivant mieux dans les plus fortes granulométries. Cette relation entre l'habitat et le phénotype des œufs est une piste pour expliquer le maintien de la variation de la taille de l'œuf en milieu naturel. Elle pourrait aussi mener à une sélection sur l'investissement gamétique dépendante de l'environnement.

L'ensemble des travaux conduits pendant cette thèse montrent que l'environnement semble affecter fortement le succès d'appariement et le succès reproducteur de la truite commune. Les effets peuvent être complexes et reposer sur plusieurs mécanismes : l'environnement physique (variabilité des débits) peut par exemple conditionner l'environnement social (réduction de l'OSR en milieu stochastique) qui à son tour influence les mécanismes de la sélection sexuelle (investissement gamétique, compétition pour l'accès au partenaire, choix de l'habitat de reproduction). Par ailleurs, toutes les populations ne présentent pas les mêmes réactions face à un changement de l'environnement. Les prédictions d'augmentation de fréquence des évènements hydrauliques extrêmes auront donc des effets multiples, à la fois sur les aspects sociaux qui gouvernent la compétition et l'offre phénotypique, mais aussi sur la survie des juvéniles, qui pourra alors modifier les coûts et bénéfices des différentes tactiques comportementales, et notamment celle de la balance entre soins parentaux et compétition intra-sexuelle chez les deux sexes. Enfin, ces modifications environnementales pourront affecter les flux de gènes entre populations, affectant la dynamique de la diversité intra-spécifique chez la truite commune.

BIBLIOGRAPHY

Abrahams, M. V. (1993). The trade-off between foraging and courting in male guppies. *Animal Behaviour*, *45*, 673–681.

Acolas, M. L. (2008). Déterminisme des tactiques de vie chez la truite commune Salmo trutta: influences maternelles et environnementales sur le comportement migratoire des juvéniles. Repport, 241p.

Alberto, F. (2009). MsatAllele_1.0: An R package to visualize the binning of microsatellite alleles. *The Journal of Heredity*, *100*(3), 394–7. doi:10.1093/jhered/esn110

Allendorf, F. W., & Thorgaard, G. H. (1984). *Tetraploidy and the Evolution of Salmonid Fishes*. *The evolutionary genetics of fishes* (Turner BJ., pp. 1–53). New York.

Anderson, R. A., & Karasov, W. H. (1988). Energetics of the lizard Cnemidophorus Tigris and life history consequences of food-acquisition mode. *Ecological Monographs*, *58*(2), 79. doi:10.2307/1942462

Anderson, S. S., & Fedak, M. A. (1985). Grey seal males: energetic and behavioural links between size and sexual success. *Animal Behaviour*, *33*(3), 829–838. doi:10.1016/S0003-3472(85)80017-8

Andersson, M. B. (1994). *Sexual selection* (Princeton University Press.).

Angilletta Jr, M. J., Niewiarowski, P. H., Dunham, A. E., Leaché, A. D., & Porter, W. P. (2004). Bergmann's clines in ectotherms: illustrating a life-history perspective with sceloporine lizards. *The American Naturalist*, *164*(6), E168–E183.

Argent, D. G., & Flebbe, P. A. (1999). Fine sediment effects on brook trout eggs in laboratory streams. *Fisheries Research*, *39*(3), 253–262. doi:10.1016/S0165-7836(98)00188-X

Arnold, S. J. (1994). Is there a unifying concept of sexual selection that applies to both plants and animals? *American Naturalist*, *144*, S1–S12.

Arnold, S. J., & Duvall, D. (1994). Animal mating systems : a synthesis based on selection theory. *The American Naturalist*, *143*(2), 317–348.

Arnold, S. J., & Wade, M. J. (1984a). On the measurement of natural and sexual selection: applications. *Evolution*, *38*(4), 720–734.

Arnold, S. J., & Wade, M. J. (1984b). On the measurment of natural and sexual selection: theory. *Evolution*, *38*(4), 709–719.

Arnqvist, G. (2013). Comment on "Bateman in nature: predation on offspring reduces the potential for sexual selection". *Science*, *340 (6*132*)*, 549.

Avise, J. C., Jones, A. G., Walker, D., & DeWoody, J. A. (2002). Genetic mating systems and reproductive natural histories of fishes: lessons for ecology and evolution. *Annual Review of Genetics*, *36(1)*, 19–45. doi:10.1146/annurev.genet.36.030602.090831

Aymes, J.-C., Larrieu, M., Tentelier, C., & Labonne, J. (2010). Occurrence and variation of egg cannibalism in brown trout *Salmo trutta*. *Die Naturwissenschaften*, *97*(4), 435–9. doi:10.1007/s00114-010-0656-0

Backwell, P. R. Y., & Passmore, N. I. (1996). Time constraints and multiple choice criteria in the sampling behaviour and mate choice of the fiddler crab, Uca annulipes. *Behavioral Ecology and Sociobiology*, *38*(6), 407–416. doi:10.1007/s002650050258

Bagenal, T. B. (1969). Relationship between egg size and fry survival in Brown Trout *Salmo trutta L*. *Journal of Fish Biology*, *1*(4), 349–353. doi:10.1111/j.1095-8649.1969.tb03882.x

Barboza, P. S., & Jorde, D. G. (2001). Intermittent feeding in a migratory omnivore: digestion and body composition of American black duck during autumn. *Physiological and Biochemical Zoology : PBZ*, *74*(2), 307–17. doi:10.1086/319658

Bardsen, B.-J., Næss, M. W., Tveraa, T., Langeland, K., & Fauchald, P. (2014). Risk-sensitive reproductive allocation: fitness consequences of body mass losses in two contrasting environments. *Ecology and Evolution*, *4*(7), 1030–8. doi:10.1002/ece3.1010

Bardsen, B.-J., Tveraa, T., Fauchald, P., & Langeland, K. (2010). Observational evidence of risk-sensitive reproductive allocation in a long-lived mammal. *Oecologia*, *162*(3), 627–39. doi:10.1007/s00442-009-1537-0

Bateman, A. J. (1948). Intra-sexual selection in Drosophila. *Heredity*, *2*, 349–368.

Beall, E., & Marty, C. (1983). Reproduction du saumon atlantique , Salmo salar L. En milieu semi-naturel contrôlé. *Bulletin Français de Pisciculture*, *289*, 77–93.

Beechie, T., Buhle, E., Ruckelshaus, M., Fullerton, A., &, & Holsinger, L. (2006). Hydrologic regime and the conservation of salmon life history diversity. *Biological Conservation*, *130*(4), 560–572.

Bell, G., & Gonzalez, A. (2011). Adaptation and evolutionary rescue in metapopulations experiencing environmental deterioration. *Science (New York, N.Y.)*, *332*(6035), 1327–30. doi:10.1126/science.1203105

Berg, O. K., Throanes, E., & Bremset, G. (1998). Energetics and survival of virgin and repeat spawning brown trout (Salmo trutta). *Canadian Journal of Fisheries and Aquatic Sciences*, *55*(1), 47–53.

Black, D., & Skinner, E. R. (1986). Features of the lipid transport system of fish as demonstrated by studies on starvation in the rainbow trout. *Journal of Comparative Physiology B*, *156*(4), 497–502. doi:10.1007/BF00691035

Blair, G. R., Rogers, D. E., & Quinn, T. P. (1993). Variation in life history characteristics and morphology of Sockeye Salmon in the Kvichak river system, Bristol Bay, Alaska. *Transactions of the American Fisheries Society*, *122*(4), 550–559. doi:10.1577/1548-8659(1993)122<0550:VILHCA>2.3.CO;2

Blanchfield, P., & Ridgway, M. (1999). The cost of peripheral males in a brook trout mating system. *Animal Behaviour*, *57*(3), 537–544. doi:10.1006/anbe.1998.1014

Blanckenhorn, W. U., Stillwell, R. C., Young, K. A., Fox, C. W., & Ashton, K. G. (2006). When Rensch meets Bergmann: does sexual size dimorphism change systematically with latitude? *Evolution*, *60*(10), 2004–2011. doi:10.1111/j.0014-3820.2006.tb01838.x

Bolnick, D. I., & Kirkpatrick, M. (2012). The relationship between intraspecific assortative mating and reproductive isolation between divergent populations. *Current Zoology*, *58*(3), 484–492.

Both, C., Bouwhuis, S., Lessells, C. M., & Visser, M. E. (2006). Climate change and population declines in a long-distance migratory bird. *Nature*, *441*(7089), 81–3. doi:10.1038/nature04539

Boughman, J. W. (2002). How sensory drive can promote speciation. *Trends in Ecology & Evolution*, *17*(12), 571–577. doi:10.1016/S0169-5347(02)02595-8

Cairney, M., Taggart, J. B., & HOyheim, B. (2000). Characterization of microsatellite and minisatellite loci in Atlantic salmon (Salmo salar L.) and cross-species amplification in other salmonids. *Molecular Ecology*, *9*(12), 2175–2178. doi:10.1046/j.1365-294X.2000.105312.x

Candolin, U., Salesto, T., & Evers, M. (2007). Changed environmental conditions weaken sexual selection in sticklebacks. *Journal of Evolutionary Biology*, *20*(1), 233–9. doi:10.1111/j.1420-9101.2006.01207.x

Chaine, A. S., & Lyon, B. E. (2008). Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. *Science (New York, N.Y.)*, *319*(5862), 459–62. doi:10.1126/science.1149167

Charnov, E. L., & Gillooly, J. F. (2004). Size and temperature in the evolution of fish life histories. *Integrative and Comparative Biology*, *44*(6), 494–7. doi:10.1093/icb/44.6.494

Cherel, Y., Robin, J. P., Walch, O., Karmann, H., Netchitailo, P., & Le Maho, Y. (1988). Fasting in king penguin. I. Hormonal and metabolic changes during breeding. *Am J Physiol Regulatory Integrative Comp Physiol*, *254*(2), R170–177.

Clutton-Brock, T. H. (1988). *Reproductive Success: studies of individual variation in contrasting breeding systems*. University of Chicago Press.

Clutton-Brock, T. H., & Guiness, F. E. (1982). *Red deer: behavior and ecology of two sexes*. University of Chicago Press.

Clutton-Brock, T. H., & Parker, G. A. (1992). Potential reproductive rates and the operation of sexual selection. *Quarterly Review of Biology*, 437–456.

Collet, J. M., Dean, R. F., Worley, K., Richardson, D. S., & Pizzari, T. (2014). The measure and significance of Bateman's principles. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1782), 20132973–20132973. doi:10.1098/rspb.2013.2973

Collet, J., Richardson, D. S., Worley, K., & Pizzari, T. (2012). Sexual selection and the differential effect of polyandry. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(22), 8641–5. doi:10.1073/pnas.1200219109

Coltman, D. W., Bancroft, D. R., Robertson, A., Smith, J. A., Clutton-brock, T. H., & Pemberton, J. M. (1999). Male reproductive success in a promiscuous mammal: behavioural estimates compared with genetic paternity. *Molecular Ecology*, *8*(7), 1199–1209. doi:10.1046/j.1365-294x.1999.00683.x

Coyne, J. A., & Orr, H. A. (2004). *Speciation* (Vol. 37). Sunderland, MA: Sinauer Associates.

Crow, J. F. (1958). Some possibilities for measuring selection intensities in man. *Human Biology*, *30*(1), 1–13.

Cureton, J. C., &, & Broughton, R. E. (2014). Rapid morphological divergence of a stream fish in response to changes in water flow. *Biology Letters*, *10*(6), 20140352.

Dankers, R., & Feyen, L. (2008). Climate change impact on flood hazard in Europe: An assessment based on high-resolution climate simulations. *Journal of Geophysical Research*, *113*(D19), D19105. doi:10.1029/2007JD009719

Darwin. (1871). *The Descent of Man and Selection in Relation to sex.* London.

Davis, M. B., Shaw, R. G., & Etterson, J. R. (2005). Evolutionary responses to changing climate. *Ecology*, *86*(7), 1704–1714. doi:10.1890/03-0788

Dobzhansky, T. (1937). Genetic nature of species differences. *American Naturalist*, *71*, 404–420.

Doucett, R. R., Booth, R. K., Power, G., & Mckinley, R. S. (1999). Effects of the spawning migration on the nutritional status of anadromous Atlantic salmon (Salmo salar) : insights from stable-isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, *56*(11), 2172–2180.

Dumas, J., & Marty, S. (2006). A new method to evaluate egg-to-fry survival in salmonids, trials with Atlantic salmon. *Journal of Fish Biology*, *68*(1), 284–304. doi:10.1111/j.1095-8649.2005.00907.x

Dumas, J., Olaïzola, M., & Barriere, L. (2007). (SALMO SALAR L .) Dans un cours d ' eau du sud de son aire de répartition , la nivelle. *BFPP/Bull.Fr.Pêche Piscic.*, *384*, 39–60.

Einum, S., & Fleming, I. A. (1999). Maternal effects of egg size in brown trout (Salmo trutta): norms of reaction to environmental quality. *Proceedings of the Royal Society B: Biological Sciences*, *266*(1433), 2095–2100. doi:10.1098/rspb.1999.0893

Einum, S., & Fleming, I. A. (2000). Selection against late emergence and small offspring in atlantic salmon (Salmo Salar). *Evolution*, *54*(2), 628–639. doi:10.1111/j.0014-3820.2000.tb00064.x

Einum, S., Hendry, A. P., & Fleming, I. A. (2002). Egg-size evolution in aquatic environments: does oxygen availability constrain size? *Proceedings. Biological Sciences / The Royal Society*, *269*(1507), 2325–30. doi:10.1098/rspb.2002.2150

Einum, S., & Nislow, K. H. (2005). Local-scale density-dependent survival of mobile organisms in continuous habitats: an experimental test using Atlantic salmon. *Oecologia*, *143*(2), 203–10. doi:10.1007/s00442-004-1793-y

Einum, S., Nislow, K. H., Mckelvey, S., & Armstrong, J. D. (2008). Nest distribution shaping within-stream variation in Atlantic salmon juvenile abundance and competition over small spatial scales. *The Journal of Animal Ecology*, *77*(1), 167–72. doi:10.1111/j.1365-2656.2007.01326.x

Elliott, J. M. (1994). Quantitative ecology and the brown trout. *Transactions of the American Fisheries Society*, 123(6), 1006-1008.

Emlen, S. T., & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science*, *197*(4300), 215–223.

Enes, P., Panserat, S., Kaushik, S., & Oliva-Teles, A. (2009). Nutritional regulation of hepatic glucose metabolism in fish. *Fish Physiology and Biochemistry*, *35*(3), 519–39. doi:10.1007/s10695-008-9259-5

Estay, F., Neira, R., Diaz, N. F., Valladares, L., & Torres, A. (1998). Gametogenesis and sex steroid profiles in cultured coho salmon (Oncorhynchus kisutch, Walbaum). *The Journal of Experimental Zoology*, *280*(6), 429–438. doi:10.1002/(SICI)1097-010X(19980415)280:6<429::AID-JEZ7>3.0.CO;2-P

Esteve, M. (2005). Observations of Spawning Behaviour in Salmoninae: Salmo, Oncorhynchus and Salvelinus. *Reviews in Fish Biology and Fisheries*, *15*(1-2), 1–21. doi:10.1007/s11160-005-7434-7

Estoup, A., Presa, P., Krieg, F., Vaiman, D., & Guyomard, R. (1993). (CT)n and (GT)n microsatellites: a new class of genetic markers for Salmo trutta L. (brown trout). *Heredity*, *71*(5), 488–496. doi:10.1038/hdy.1993.167

Fisher, R. A. (1930). *The Genetical Theory of Natural Selection.* (Oxford.). Clarendon Press.

Fleming, I. A., & Gross, M. R. (1994). Breeding competition in a pacifis salmon (Coho: Onchorhynchus Kisutch): measures of natural and sexual selection. *Evolution*, *48*(3), 637–657.

Forsberg, L. A., Dannewitz, J., Petersson, E., & Grahn, M. (2007). Influence of genetic dissimilarity in the reproductive success and mate choice of brown trout - females fishing for optimal MHC dissimilarity. *Journal of Evolutionary Biology*, *20*(5), 1859–69. doi:10.1111/j.1420-9101.2007.01380.x

Fox, C. W., & Czesak, M. E. (2000). Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology*, *45*, 341–69. doi:10.1146/annurev.ento.45.1.341

Freychet, M. (2011). Soins paternels et cannibalisme filial chez la truite commune (*Salmo trutta L*).

Fukushima, M., Quinn, T. J., & Smoker, W. W. (1998). Estimation of eggs lost from superimposed pink salmon (Oncorhynchus gorbuscha) redds. *Journal Canadien Des Sciences Halieutiques et Aquatiques*, *55*(3), 618–625.

Funk, D. J. (1998). Isolating a role for natural selection in speciation: host adaptation and sexual isolation in Neochlamisus bebbianae leaf beetles. *Evolution*, *52*(6), 1744–1759.

Funk, D. J., Nosil, P., & Etges, W. J. (2006). Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(9), 3209–13. doi:10.1073/pnas.0508653103

Garant, D. (2001). A genetic evaluation of mating system and determinants of individual reproductive success in atlantic salmon (Salmo salar L.). *Journal of Heredity*, *92*(2), 137–145. doi:10.1093/jhered/92.2.137

Garant, D., Dodson, J. J., & Bernatchez, L. (2001). A genetic evaluation of mating system and determinants of individual reproductive success in Atlantic salmon (Salmo salar L.). *Journal of Heredity*, *92*(2), 137–145.

Garcia-Vazquez, E., Moran, P., Martinez, J. L., Perez, J., de Gaudemar, B., & Beall, E. (2001). Alternative mating strategies in Atlantic salmon and brown trout. *The Journal of Heredity*, *92*(2), 146–9.

Gaudemar, B., & Beall, E. (1998). Effects of overripening on spawning behaviour and reproductive success of Atlantic salmon females spawning in. *Journal of Fish Biology*, *33*, 434–446.

Gaudemar, B., Bonzom, J. M., & Beall, E. (2000). Effects of courtship and relative mate size on sexual motivation in Atlantic salmon. *Journal of Fish Biology*, *57*, 502–515. doi:10.1006/jfbi.2000.1331

Gelman, A., & Rubin, D. B. (1992). Inference from Iterative Simulation Using Multiple Sequences. *Statistical Science*, *7*(4), 457–472.

Gienapp, P., Leimu, R., & Merilä, J. (2007). Responses to climate change in avian migration time Microevolution versus phenotypic plasticity. *Climate Research (Open Access for Articles 4 Years Old and Older)*, *35*(1), 25.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science (New York, N.Y.)*, *293*(5538), 2248–51. doi:10.1126/science.1061967

Gordo, O. (2007). Why are bird migration dates shifting? A review of weather and climate effects on avian migratory phenology. *Climate Research (Open Access for Articles 4 Years Old and Older)*, *35*(1), 37–58.

Gosset, C., Rives, J., & Labonne, J. (2006). Effect of habitat fragmentation on spawning migration of brown trout (Salmo trutta L.). *Ecology of Freshwater Fish*, *15*(3), 247–254. doi:10.1111/j.1600-0633.2006.00144.x

Gowaty, P. A., Kim, Y.-K., & Anderson, W. W. (2012). No evidence of sexual selection in a repetition of Bateman's classic study of Drosophila melanogaster. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(29), 11740–5. doi:10.1073/pnas.1207851109

Gray, S. M., Dill, L. M., & McKinnon, J. S. (2007). Cuckoldry incites cannibalism: male fish turn to cannibalism when perceived certainty of paternity decreases. *The American Naturalist*, *169*(2), 258–63. doi:10.1086/510604

Greeley, J. R. (1932). The spawning habits of brook, brown and rain-browntrout, and the problem of egg predator. *American Fisheries*, *62*(239-248).

Gregersen, F., Haugen, T. O., & Vøllestad, L. A. (2008). Contemporary egg size divergence among sympatric grayling demes with common ancestors. *Ecology of Freshwater Fish*, *17*(1), 110–118. doi:10.1111/j.1600-0633.2007.00264.x

Hance, T., van Baaren, J., Vernon, P., & Boivin, G. (2007). Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology*, *52*, 107–26. doi:10.1146/annurev.ento.52.110405.091333

Harshman, L. G., & Zera, A. J. (2007). The cost of reproduction: the devil in the details. *Trends in Ecology & Evolution*, *22*(2), 80–6. doi:10.1016/j.tree.2006.10.008

Hartmann, H.C., Bales,R. & Sorooshian, S. (2002). Weather, climate, and hydrologic forecasting for the US Southwest: a survey.. *Climate Research*, *21*(3), 239-258.

Hayward, A., & Gillooly, J. F. (2011). The cost of sex: quantifying energetic investment in gamete production by males and females. *PloS One*, *6*(1), e16557. doi:10.1371/journal.pone.0016557

Head, M. L., Wong, B. B. M., & Brooks, R. (2010). Sexual display and mate choice in an energetically costly environment. *PloS One*, *5*(12), e15279. doi:10.1371/journal.pone.0015279

Healey, M. C., & Prince, A. (1998). Alternative tactics in the breeding behaviour of male Coho Salmon. *Behaviour*, *135*(8), 1099–1124.

Hemre, G.-I., Mommsen, T. P., & Krogdahl, A. (2002). Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquaculture Nutrition*, *8*(3), 175–194. doi:10.1046/j.1365-2095.2002.00200.x

Hendry, A., & Beall, E. (2004). Energy use in spawning Atlantic salmon. *Ecology of Freshwater Fish*, *13*, 185–196.

Hendry, A. P. (2009). Ecological speciation! Or the lack thereof? *Canadian Journal of Fisheries and Aquatic Sciences*, *66*(8), 1383–1398.

Hendry, A. P., & Day, T. (2003). Revisiting the positive correlation between female size and egg size. *Evolutionary Ecology Research*, *5*(3), 421–429.

Hendry, A. P., Day, T., & Cooper, A. B. (2001). Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. *The American Naturalist*, *157*(4), 387–407.

Hendry, A. P., Huber, S. K., De Leon, L. F., Herrel, A., & Podos, J. (2009). Disruptive selection in a bimodal population of Darwin's finches. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1657), 753–759.

Hendry, A. P., Wenburg, J. K., Bentzen, P., Volk, E. C., & Quinn, T. P. (2000). Rapid evolution of reproductive isolation in the wild: Evidence from introduced salmon. *Science*, *290*(5491), 516–518.

Hoegg, S., Brinkmann, H., Taylor, J., & Meyer, A. (2004). Phylogenetic timing of the fish-specific genome duplication correlates with the diversification of teleost fish. *Journal of molecular evolution*, *59*(2), 190-203.

Höjesjö, J., Johnsson, J., & Bohlin, T. (2004). Habitat complexity reduces the growth of aggressive and dominant brown trout (Salmo trutta) relative to subordinates. *Behavioral Ecology and Sociobiology*, *56*(3), 286–289. doi:10.1007/s00265-004-0784-7

Holt, R. D. (1990). The microevolutionary consequences of climate change. *Trends in Ecology & Evolution*, *5*(9), 311–315. doi:10.1016/0169-5347(90)90088-U

Houston, A. I., Székely, T. & McNamara, J.M. (2013). The parental investment models of Maynard Smith: a restrospective and prospective vue. *Animal Behaviour*, *86*, 667–674.

Imre, I., McLaughlin, R. L., &, & Noakes, D. L. G. (2002). Phenotypic plasticity in brook charr: changes in caudal fin induced by water flow. *Journal of Fish Biology*, *61*(5), 1171–1181.

Jacob, A., Evanno, G., Von Siebenthal, B. a, Grossen, C., & Wedekind, C. (2010). Effects of different mating scenarios on embryo viability in brown trout. *Molecular Ecology*, *19*(23), 5296–307. doi:10.1111/j.1365-294X.2010.04884.x

Jacob, A., Nusslé, S., Britschgi, A., Evanno, G., Müller, R., & Wedekind, C. (2007). Male dominance linked to size and age, but not to "good genes" in brown trout (Salmo trutta). *BMC Evolutionary Biology*, *7*, 207. doi:10.1186/1471-2148-7-207

Jann, P., Blanckenhorn, W. U., & Ward, P. I. (2000). Temporal and microspatial variation in the intensities of natural and sexual selection in the yellow dung fly Scathophaga stercoraria. *Journal of Evolutionary Biology*, *13*(6), 927–938. doi:10.1046/j.1420-9101.2000.00230.x

Jones, A. G. (2009). On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. *Evolution; International Journal of Organic Evolution*, *63*(7), 1673–1684. doi:10.1111/j.1558-5646.2009.00664.x

Jones, A. G., & Ardren, W. R. (2003). Methods of parentage analysis in natural populations. *Molecular Ecology*, *12*(10), 2511–2523. doi:10.1046/j.1365-294X.2003.01928.x

Jones, A. G., Moore, G. I., Kvarnemo, C., Walker, D., & Avise, J. C. (2003). Sympatric speciation as a consequence of male pregnancy in seahorses. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(11), 6598–603. doi:10.1073/pnas.1131969100

Jones, A. G., & Ratterman, N. L. (2009). Mate choice and sexual selection: what have we learned since Darwin? *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 10001–8. doi:10.1073/pnas.0901129106

Jones, A. G., Rosenqvist, G., Berglund, A., Arnold, S. J., & Avise, J. C. (2000). The Bateman gradient and the cause of sexual selection in a sex-role-reversed pipefish. *Proceedings. Biological Sciences / The Royal Society*, *267*(1444), 677–80. doi:10.1098/rspb.2000.1055

Kakisawa, S., Kaneko, T., Hasegawa, S., & Hirano, T. (1995). Effects of feeding, fasting, background adaptation, acute stress, and exhaustive exercise on the plasma somatolactin concentrations in Rainbow Trout. *General and Comparative Endocrinology*, *98*(2), 137–146.

Kalinowski, S. T. (2002). Evolutionary and statistical properties of three genetic distances. *Molecular Ecology*, *11*(8), 1263–1273. doi:10.1046/j.1365-294X.2002.01520.x

Kamalam, B. S., Medale, F., Kaushik, S., Polakof, S., Skiba-Cassy, S., & Panserat, S. (2012). Regulation of metabolism by dietary carbohydrates in two lines of rainbow trout divergently selected for muscle fat content. *The Journal of Experimental Biology*, *215*(Pt 15), 2567–78. doi:10.1242/jeb.070581

Kamler, E. (1992). *Early life history of fish: an energetics approach*. London: Chapman & Hall.

Kasumovic, M. M., Bruce, M. J., Andrade, M. C. B., & Herberstein, M. E. (2008). Spatial and temporal demographic variation drives within-season fluctuations in sexual selection. *Evolution; International Journal of Organic Evolution*, *62*(9), 2316–25. doi:10.1111/j.1558-5646.2008.00446.x

Keenleyside, M. H. A., & Dupuis, H. M. C. (1988). Courtship and spawning competition in pink salmon (Oncorhynchus gorbuscha). *Canadian Journal of Zoology*, *66*(1), 262–265. doi:10.1139/z88-037

Kerley, L. L., Goodrich, J. M., Miquelle, D. G., Smirnov, E. N., Quigley, H. B., & Hornocker, M. G. (2002). Effects of Roads and Human Disturbance on Amur Tigers. *Conservation Biology*, *16*(1), 97–108. doi:10.1046/j.1523-1739.2002.99290.x

Kilgas, P., Mänd, R., Mägi, M., & Tilgar, V. (2006). Hematological parameters in brood-rearing great tits in relation to habitat, multiple breeding and sex. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *144*(2), 224–231.

Kirkpatrick, M., & Barton, N. H. (1997). Evolution of a species' range. *The American Naturalist*, *150*(1), 1–23.

Klemetsen, A., Amundsen, P.-A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F., & Mortensen, E. (2003). Atlantic salmon Salmo salar L., brown trout Salmo trutta L. and Arctic charr Salvelinus alpinus (L.): a review of aspects of their life histories. *Ecology of Freshwater Fish*, *12*(1), 1–59. doi:10.1034/j.1600-0633.2003.00010.x

Klug, H., Heuschele, J., Jennions, M. D., & Kokko, H. (2010). The mismeasurement of sexual selection. *Journal of Evolutionary Biology*, *23*(3), 447–62. doi:10.1111/j.1420-9101.2009.01921.x

Kokko, H., Brooks, R., Jennions, M. D., & Morley, J. (2003). The evolution of mate choice and mating biases. *Proceedings. Biological Sciences / The Royal Society*, *270*(1515), 653–64. doi:10.1098/rspb.2002.2235

Kokko, H., & Jennions, M. D. (2008). Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology*, *21*(4), 919–48. doi:10.1111/j.1420-9101.2008.01540.x

Kokko, H., & Monaghan, P. (2001). Predicting the direction of sexual selection. *Ecology Letters*, *4*(2), 159–165. doi:10.1046/j.1461-0248.2001.00212.x

Krause, J., Krause, S., Arlinghaus, R., Psorakis, I., Roberts, S., & Rutz, C. (2013). Reality mining of animal social systems. *Trends in Ecology & Evolution*, *28*(9), 541–51. doi:10.1016/j.tree.2013.06.002

Kvarnemo, C., & Ahnesjö, I. (1996). The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution*, *11*(10), 404–408. doi:10.1016/0169-5347(96)10056-2

Labonne, J., Augery, M., Parade, M., Brinkert, S., Prevost, E., Héland, M., & Beall, E. (2009). Female preference for male body size in brown trout, Salmo trutta: is big still fashionable? *Animal Behaviour*, *77*(1), 129–137. doi:10.1016/j.anbehav.2008.09.018

Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution*, 1210–1226.

Landry, C., Garant, D., Duchesne, P., & Bernatchez, L. (2001). "Good genes as heterozygosity": the major histocompatibility complex and mate choice in Atlantic salmon (Salmo salar). *Proceedings. Biological Sciences / The Royal Society*, *268*(1473), 1279–85. doi:10.1098/rspb.2001.1659

Langerhans, R. B., & Makowicz, A. M. (2013). Sexual selection paves the road to sexual isolation during ecological speciation. *Evolutionary Ecology Research*, *15*, 633–651.

Lecomte, F., Beall, E., Chat, J., Davaine, P., & Gaudin, P. (2013). The complete history of salmonid introductions in the Kerguelen Islands, Southern Ocean. *Polar Biology*, *36*(4), 457–475. doi:10.1007/s00300-012-1281-5

Lerceteau-Köhler, E., & Weiss, S. (2006). Development of a multiplex PCR microsatellite assay in brown trout Salmo trutta, and its potential application for the genus. *Aquaculture*, *258*(1-4), 641–645. doi:10.1016/j.aquaculture.2006.04.028

Levins, R. (1968). *Evolution in changing environments: some theoretical explorations.* Princeton University Press.

Lourdais, O., Brischoux, F., Shine, R., & Bonnet, X. (2005). Adaptive maternal cannibalism in snakes (Epicrates cenchria maurus, Boidae). *Biological Journal of the Linnean Society*, *84*(4), 767–774. doi:10.1111/j.1095-8312.2005.00436.x

Maan, M. E., Seehausen, O., & Van Alphen, J. J. M. (2010). Female mating preferences and male coloration covary with water transparency in a Lake Victoria cichlid fish. *Biological Journal of the Linnean Society*, *99*(2), 398–406. doi:10.1111/j.1095-8312.2009.01368.x

Manica, A. (2002). Filial cannibalism in teleost fish. *Biological Reviews of the Cambridge Philosophical Society*, *77*(2), 261–77.

Marie-Orleach, L., Roussel, J.-M., Bugeon, J., Tremblay, J., Ombredane, D., & Evanno, G. (2014). Melanin-based coloration of sneaker male Atlantic salmon is linked to viability and emergence timing of their offspring. *Biological Journal of the Linnean Society*, *111*(1), 126–135. doi:10.1111/bij.12187

Martin, T. G., Wintle, B. A., Rhodes, J. R., Kuhnert, P. M., Field, S. A., Low-Choy, S. J., … Possingham, H. P. (2005). Zero tolerance ecology: improving ecological inference by modelling the source of zero observations. *Ecology Letters*, *8*(11), 1235–46. doi:10.1111/j.1461-0248.2005.00826.x

Masello, J. F., & Quillfeldt, P. (2004). Are haematological parameters related to body condition, ornamentation and breeding success in wild burrowing parrots Cyanoliseus patagonus ? *Journal of Avian Biology*, *35*(5), 445–454. doi:10.1111/j.0908-8857.2004.03278.x

McCue, M. D. (2010). Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *156*(1), 1–18. doi:10.1016/j.cbpa.2010.01.002

McDonald, G. C., James, R., Krause, J., & Pizzari, T. (2013). Sexual networks: measuring sexual selection in structured, polyandrous populations. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *368*(1613), 20120356. doi:10.1098/rstb.2012.0356

McKinnon, J. S., Mori, S., Blackman, B. K., David, L., Kingsley, D. M., Jamieson, L., Chou, J. & Schluter, D. (2004). Evidence for ecology's role in speciation. *Nature*, *429*(6989), 294–298.

McNamara, J. M., & Houston, A. I. (1996). State-dependent life histories. *Nature*, *380*(6571), 215–221.

Merilä, J., & Svensson, E. (1995). Fat reserves and health state in migrant Goldcrest Regulus regulus. *Functional Ecology*, *9*(6), 842–848.

Meyers, L. A., & Bull, J. J. (2002). Fighting change with change: adaptive variation in an uncertain world. *Trends in Ecology & Evolution*, *17*(12), 551–557. doi:10.1016/S0169-5347(02)02633-2

Milinski, M. (2006). The major histocompatibility complex, sexual selection, and mate choice. *Annual Review of Ecology, Evolution, and Systematics*, *37*, 159–186.

Mills, S. C., Grapputo, A., Koskela, E., & Mappes, T. (2007). Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices. *Proceedings. Biological Sciences / The Royal Society*, *274*(1606), 143–50. doi:10.1098/rspb.2006.3639

Milly, P. C. D., Dunne, K. A., & Vecchia, A. V. (2005). Global pattern of trends in streamflow and water availability in a changing climate. *Nature*, *438*(7066), 347–50. doi:10.1038/nature04312

Milly, P. C. D., Wetherald, R. T., Dunne, K. A., & Delworth, T. L. (2002). Increasing risk of great floods in a changing climate. *Nature*, *415*(6871), 514–7. doi:10.1038/415514a

Moller, A. P. (2004). Protandry, sexual selection and climate change. *Global Change Biology*, *10*(12), 2028–2035. doi:10.1111/j.1365-2486.2004.00874.x

Møller, A. P., & Jennions, M. D. (2001). How important are direct fitness benefits of sexual selection? *Naturwissenschaften*, *88*(10), 401–415. doi:10.1007/s001140100255

Moorad, J. A., & Wade, M. J. (2013). Selection gradients, the opportunity for selection, and the coefficient of determination. *The American Naturalist*, *181*(3), 291–300. doi:10.1086/669158

Moore, S. (1968). Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. *The Journal of Biological Chemistry*, *243*(23), 6281–3.

Morbey, Y. (2000). Protandry in Pacific sallmon. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(6), 1252–1257.

Müllenbach, R., Lagoda, P. J., & Welter, C. (1989). An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends in Genetics : TIG*, *5*(12), 391.

Murchie, K. J., Cooke, S. J., & Danylchuk, A. J. (2010). Seasonal Energetics and Condition of Bonefish from Different Subtropical Tidal Creeks in Eleuthera, the Bahamas. *Marine and Coastal Fisheries*, *2*(1), 249–262. doi:10.1577/C09-042.1

Nosil, P. (2002). Transition rates between specialization and generalization in phytophagous insects. *Evolution*, *56*(8), 1701–1706. doi:10.1111/j.0014-3820.2002.tb01482.x

Nosil, P., Harmon, L. J., & Seehausen, O. (2009). Ecological explanations for (incomplete) speciation. *Trends in Ecology & Evolution*, *24*(3), 145–56. doi:10.1016/j.tree.2008.10.011

Nosil, P., Vines, T. H., & Funk, D. J. (2005). Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*, *59*(4), 705–719. doi:10.1111/j.0014-3820.2005.tb01747.x

O'Reilly, P. T., Hamilton, L. C., McConnell, S. K., & Wright, J. M. (1996). Rapid analysis of genetic variation in Atlantic salmon (Salmo salar) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(10), 2292–2298. doi:10.1139/f96-192

Ojanguren, A. F., Reyes-Gavilán, F. G., & Braña, F. (1996). Effects of egg size on offspring development and fitness in brown trout, Salmo trutta L. *Aquaculture*, *147*(1-2), 9–20. doi:10.1016/S0044-8486(96)01398-1

Orsini, L., Vanoverbeke, J., Swillen, I., Mergeay, J., & Meester, L. (2013). Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. *Molecular Ecology*, *22*(24), 5938–5999.

Panserat, S., Perrin, A., & Kaushik, S. (2002). High dietary lipids induce liver glucose-6-phosphatase expression in rainbow trout (Oncorhynchus mykiss). *The Journal of Nutrition*, *132*(2), 137–141.

Parker, G. A. (1982). Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. *Journal of Theoretical Biology*, *96*(2), 281–294.

Parker, P. G., & Tang-Martinez, Z. (2005). Bateman gradients in field and laboratory studies: a cautionary tale. *Integrative and Comparative Biology*, *45*(5), 895–902. doi:10.1093/icb/45.5.895

Paterson, S., Piertney, S. B., Knox, D., Gilbey, J., & Verspoor, E. (2004). Characterization and PCR multiplexing of novel highly variable tetranucleotide Atlantic salmon (Salmo salar L.) microsatellites. *Molecular Ecology Notes*, *4*, 160–162. doi:10.1111/j.1471-8286.2004.00598.x

Pearse, D. E., Hayes, S. A., Bond, M. H., Hanson, C. V., Anderson, E. C., Macfarlane, R. B., & Garza, J. C. (2009). Over the Falls? Rapid Evolution of Ecotypic Differentiation in Steelhead/Rainbow Trout (Oncorhynchus mykiss). *Journal of Heredity*, *100*(5), 515–525.

Pélissié, B., Jarne, P., Sarda, V., & David, P. (2014). Disentangling precopulatory and postcopulatory sexual selection in polyandrous species. *Evolution; International Journal of Organic Evolution*, *68*(5), 1320–31. doi:10.1111/evo.12353

Pemberton, J. M., Albon, S. D., Guinness, F. E., Clutton-Brock, T. H., & Dover, G. A. (1992). Behavioral estimates of male mating success tested by DNA fingerprinting in a polygynous mammal. *Behavioral Ecology*, *3*(1), 66–75. doi:10.1093/beheco/3.1.66

Perkins, D. M., Reiss, J., Yvon-Durocher, G., & Woodward, G. (2010). Global change and food webs in running waters. *Hydrobiologia*, *657*(1), 181–198. doi:10.1007/s10750-009-0080-7

Peterson, D. A., Hilborn, R., &, & Hauser, L. (2014). Local adaptation limits lifetime reproductive success of dispersers in a wild salmon metapopulation. *Nature Communications*, *5*(3696).

Petersson, E., & Järvi, T. (2001). "False orgasm" in female brown trout: trick or treat? *Animal Behaviour*, *61*(2), 497–501. doi:10.1006/anbe.2000.1585

Petersson, E., Järvi, T., Olsén, H., Mayer, I., & Hedenskog, M. (1999). Male-male competition and female choice in brown trout. *Animal Behaviour*, *57*(4), 777–783. doi:10.1006/anbe.1998.1043

Pischedda, A., & Rice, W. (2012). Partitioning sexual selection into its mating success and fertilization success components. *Proceedings of the National Academy of Sciences*, *109*(6), 2049-2053.

Price, G. R. (1970). Selection and Covariance. *Nature*, *227*(5257), 520–521. doi:10.1038/227520a0

Pritchard, A. L. (1937). Variation in the Time of Run, Sex Proportions, Size and Egg Content of adult pink salmon (Oncorhynchus gorbuscha) at McClinton Creek, Masset Inlet, B.C. *Journal of the Biological Board of Canada*, *3*(5), 403–416. doi:10.1139/f37-023

Régnier, T. (2011). *Influences parentales sur le statut énergétique des jeunes stades de truite commune (Salmo trutta): conséquences sur les comportements précoces et l'aptitude phénotypique*.

Régnier, T., Bolliet, V., Gaudin, P., & Labonne, J. (2013). Bigger is not always better: egg size influences survival throughout incubation in brown trout (Salmo trutta). *Ecology of Freshwater Fish*, *22*(2), 169–177. doi:10.1111/eff.12018

Relyea, R. A. (2001). Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology*, *82*(2), 523–540.

Reynolds, J. D. (1996). Animal breeding systems. *TREE*, *11*, 68–72.

Reznick, D. (1985). Costs of reproduction: an evaluation of the empirical evidence. *Oikos*, 44(2), 257–267.

Rice, W. R., & Hostert, E. E. (1993). Laboratory experiments on speciation: What have we learned in 40 years? *Evolution*, *47*, 1637–1653.

Rice, W. R., & Salt, G. W. (1990). The evolution of reproductive isolation as a correlated character under sympatric conditions: experimental evidence. *Evolution*, *44*(5), 1140–1152.

Rideout, R. M., & Tomkiewicz, J. (2011). Skipped spawning in fishes: more common than you migth think. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science*, *3*, 176–189.

Roff, D. A. (1992). *Evolution of life histories: theory and analysis*. Springer Science & Business Media.

Roff, D. A. (2002). *Life History Evolution* (Sinauer As.). Sunderland.

Rose, E., Paczolt, K. A., & Jones, A. G. (2013). The contributions of premating and postmating selection episodes to total selection in sex-role-reversed Gulf pipefish. *The American Naturalist*, *182*(3), 410–420.

Rose, M. R., & Charlesworth, B. (1981a). Genetics of life history in Drosophila melanogaster. I. Sib analysis of adult females. *Genetics*, *97*(1), 173–186.

Rose, M. R., & Charlesworth, B. (1981b). Genetics of life history in Drosophila melanogaster. II. Exploratory selection experiments. *Genetics*, *97*(1), 187–196.

Rousset, F. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103–6. doi:10.1111/j.1471-8286.2007.01931.x

Roussille, V. (2014). *Coloration mélanique et stratégies comportementales chez la truite fario (salmo trutta).*

Rundle, H. D. (2000). Natural Selection and Parallel Speciation in Sympatric Sticklebacks. *Science*, *287*(5451), 306–308. doi:10.1126/science.287.5451.306

Rundle, H. D. (2003). How divergent environnements and population bottlenecks fail to generate premating isolation in Drosophila pseudoobscura. *Evolution*, *57*, 2257–2565.

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, *8*(3), 336–352. doi:10.1111/j.1461-0248.2004.00715.x

Salem, M., Kenney, P. B., Rexroad, C. E., & Yao, J. (2006). Molecular characterization of muscle atrophy and proteolysis associated with spawning in rainbow trout. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *1*(2), 227–237.

Sargent, J., Tocher, D., & Bell, J. (2002). Fish Nutrition. In *Fish Nutrition* (pp. 181–257).

Schluter, D. T. E. of A. R. (2000). *The Ecology of Adaptive Radiation* (Oxford Uni.). Oxford.

Schmid, B., Tam-Dafond, L., Jenni-Eiermann, S., Arlettaz, R., Schaub, M., & Jenni, L. (2013). Modulation of the adrenocortical response to acute stress with respect to brood value, reproductive success and survival in the Eurasian hoopoe. *Oecologia*. doi:10.1007/s00442-013-2598-7

Schreck, C. B., Contreras-Sanchez, W., & Fitzpatrick, M. S. (2001). Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture*, *197*(1-4), 3–24. doi:10.1016/S0044-8486(01)00580-4

Schroder, S. L. (1981). *The role of sexual selection in determining the overall mating patterns and mate choice in chum salmon.* University of Washington, Seattle.

Schulte-Hostedde, A. I., Millar, J. S., & Hickling, G. J. (2001). Evaluating body condition in small mammals. *Canadian Journal of Zoology*, *79*(6), 1021–1029.

Schwartz, A. K., Weese, D. J., Bentzen, P., Kinnison, M. T., & Hendry, A. P. (2010). Both Geography and Ecology Contribute to Mating Isolation in Guppies. *Plos One*, *5*(12), e15659.

Seehausen, O. (1997). Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, *277*(5333), 1808–1811. doi:10.1126/science.277.5333.1808

Seiliez, I., Gabillard, J.-C., Riflade, M., Sadoul, B., Dias, K., Avérous, J., Tesseraud, S. Skiba, S. & Panserat, S. (2012). Amino acids downregulate the expression of several autophagy-related genes in rainbow trout myoblasts. *Autophagy*, *8*(3), 364–75. doi:10.4161/auto.18863

Serbezov, D., Bernatchez, L., Olsen, E. M., & Vøllestad, L. A. (2010). Mating patterns and determinants of individual reproductive success in brown trout (Salmo trutta) revealed by parentage analysis of an entire stream living population. *Molecular Ecology*, *19*(15), 3193–205. doi:10.1111/j.1365-294X.2010.04744.x

Servedio, M. R., & Kopp, M. (2012). Sexual selection and magic traits in speciation with gene flow. *Current Zoology*, *58*(3), 510–516.

Sexton, J. P., Hangartner, S. B., & Hoffmann, A. A. (2014). Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution; 68*(1), 1–15. doi:10.1111/evo.12258

Shafer, A. B. A., & Wolf, J. B. W. (2013). Widespread evidence for incipient ecological speciation: a meta-analysis of isolation-by-ecology. *Ecology Letters*, *16*(7), 940–50. doi:10.1111/ele.12120

Shuster, S. M., & Wade, M. J. (2003). *Mating systems and strategies* (Princeton .).

Silbergeld, E. K. (1974). Blood glucose: A sensitive indicator of environmental stress in fish. *Bulletin of Environmental Contamination & Toxicology*, *11*(1), 20-25.

Skiba-Cassy, S., Panserat, S., Larquier, M., Dias, K., Surget, A., Plagnes-Juan, E., Kaushik, S. & Seiliez, I. (2013). Apparent low ability of liver and muscle to adapt to variation of dietary carbohydrate:protein ratio in rainbow trout (Oncorhynchus mykiss). *The British Journal of Nutrition*, *109*(8), 1359–72. doi:10.1017/S0007114512003352

Slettan, A., Olsaker, I., & Lie, Ø. (1995). Atlantic salmon, Salmo salar , microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. *Animal Genetics*, *26*(4), 281–282. doi:10.1111/j.1365-2052.1995.tb03262.x

Smith, C. C., & Fretwell, S. D. (1974). The optimal balance between size and number of offspring. *American Naturalist*, *108*, 499–506.

Snyder, B. F., & Gowaty, P. A. (2007). A reappraisal of Bateman's classic study of intrasexual selection. *Evolution; 61*(11), 2457–68. doi:10.1111/j.1558-5646.2007.00212.x

Sobel, J. M., & Chen, G. F. (2014). Unification of methods for estimating the strength of reproductive isolation. *Evolution; International Journal of Organic Evolution*, *68*(5), 1511–22. doi:10.1111/evo.12362

Stahl, K., Hisdal, H., Hannaford, J., Tallaksen, L. M., Van Lanen, H. A. J., Sauquet, E., Demuth, M., Fendekova, M. & Jódar, J. (2010). Streamflow trends in Europe: evidence from a dataset of near-natural catchments. *Hydrology and Earth System Sciences Discussions*, *7*(4), 5769–5804.

Stahl, K., Tallaksen, L. M., Hannaford, J., & van Lanen, H. A. J. (2012, July 23). Filling the white space on maps of European runoff trends: estimates from a multi-model ensemble. *Hydrology and Earth System Sciences*. doi:10.5194/hess-16-2035-2012 <http://dx.doi.org/10.5194/hess-16-2035-2012>

Stearns, S. (1992). *The evolution of life histories Oxford University Press (Oxford University Press)*

Stevenson, R. D., & Woods, W. A. (2006). Condition indices for conservation: new uses for evolving tools. *Integrative and Comparative Biology*, *46*(6), 1169–90. doi:10.1093/icb/icl052

Stevenson, R. J., & Sabater, S. (2010). Understanding effects of global change on river ecosystems: science to support policy in a changing world. *Hydrobiologia*, *657*(1), 3–18. doi:10.1007/s10750-010-0392-7

Stone, D. A. J. (2003). Dietary carbohydrate utilization by fish. *Reviews in Fisheries Science*, *11*(4), 337–369. doi:10.1080/10641260390260884

Sutherland, W. J. (1985). Chance can produce a sex difference in variance in mating success and explain Bateman's data. *Animal Behaviour*, *33*(4), 1349–1352. doi:10.1016/S0003-3472(85)80197-4

Tappel, P. D., & Bjornn, T. C. (1983). A New Method of Relating Size of Spawning Gravel to Salmonid Embryo Survival. *North American Journal of Fisheries Management*, *3*(2), 123–135. doi:10.1577/1548-8659(1983)3<123:ANMORS>2.0.CO;2

Tentelier, C., Larrieu, M., Aymes, J.-C., & Labonne, J. (2011). Male antagonistic behaviour after spawning suggests paternal care in brown trout, Salmo trutta. *Ecology of Freshwater Fish*, *20*(4), 580–587. doi:10.1111/j.1600-0633.2011.00507.x

Thibert-Plante, X., & Hendry, A. P. (2010). When can ecological speciation be detected with neutral loci? *Molecular Ecology*, *19*(11), 2301–2314.

Thomas, L. K., & Manica, A. (2003). Filial cannibalism in an assassin bug. *Animal Behaviour*, *66*(2), 205–210. doi:10.1006/anbe.2003.2202

Toonen, R. J., & Hughes, S. (2001). Increased throughput for fragment analysis on an ABI Prism 377 automated sequencer using a 1 membrane comb and STRand software. *BioTechniques*, *31*, 1320–1324.

Trivers, R. (1972). *Parental investment and sexual selection*. Chicago University Press.

Tuljapurkar, S. (1990). Delayed reproduction and fitness in variable environments. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(3), 1139–1143.

Tuomainen, U., & Candolin, U. (2011). Behavioural responses to human-induced environmental change. *Biological Reviews of the Cambridge Philosophical Society*, *86*(3), 640–57. doi:10.1111/j.1469-185X.2010.00164.x

Tyler, C. R. (1990). The dynamics of oocyte growth during vitellogenesis in the rainbow trout (Oncorhynchus mykiss). *Biology of Reproduction*, *43*(2), 202–209. doi:10.1095/biolreprod43.2.202

Uller, T., & Olsson, M. (2008). Multiple paternity in reptiles: patterns and processes. *Molecular Ecology*, *17*(11), 2566–80. doi:10.1111/j.1365-294X.2008.03772.x

Vézina, F., & Williams, T. D. (2005). The metabolic cost of egg production is repeatable. *The Journal of Experimental Biology*, *208*(Pt 13), 2533–8. doi:10.1242/jeb.01675

Vincent, A., Ahnesjö, I., Berglund, A., & Rosenqvist, G. (1992). Pipefishes and seahorses: Are they all sex role reversed? *Trends in Ecology & Evolution*, *7*(7), 237–41. doi:10.1016/0169-5347(92)90052-D

Vitousek, P. M. (1994). Beyond global warming: ecology and global change. *Ecology*, *75*(7), 1861. doi:10.2307/1941591

Wade, M. J. (1979). Sexual selection and variance in reproductive success. *American Naturalist*, *114*, 742–747.

Wade, M. J., & Arnold, S. J. (1980). The intensity of sexual selection in relation to male sexual behaviour, female choice, and sperm precedence. *Animal Behaviour*, *28*(2), 446–461. doi:10.1016/S0003-3472(80)80052-2

Wedekind, C., Jacob, A., Evanno, G., Nusslé, S., & Müller, R. (2008). Viability of brown trout embryos positively linked to melanin-based but negatively to carotenoid-based colours of their fathers. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1644), 1737–44. doi:10.1098/rspb.2008.0072

Wedekind, C., Seebeck, T., Bettens, F., & Paepke, A. J. (1995). MHC-dependent mate preferences in humans. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *260*(1359), 245–9. doi:10.1098/rspb.1995.0087

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*(6), 1358–1370.

Weir, L. K., Grant, J. W., & Hutchings, J. A. (2011). The influence of operational sex ratio on the intensity of competition for mates. *The American Naturalist*, *177*(2), 167–176.

Williams, G. C. (1966). Natural selection, the costs of reproduction, and a Refinement of Lack's principle. *American Naturalist*, *100*(916), 687–690.

Wilson, A. B., Ahnesjo, I., Vincent, A. C. J., & Meyer, A. (2003). The dynamics of male brooding, matting patterns, and sex roles in pipefishes and seahorses (Family Syngnathidae). *Evolution*, *57*(6), 1374–1386. doi:10.1111/j.0014-3820.2003.tb00345.x

Wilson, R. P. (1994). Utilization of dietary carbohydrate by fish. *Aquaculture*, *124*(1), 67–80.

Winkler, D. W., Dunn, P. O., & McCulloch, C. E. (2002). Predicting the effects of climate change on avian life-history traits. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(21), 13595–9. doi:10.1073/pnas.212251999

Yano, A., Guyomard, R., Nicol, B., Jouanno, E., Quillet, E., Klopp, C.& Guiguen, Y. (2012). An immune-related gene evolved into the master sex-determining gene in rainbow trout, Oncorhynchus mykiss. *Current Biology : CB*, *22*(15), 1423–8. doi:10.1016/j.cub.2012.05.045

Yano, A., Nicol, B., Jouanno, E., Quillet, E., Fostier, A., Guyomard, R., & Guiguen, Y. (2013). The sexually dimorphic on the Y-chromosome gene (sdY) is a conserved male-specific Y-chromosome sequence in many salmonids. *Evolutionary Applications*, *6*(3), 486–96. doi:10.1111/eva.12032

SUPPLEMENTARY INFORMATIONS

Supplementary Information 1:

A publication accepted in Comparative Physiology and Biochemistry (Part A).

The concentration of plasma metabolites varies throughout reproduction and affects offspring number in wild brown trout (Salmo trutta)

Zoé Gauthey[a,d], Marine Freychet[a,d], Aurélie Manicki[a,d], Alexandre Herman[b], Olivier Lepais[a,d,] Stéphane Panserat[b], Arturo Elosegi[c], Cédric Tentelier[a,d] & Jacques Labonne[a,d*]

[a] INRA, UMR 1224, Ecologie Comportementale et Biologie des Populations de Poissons, Aquapôle, quartier Ibarron, 64310, Saint-Pée sur Nivelle, France.

[b] INRA, UR 107, Nutrition Metabolism Aquaculture, Aquapôle, 64310, Saint Pée sur Nivelle, France.

[c] Faculty of Science and Technology, University of the Basque Country UPV/EHU, 48080 Bilbao, Spain.

[d] Univ Pau & Pays Adour, UMR 1224, Ecologie Comportementale et Biologie des Populations de Poissons, UFR Sciences et Techniques de la Côte Basque, Allée du parc Montaury, 64600, Anglet, France.

# These authors contributed equally to the study

Running title: plasma metabolites and reproductive investment in trout

Corresponding author:

Jacques Labonne

INRA, Laboratoire Ecobiop

Quartier Ibarron

64310, Saint-Pée sur Nivelle, FRANCE

Tel: +33 5 59 51 59 76

Email: labonne@st-pee.inra.fr

Abstract

In wild populations, measuring energy invested in the reproduction and disentangling investment in gametes versus investment in reproductive behaviour (such as intrasexual competition or intersexual preference) remains challenging. In this study, we investigated the energy expenditure in brown trout reproductive behaviour by using two proxies: variation in weight and variation of plasma metabolites involved in energy production, over the course of reproductive season in a semi natural experimental river. We estimated overall reproductive success using genetic assignment at the end of the reproductive season. Results show that triglycerides and free fatty acids concentrations vary negatively during reproduction, while amino-acids and glucose concentrations remain stable. Decrease in triglycerides and free fatty acids concentrations during reproduction is not related to initial concentrations levels or to weight variation. Both metabolites concentrations variation and weight variation are correlated to the number of offspring produced, which could indicate that gametic and behavioural reproductive investments substantially contribute to reproductive success in wild brown trout. This study opens a path to further investigate variations in reproductive investment in wild populations.

Key words : plasma metabolites, reproductive investment, reproductive success, metabolic status, salmonid.

1. Introduction

Reproductive effort, or the amount of energy invested by an individual in reproduction, can be partitioned in three essential functions: production of gametes, intra-sexual competition, and parental care (Williams, 1966). While the study of these functions are of interest to understand how individuals adapt to their environment and maximize their number of offspring (Clutton-Brock, 1998), it remains a challenge to measure and partition reproductive effort between these functions in natural populations. Indeed, the cost of reproduction has been estimated in multiple ways, including gamete production (Vézina and Williams, 2005; Hayward and Gillooly, 2011), hormonal regulation, immune functions, proteins, and defense against stress and toxicity (Harshman and Zera, 2007). Another widely-used surrogate for energy expenditure is the decrease of weight or condition index during the reproduction (Schulte-Hostedde et al., 2001; Stevenson and Woods, 2006). However, in many taxa such as fish, insect or bird, weight loss during reproduction often results from the combined effect of gamete release and other functions such as breeding behaviors, making it too integrative an estimate of reproductive effort. Finally other methods to measure energy loss have also described the loss of energy in relation with breeding behaviors (Anderson and Fedak, 1985; Hendry and Beall, 2004; Murchie et al., 2010) but these methods are often either lethal or highly invasive and thus cannot be implemented in wild populations.

A key possibility to tackle these problems is to turn to plasma metabolites involved in energy production through catabolism (i.e. glucose, triglycerides, free fatty acids and amino acids). On the one hand plasma metabolites are based on simple blood tests and therefore could be used as a non-lethal method and can inform on the other hand about the energetic status of individuals. When the energy obtained through food by an animal

surpasses the metabolic expenditures, excess energy is stored in form of lipids in adipose tissues and in muscles. On the contrary, when the levels of energy readily available fall, lipids such as triglycerides are released into blood and will be later degraded into fatty acids in an energy-yielding process called muscle lipolysis (Sargent et al., 2002). Then, when energy is required, plasma triglyceride concentration globally decreases steadily (Kakisawa et al., 1995) whereas plasma free fatty acids first show a plasma peak before decreasing (McCue, 2010). High muscle proteolysis denotes a poor metabolic condition of individuals since they consume amino acids when fat reserves are spent. Finally, depending on the species, glucose may be also used to produce energy when it is readily available from food. In other species such as in fishes, it is secondarily produced via neoglycogenesis and used to maintain brain activity. Therefore, plasma metabolites can be analyzed to infer energy expended by organisms in period of intense activity such as reproduction. For instance, the concentration of triglycerides in plasma is a good indicator of bird health and reproductive success (Merilä and Svensson, 1995; Masello and Quillfeldt, 2004).

In species in which gametogenesis occurs before the reproductive season, the measure of plasma metabolites during the reproduction should not account for gametic investment thereby giving information on behavioral investment especially in species that do not feed during the reproductive season. Assuming that reproductive effort involves costs, there should be a correlation between i) the proportion of energy invested in breeding behaviors relative to the initial amount of energy stored at the onset of the reproduction, ii) the metabolic status of individuals at the onset of the reproduction and iii) the benefit as measured by the number of offspring produced.

To explore the possible relationship between variation in metabolite consumption over reproductive season and access to fertilization and number of offspring, we turned to the

brown trout, an iteroparous species belonging to the salmonid family. In sedentary brown trout, females start vitellogenesis in summer whereas the reproductive season occurs in December-January (Tyler, 1990; Estay et al., 1998). During this period, females compete for spawning sites and dig nests in gravel bars to protect eggs against predation, whereas males display intense and fierce agonistic behavior with conspecifics to gain access to sexual partners (Schroder, 1981; Beall and Marty, 1983; Berg et al., 1998; Gaudemar and Beall, 1998; Garcia-Vazquez et al., 2001; Esteve, 2005) or to provide paternal care (Tentelier et al., 2011). As these behavioral activities differ between sexes and are presumed to have a high energetic cost, and because feeding resources are rare at that period (Rincón and Lobón-Cerviá 1997), the metabolic status at the onset of the breeding season and the variation of metabolites concentrations can potentially differ between sexes and are expected to play an important role on offspring production.

To explore the above mentioned correlations between the proportion of energy invested in breeding behaviors, the metabolic status at the onset of the reproduction and the number of offspring produced, we set up a spawning experiment using wild brown trout in a semi-natural channel. We combined weight and plasma metabolite measurements at the onset and at the end of the spawning period, and genetic assignment of fry subsequently emerging from the channel to assess reproductive success. This allowed 1) describing both the initial energetic status of spawners and its variation over the spawning season (measures of triglycerides, free fatty acids, amino-acids and glucose) and 2) relating these variables to individual reproductive success.

2. Material and methods

2.1. Study site and fish measurements

The study was conducted from December 2010 to the end of March 2011 in an experimental channel beside Lapitxuri Stream, a tributary to the Nivelle River in south-western France (+43° 16' 59", -1° 28' 54") (Gaudemar et al., 2000). This experimental channel, fed with natural river water has already been used for many experiments (Hendry and Beall, 2004). Five communicating and linear sections were used during the experiment, each measuring 10 meters long and 2.80 meters wide. Traps were placed downstream of the fifth section to catch drifting individuals, and upstream movement was prevented. The stream bed was covered with coarse gravel (1-2 cm diameter). Each section provided a spawning ground in its upstream part with a mean depth of 15 cm, as well as a shelter area with visual obstacles (mean depth of 30 cm). Wild brown trout (29 females and 20 males ranging in size from 18 cm to 38 cm) were sampled by electrofishing in the River Bastan (+43° 16' 2.51", -1° 22' 32.46") in November and transferred to the experimental channel. More females than males were selected for this experiment in order to maximize the number of reproductions during the reproductive season. However, because females reproduce at different times, the operational sex ratio was comparable to natural conditions. All fish were diagnosed as mature to semi-mature with different maturity degrees for females. To do so, abdomen of each individual was gently squeezed and males releasing sperm and females carrying eggs were selected. Males and females were mixed and acclimatized all together during 48h in tanks without food, then individually anesthetized (0.3mL/L of 2-phenoxyethanol), measured, weighed and photographed for individual recognition. No tagging of any sort was used to avoid interference with either survival or behavior, because salmonids in general and trout in particular trout are thought to use visual cues in both intrasexual competition and mate

choice (Takeuchi et al., 1987; Gil et al., in press). Fish recognition was possible from inter-individual phenotypic variation: the density and the position of both black and red spots vary consistently from one individual to another and does not change during over the time in reproductive season (Appendix A). A blood sample (500 µL) was taken from the caudal vein of each individual with a disposable heparinized syringe. Fish were then maintained in tanks for 24 hours before being released in the experimental channel. Because the experimental channel is a derivation from a natural river, natural food such as macro-invertebrates or snails is readily available by drift from incoming water. All fish were free to move between sections, and downstream traps were checked every morning in order to release fish that moved downstream back into the experimental sections. Few fish were found in the traps during the first week of the experiment, and this process ceased afterwards (note that spawners could leave the trap to return into the experimental sections without much difficulty).

After the last reproduction (14th of January 2011), trout were removed from the experimental channel, anesthetized, identified (from pictures taken before reproduction), measured, weighed, a blood sample was taken and a small piece of their caudal fin was cut and placed in absolute ethanol for genetic analysis. Fish were stripped to assess if there was any remaining eggs or sperm. They were then kept in a tank and released 48 hours later in their original river. After the removal of adults, traps were checked every day to capture the emergent juveniles (Argent and Flebbe, 1999). At the end of the experiment (800 degree.days and about two months after the last reproduction), all remaining juveniles were captured by electrofishing. A subsample of the total juveniles was kept for the genetic analysis: 20 individuals were taken randomly each day from the traps irrespective of the number of juveniles trapped and 20% of the electrofished individuals were kept randomly. Therefore 1088 juveniles (689 from traps, 399

electrofished) were subsampled for parentage analysis which represents about 35% of the total number of juveniles. Bigger juveniles were sampled for a piece of caudal fin after being anesthetized. Other juveniles were killed with a lethal dose of 2-phenoxyethanol and placed individually in a tube of absolute ethanol (90°). The remaining juveniles were released in the River Bastan.

2.2. Ethics Statement

Our experiment involved capture of fish by classical electrofishing methods that are well handled by our lab (Gosset et al., 1971) (authorization N°2010-252-16 provided by 'Direction Départementale des Territoires et de la Mer', 'Association des Pêcheurs Riverains de la Nive' and 'Office National de l'Eau et des Milieux Aquatiques'). The experimental channel was covered with nets to prevent attacks from birds and to hide from experimenter passages. The experiment did not require any tagging, that may affect behavior, growth, or sexual recognition: we only relied on individual variation in the number and position of red and black spots to achieve individual recognition when required. During the experiment, an extra adult fish accidentally entered the channel from outside and another could not be recovered and has probably escaped from the experimental channel. These two fish were included in the different statistical analyses and for the reproductive success assessment. All adults were released in their river of origin, and a large part of their progeny was also released there. A part of the juveniles were killed with a lethal dose of 2-phenoxyethanol and placed individually in a tube of absolute ethanol (90°). As described in results, the physiological status of fish (external aspect, metabolic concentration of amino-acids and especially glucose) indicated that they

were not particularly stressed compared to data obtained from captive individuals (López-Patiño et al., 2014). From a behavioral point of view, fish were initially very shy, with no reproductive activity, for the first two weeks. Then reproductive activity began and nearly all fish spawned. We did not approach any animal ethics committee or equivalent committee prior to the beginning of our study. Indeed, no competent animal ethics committee was constituted in our country at the time of the experiment (2010) but our experimental procedures fully comply with our institute's ethical rules as well as with French laws.

2.3. Measurement of metabolites in plasma

Blood samples were centrifuged for 5 min at 3500 rpm, 300 µl of plasma were removed and placed in a new tube, and immediately frozen at -20°C and then at -80°C. As previously measured in other studies (Panserat et al., 2002; Kamalam et al., 2012), the concentration of plasma glucose (Glucose RTU™ kit, bioMérieux, Marcy l'Etoile, France), triglycerides (Sobioda kit, bioMérieux) and free fatty acids (NEFA HR kit, Wako Chemicals, Neuss, Germany) were determined using commercial kits adapted to a microplate format. Total plasma free amino acid levels were determined by the ninhydrin reaction (Moore, 1968), with glycine as standard. The metabolite concentration for each adult was measured in $g.l^{-1}$ before and after the reproduction. For three individuals, some concentrations could not be performed satisfactorily due to insufficient blood sample volume. They were thus excluded from the statistical analyses.

2.4. Genetic analysis and parentage assignment

DNA was extracted using a NaCl / chloroform method (see detailed protocol in Appendix B) and eight microsatellites previously developed for salmonids were selected: *Str60INRA*; *Str73INRA* (Estoup et al., 1993); *SsoSL438 and SsoSL417* (Slettan et al., 1995)*; Ssa85* (O'Reilly et al., 1996)*; SSsp2216* (Paterson et al., 2004); *Ssa410Uos* and *Ssa408Uos* (Cairney et al., 2000). We used a multiplex protocol allowing amplification of the eight loci in one polymerase chain reaction (multiplex PCR) following (Lerceteau-Köhler and Weiss, 2006). Fragments were sized on a ABI 3100-Avant (Life Technologies) using a GeneScan 500 LIZ internal size standard (Life Technologies), scored using STRand software (Toonen and Hughes, 2001) and raw allele sizes were binned into discrete allele classes using MSatAllele package (Alberto, 2009) for R version 2.13.0 (R Development Core Team 2011). Parentage analysis was performed using Cervus software (version 3.0.3, Kalinowski, 2002) to assign parents to each sampled offspring, using allele frequencies computed from the genotypes of the candidate parents. The following simulation parameters were used: 10 000 cycles, 29 candidate mothers and 20 candidate fathers, a mistyping error rate of 1%, a genotyping error rate of 1%. We used the "parents pair analysis, sexes known" option in Cervus to assign juveniles to parents. All juveniles with more than one locus missing were removed from the analysis. We accepted parentage assignment at confidence level of 80% and only when the juvenile was assigned to two parents. Hardy Weinberg equilibrium and linkage disequilibrium between loci were tested using Genepop 4.2 (Rousset, 2008) with Bonferroni correction for multiple comparisons.

2.5. Variations of weight and plasma metabolites concentrations

Weight and metabolites variations during the reproduction were studied using a Kruskal-Wallis rank sum test to compare 1) the initial concentrations of glucose, triglycerides, free fatty acids and amino acids (respectively hereafter termed as IG, IT, IFFA, IAA) between sexes 2) the final concentrations of glucose, triglycerides, free fatty acids and amino acids between sexes, 3) the average weight variations between males and females, 4) the relative variation of the four metabolites between males and females. Because individuals enter in the reproduction with different initial metabolite concentrations, we compared their relative variations instead of the absolute loss of metabolites. The relative variation of triglycerides, free fatty acids, glucose and amino acids (respectively RVG, RVT, RVFA and RVAA) were calculated as the difference of the plasma concentration before the reproduction to plasma concentration after the reproduction, divided by the plasma concentration before the reproduction. Hence, when the variation was positive, the level of plasma metabolites decreased during the reproductive season whereas it increased when the variation was negative. The relative variation of weight (RVW) was calculated in the same way.

2.6. Statistical Analysis

A Kruskal-Wallis rank sum test was also used to compare the number of mates found in the offspring between males and females. A Spearman correlation test was performed to assess the relationship between initial concentration and relative variation of plasma metabolites. Additionally, a correction by the mean was also applied to assess the level of

correlation using the method described by Kelly and Price (2005). A Spearman correlation test was applied to assess the relationship between the relative variation of weight and the different variations of metabolites: RVG, RVT, RVFA and RVAA.

To obtain a synthetic indicator of metabolites data variation, we performed a scaled principal component analysis (PCA) using the initial concentration and the relative variation of each metabolite (function prcomp, R software) on all individuals. The scores of individuals on the first two axes of the PCA (A1, A2) were kept as synthetic indicators of their metabolic profiles during reproduction season. We then fitted a negative binomial regression model with a log link function (package MASS, R software) to infer the effect of A1, A2, initial weight (IW) and relative weight variation (RVW) on offspring number N as follows:

$$log(N_i) = \beta_0 + \beta_1 \times A1_i + \beta_2 \times A2_i + \beta_3 \times IW_i + \beta_4 \times RVW_i + \beta_5 \times A1_i \times IW_i + \beta_6 \times A2_i \times IW_i + \beta_7 \times IW_i \times RVW_i + \beta_8 \times A1_i \times RVW_i + \beta_9 \times A2_i \times RVW_i + \beta_{10} \times A1_i \times IW_i \times RVW_i + \beta_{11} \times A2_i \times IW_i \times RVW_i$$

With (β_0, β_1, …, β_{11}) the parameters to estimate. The negative binomial model was chosen to account for overdispersed variance in reproductive success data that prevents to use the Poisson regression model usually adapted to count data. The interaction between A1 and A2 was previously tested and yielded no effect, it is therefore not presented for the sake of simplicity. An analysis of deviance table using a χ^2 test was applied to assess the statistical significance of each parameter.

3. Results

3.1. Variation in weight and metabolites

In the experiment, males were heavier than females both at the onset (χ^2= 7.74, df = 1, p = 0.005) and at the end of the reproductive season (χ^2= 11.62, df = 1, p = 0.0007). With respect to their initial weight, females generally did lose more weight than males (Table 1). Initial concentrations of glucose did not differ between males and females (average 1.02 and 0.95 g/L respectively, Table 1) (raw data are given in Appendix C). On the other hand, there were important differences in initial concentrations in triglycerides, free fatty acids and amino acids between males and females. Females started reproduction with higher concentrations of triglycerides and free fatty acids than males whereas males presented higher concentrations of amino acids (Table 1). The variations in metabolite concentration during the reproductive season did not differ between males and females for glucose, triglycerides and free fatty acids (Table 1). However, the levels of amino acids in plasma decreased faster in males than in females. During the experiment, small reductions were detected in the concentrations of glucose (8.7% for males and 0% for females) and amino acids (11.3% for males and -0.1% for females). In contrast, the concentration of triglycerides fell by 50% in both sexes, and that of free fatty acids by 60%.

Regarding the relationship between initial concentration and relative variation of plasma metabolites during the reproductive season, we found positive significant correlations for glucose (df = 45; $p < 0.0001$; r = 0.64) and amino-acids (df = 45; $p < 0.0001$; r = 0.75) while no significant correlations were found for free fatty acids (df = 45; p = 0.269; r = 0.16) and triglycerides (df = 44; p = 0.182; r = 0.2). However, when accounting for a possible regression to the mean bias that is usually present when dealing with such type of data (initial values and relative change with possible measurement error, see Kelly and Price 2005), it appears that only plasma triglycerides and free fatty acids show a positive

relationship between initial and final values (respectively, df = 44; $p < 0.001$; $r = 0.48$; df = 45; $p < 0.001$; $r = 0.39$) whereas amino acids and glucose do not show any effect (respectively, df = 45; $p = 0.98$; $r = 0.002$ and df = 45; $p = 0.098$; $r = 0.75$). We therefore conclude that for lipids, the higher the initial concentration, the higher the decrease in concentration throughout reproductive season.

No significant correlation was found between the relative variation of weight and each of these variations of plasma metabolites levels: glucose (df = 45; $p= 0.80$; $r = 0.04$), amino acids (df = 45; $p = 0.82$; $r = 0.03$), free fatty acids (df = 45; $p = 0.34$; $r = 0.14$) and triglycerides (df = 44; $p = 0.89$; $r = -0.02$).

3.2. Reproductive success estimation

A total of 1137 individuals (49 parents and 1088 juveniles) were genotyped for parentage analysis. Among the 1088 offspring collected, 61 were discarded from the assignment analysis: either poor quality of amplification, missing extraction due to poor DNA conservation, or more than one locus missing. Among the remaining 1027 individuals, 983 were assigned both parents whereas 44 were assigned only one parent (Appendix D). There was no genotypic linkage disequilibrium for the eight loci. Seven loci out of eight were at the Hardy Weinberg equilibrium. We found a small deficit of heterozygotes for the locus Ssa410Uos. Parentage analysis revealed that the total number of offspring varied between 0 and 258 offspring (mean = 49.15, sd = 76.25) for males and between 0 and 163 (mean =33.90, sd =41.58) for females. Only one male and one female had no offspring at all. Four males showed a high total reproductive success with more than 100 offspring. The number of mates for females ranged from 0 to 8 (mean =3.1, sd =1.83) whereas it ranged from 0 to 10 (mean =4.5, sd =3.5) for males (Table 1). Only one

female was still ovigerous at the end of the experiment and thus did not reproduce, and five other were not totally spent (few eggs remaining). Five males had not released all their sperm at the end of the reproduction.

3.3. Effect of metabolic profiles and weight on offspring number

The results of the principal component analysis oppose on the two first axes (37.7% of the total data variance) variation in amino acids on the one hand (axis A1), and triglycerides and free fatty acids on the other hand (axis A2, Fig. 1). Variation in glucose is expressed on both axes. Individuals having a high A1 score entered the reproduction season with high concentrations of amino-acids and showed an important decrease in the level of circulating plasma amino acids between the beginning and the end of the reproductive period. These individuals were generally males (Fig. 1). Individuals having a high A2 score showed an important decrease of their circulating plasma triglycerides and free fatty acids levels between the beginning and the end of the reproductive period.

The results of the negative binomial regression model indicated that the first axis A1 of the principal component analysis was not related to offspring number, while the second PCA axis A2 had a statistically very significant effect on offspring number ($p < 0.00001$). Relative weight variation also had a significant effect on offspring number ($p=0.03513$). Finally, we detected a significant interaction between initial weight and A2 ($p=0.00212$). The first PCA axis A1 had no significant effect on offspring number as well as interactions between A1 and initial weight, A2 and relative weight variation, and initial weight and relative weight variation but all ps were inferior to 0.1. Triple interactions had no significant effect with ps superior to 0.2.

Based on this model, an important loss of weight was associated to a high number of offspring (Fig. 2). The effect of high individual scores on A2 was translated also into a higher number of offspring, also depending on initial weight, but it could be overridden when relative variation of weight was too small. In that case only the initial weight positively affected the number of offspring.

4. Discussion

Our study uncovers three important novel results: i) the plasma concentration of two metabolites - triglycerides and free fatty acids - involved in ATP production shows a general decrease for all spawners ii) this decrease is uncorrelated to weight loss iii) the variation of plasma metabolites concentration strongly affects the number of offspring produced, displaying an even more important effect than weight loss.

4.1. Plasma metabolites variations during reproductive season

During our study we found no significant correlation between the variation of weight and variation of metabolites concentration, hinting that metabolites variations during spawning period are probably not strongly associated with gametes release, which is one of the major determinants of weight variation in salmonids. As previously proposed for other biological systems (Masello and Quillfeldt, 2004; Kilgas et al., 2006) this finding supports the general idea that variation in metabolites concentrations is actually a useful proxy for measuring energy investment in reproductive behavior.

The initial observed concentrations of the four metabolites were within the range of values shown by several studies in fasted rainbow trout (Panserat et al., 2002; Seiliez et al., 2012; Kamalam et al., 2012; Skiba-Cassy et al., 2013). We observed differences in the initial metabolite concentrations between males and females at the onset of the reproduction. Females showed a higher lipid concentration for both triglycerides and free fatty acids. Previous studies of several salmon species showed that males arrive earlier than females on average in the spawning grounds (Pritchard, 1937; Gosset et al., 2006). The mate opportunity hypothesis proposes that males who arrive first will increase their mating opportunity (Morbey, 2000). In our case, it is possible that we missed a part of male competition because it started before our experiment, males may have already consumed their lipid reserves (i.e. free fatty acids).

In many species, food intake is reduced during the reproduction because animals allocate preferentially their time and energy to different activities such as looking for mates or territorial defense (Doucett et al.,; Cherel et al., 1988; Anderson and Karasov, 1988; Barboza and Jorde, 2001; Esteve, 2005). Thus, during reproduction the energetic demand is expected to be high whereas feeding is reduced. We therefore expected a decrease of triglycerides, but also a potential variation of amino acids and glucose if individuals are in distress at the end of the reproduction. Our goal was to study the trade-off between energy investment and stress conditions of individuals to understand at which point reproduction is costly. The results give insights on several aspects of trout performance. Firstly, there were no significant variations in glucose level during the experiment neither for males nor for females. Glucose is absent from food intake, but neoglucogenesis is especially used for maintenance activity or during long periods of food deprivation (Wilson, 1994; Hemre et al., 2002; Stone 2003; Enes et al., 2009), and as such, variation in glucose is related to long term stress in fish (Silbergeld, 1974). Hence, the steady plasma concentration of

glucose in our experiment indicates that individuals did not suffer from stress. Secondly, plasma amino acids did not vary a lot from their initial concentration which indicates that our fish had not engaged proteolysis by the end of the reproduction. Therefore, it seems that energy invested in reproductive effort does not impact individuals' immediate survival since almost all individuals reproduced without showing any sign of distress at the end of the reproduction. However, a high variation was observed in some females especially in the relative variation of amino acids. Salem *et al.* (2006) have described that during spawning, rainbow trout females could show a high proteolysis. Hence, asynchronous spawning in our experiment could explain this variation in amino acids loss between females.

During reproduction, the concentration of triglycerides fell by 50% on average and that of free fatty acids by 60%. Therefore, despite some scarce food availability and potential oophagy (Aymes et al., 2010), lipid reserves seem to be actively utilized during reproduction. It is well known that investment in reproduction can affect lifetime reproductive success in individuals (Williams, 1966; Stearns, 1992). Consequently, an actual measure of the trade-off between current investment and future survival and reproduction opportunities (as both expected by theory and shown for many species) would require monitoring the individuals over a long period with knowledge of their reproductive investment. This could now potentially be undertaken in wild populations since the plasma metabolites are accessible via simple blood samples and fish can be released afterward.

4.2. Do metabolites and weight variations affect offspring number?

We found that relative weight variation increased the number of offspring. This result seems logical since weight loss strongly correlates with the number of gametes released (Healey and Prince, 1998), which is in direct link with fertility in females and with the outcome of sperm competition in males (Parker, 1982). While metabolite level variation was uncorrelated to weight variation, metabolite level variation displayed an even greater effect on the measure of the number of offspring. In salmonids, fierce competition between males to get access to females is the norm (Fleming and Gross, 1994; Höjesjö et al., 2004). Additionally, paternal care in brown trout occurs when egg cannibalism pressure is high (Aymes et al., 2010; Tentelier et al., 2011). Likewise, an efficient nest digging by females is expected to provide benefits such as protection against predators and environmental stochasticity (Tappel and Bjornn, 1983; Fukushima et al., 1998; Møller and Jennions, 2001). We also investigated further if sex could influence this trade-off between energy invested and offspring number produced. However, whereas some differences between sexes were reflected on the A1 axis of the PCA, this same axis had no effect on offspring number produced, nor were the residuals of the model dependent on sex. Additionally, we also fitted a more complex model (not presented here) accounting for the effect of sex in interaction with the other factors, but this model presented a poorer fit to the data (as measured by information criterion).

All these behavioural activities related to reproduction can be costly and this cost appears to be well reflected by variations of plasma metabolites such as triglycerides and free fatty acids.

This is exemplified by the fact that metabolic profiles, as synthesized by the A2 individual scores, interact with initial weight. There is therefore a synergistic effect of initial weight and metabolites levels variations, especially when weight loss over the reproduction period is important: initial weight can be for instance a good proxy of

intrasexual competitive ability (Jacob et al., 2007), but it is efficient only if one invests in both gametes production *and* active behavior such as agonistic interactions or parental care. This finding implies that gametic investment, as approached by weight variation, cannot be used as the sole measure of reproductive investment and that it is possible in wild fish to efficiently complete the picture by a proxy of behavioural investment in reproduction, as approached by metabolites variation.

5. Conclusion

The present study demonstrates that variations of plasma metabolites such as triglycerides and free fatty acids affect reproductive success of wild brown trout. The measure of plasma metabolites only requires blood samples and therefore allows monitoring wild populations for behavioural reproductive investment in relationship with gametic investment as approached by weight variation. Our results also confirm a crucial link often evoked but rarely demonstrated in sexual selection between energy invested and number of offspring produced: in brown trout, energy investment over the reproduction season is on average rewarded by increased reproductive success.

Acknowledgments

We thank the guards of APRN (‘Association des Pêcheurs Riverains de la Nive’) for allowing us to capture and use genitors of Bastan river (authorization N°2010-252-16). We are grateful to J.C Aymes, F. Guéraud and S. Glise for providing us help and

assistance during the field study. This study was funded thanks to a BQR grant from the Université de Pau et des Pays de l'Adour.

References

Alberto, F., 2009. MsatAllele_1.0: An R package to visualize the binning of microsatellite alleles. J. Hered. 100, 394–7. doi:10.1093/jhered/esn110

Anderson, R.A., Karasov, W.H., 1988. Energetics of the Lizard Cnemidophorus Tigris and Life History Consequences of Food-Acquisition Mode. Ecol. Monogr. 58, 79. doi:10.2307/1942462

Anderson, S.S., Fedak, M.A., 1985. Grey seal males: energetic and behavioural links between size and sexual success. Anim. Behav. 33, 829–838. doi:10.1016/S0003-3472(85)80017-8

Argent, D.G., Flebbe, P.A., 1999. Fine sediment effects on brook trout eggs in laboratory streams. Fish. Res. 39, 253–262. doi:10.1016/S0165-7836(98)00188-X

Aymes, J.-C., Larrieu, M., Tentelier, C., Labonne, J., 2010. Occurrence and variation of egg cannibalism in brown trout Salmo trutta. Naturwissenschaften 97, 435–9. doi:10.1007/s00114-010-0656-0

Barboza, P.S., Jorde, D.G., 2001. Intermittent feeding in a migratory omnivore: digestion and body composition of American black duck during autumn. Physiol. Biochem. Zool. 74, 307–17. doi:10.1086/319658

Beall, E., Marty, C., 1983. Reproduction du saumon atlantique , Salmo salar L. En milieu semi-naturel contrôlé. Bull. Français Piscic. 289, 77–93.

Berg, O.K., Throanes, E., Bremset, G., 1998. Energetics and survival of virgin and repeat spawning brown trout (Salmo trutta). Can. J. Fish. Aquat. Sci. 55, 47–53.

Cairney, M., Taggart, J.B., HOyheim, B., 2000. Characterization of microsatellite and minisatellite loci in Atlantic salmon (Salmo salar L.) and cross-species amplification in other salmonids. Mol. Ecol. 9, 2175–2178. doi:10.1046/j.1365-294X.2000.105312.x

Cherel, Y., Robin, J.P., Walch, O., Karmann, H., Netchitailo, P., Le Maho, Y., 1988. Fasting in king penguin. I. Hormonal and metabolic changes during breeding. Am J Physiol Regul. Integr. Comp Physiol 254, R170–177.

Clutton-Brock, T.H., 1988. Reproductive Success: studies of individual variation in contrasting breeding systems, University. ed. Chicago.

Doucett, R.R., Booth, R.K., Power, G., Mckinley, R.S., n.d. Effects of the spawning migration on the nutritional status of anadromous Atlantic salmon (Salmo salar) : insights from stable-isotope analysis. Can. J. Fish. Aquat. Sci. 56, 2172–2180.

Enes, P., Panserat, S., Kaushik, S., Oliva-Teles, A., 2009. Nutritional regulation of hepatic glucose metabolism in fish. Fish Physiol. Biochem. 35, 519–39. doi:10.1007/s10695-008-9259-5

Estay, F., Neira, R., Diaz, N.F., Valladares, L., Torres, A., 1998. Gametogenesis and sex steroid profiles in cultured coho salmon (Oncorhynchus kisutch, Walbaum). J. Exp. Zool. 280, 429–438. doi:10.1002/(SICI)1097-010X(19980415)280:6<429::AID-JEZ7>3.0.CO;2-P

Esteve, M., 2005. Observations of Spawning Behaviour in Salmoninae: Salmo, Oncorhynchus and Salvelinus. Rev. Fish Biol. Fish. 15, 1–21. doi:10.1007/s11160-005-7434-7

Estoup, A., Presa, P., Krieg, F., Vaiman, D., Guyomard, R., 1993. (CT)n and (GT)n microsatellites: a new class of genetic markers for Salmo trutta L. (brown trout). Heredity (Edinb). 71, 488–496. doi:10.1038/hdy.1993.167

Fleming, I.A., Gross, M.R., 1994. Breeding competition in a pacifis salmon (Coho: Onchorhynchus Kisutch): measures of natural and sexual selection. Evolution (N. Y). 48, 637–657.

Fukushima, M., Quinn, T.J., Smoker, W.W., 1998. Estimation of eggs lost from superimposed pink salmon (Oncorhynchus gorbuscha) redds. J. Can. des Sci. halieutiques Aquat. 55, 618–625.

Garcia-Vazquez, E., Moran, P., Martinez, J.L., Perez, J., de Gaudemar, B., Beall, E., 2001. Alternative mating strategies in Atlantic salmon and brown trout. J. Hered. 92, 146–9.

Gaudemar, B., Beall, E., 1998. Effects of overripening on spawning behaviour and reproductive success of Atlantic salmon females spawning in. J. Fish Biol. 33, 434–446.

Gaudemar, B. de, Schroder, S.L., Beall, E.P., 2000. Nest placement and egg distribution in Atlantic Salmon redds. Environ. Biol. Fishes 57, 37–47. doi:10.1023/A:1007562508973

Gil, J., Caudron, A., Labonne, J., (in press) Can female preference drive intra-specific diversity dynamics in brown trout (*Salmo trutta*, L.)? Ecol. Freshw. Fish.

Gosset, C., Lamarque, P., Charlon, N., Garaïcoechea, C., 1971. Un nouvel appareil de pêche électrique portable : Le “Martin-Pêcheur”. Bull. Français Piscic. 242, 33–46. doi:10.1051/kmae:1971004

Gosset, C., Rives, J., Labonne, J., 2006. Effect of habitat fragmentation on spawning migration of brown trout (Salmo trutta L.). Ecol. Freshw. Fish 15, 247–254. doi:10.1111/j.1600-0633.2006.00144.x

Harshman, L.G., Zera, A.J., 2007. The cost of reproduction: the devil in the details. Trends Ecol. Evol. 22, 80–6. doi:10.1016/j.tree.2006.10.008

Hayward, A., Gillooly, J.F., 2011. The cost of sex: quantifying energetic investment in gamete production by males and females. PLoS One 6, e16557. doi:10.1371/journal.pone.0016557

Healey, M.C., Prince, A., 1998. Alternative tactics in the breeding behaviour of male Coho Salmon. Behaviour 135, 1099–1124.

Hemre, G.-I., Mommsen, T.P., Krogdahl, A., 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. Aquac. Nutr. 8, 175–194. doi:10.1046/j.1365-2095.2002.00200.x

Hendry, A., Beall, E., 2004. Energy use in spawning Atlantic salmon. Ecol. Freshw. Fish 13, 185–196.

Höjesjö, J., Johnsson, J., Bohlin, T., 2004. Habitat complexity reduces the growth of aggressive and dominant brown trout (Salmo trutta) relative to subordinates. Behav. Ecol. Sociobiol. 56, 286–289. doi:10.1007/s00265-004-0784-7

Jacob, A., Nusslé, S., Britschgi, A., Evanno, G., Müller, R., Wedekind, C., 2007. Male dominance linked to size and age, but not to "good genes" in brown trout (Salmo trutta). BMC Evol. Biol. 7, 207. doi:10.1186/1471-2148-7-207

Kakisawa, S., Kaneko, T., Hasegawa, S., Hirano, T., 1995. Effects of feeding, fasting, background adaptation, acute stress, and exhaustive exercise on the plasma somatolactin concentrations in Rainbow Trout. Gen. Comp. Endocrinol. 98, 137–146.

Kalinowski, S.T., 2002. Evolutionary and statistical properties of three genetic distances. Mol. Ecol. 11, 1263–1273. doi:10.1046/j.1365-294X.2002.01520.x

Kamalam, B.S., Medale, F., Kaushik, S., Polakof, S., Skiba-Cassy, S., Panserat, S., 2012. Regulation of metabolism by dietary carbohydrates in two lines of rainbow trout divergently selected for muscle fat content. J. Exp. Biol. 215, 2567–78. doi:10.1242/jeb.070581

Kelly, C., Price, T.D., 2005. Correcting for regression to the mean in behaviour and ecology. Am. Nat. 166, 700–707.

Kilgas, P., Mänd, R., Mägi, M., Tilgar, V., 2006. Hematological parameters in brood-rearing great tits in relation to habitat, multiple breeding and sex. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 144, 224–231.

Lerceteau-Köhler, E., Weiss, S., 2006. Development of a multiplex PCR microsatellite assay in brown trout Salmo trutta, and its potential application for the genus. Aquaculture 258, 641–645. doi:10.1016/j.aquaculture.2006.04.028

López-Patiño, M.A., Hernández-Pérez, J., Gesto, M., Librán-Pérez, M., Míguez, J.M., Soengas, J.L., 2014. Short-term time course of liver metabolic response to acute handling stress in rainbow trout, Oncorhynchus mykiss. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 168, 40–49.

Martin, T.G., Wintle, B.A., Rhodes, J.R., Kuhnert, P.M., Field, S.A., Low-Choy, S.J., Tyre, A.J., Possingham, H.P., 2005. Zero tolerance ecology: improving ecological inference by modelling the source of zero observations. Ecol. Lett. 8, 1235–46. doi:10.1111/j.1461-0248.2005.00826.x

Masello, J.F., Quillfeldt, P., 2004. Are haematological parameters related to body condition, ornamentation and breeding success in wild burrowing parrots Cyanoliseus patagonus ? J. Avian Biol. 35, 445–454. doi:10.1111/j.0908-8857.2004.03278.x

McCue, M.D., 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 156, 1–18. doi:10.1016/j.cbpa.2010.01.002

Merilä, J., Svensson, E., 1995. Fat reserves and health state in migrant Goldcrest Regulus regulus. Funct. Ecol. 9, 842–848.

Møller, A.P., Jennions, M.D., 2001. How important are direct fitness benefits of sexual selection? Naturwissenschaften 88, 401–415. doi:10.1007/s001140100255

Moore, S., 1968. Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. J. Biol. Chem. 243, 6281–3.

Morbey, Y., 2000. Protandry in Pacific sallmon. Can. J. Fish. Aquat. Sci. 57, 1252–1257.

Murchie, K.J., Cooke, S.J., Danylchuk, A.J., 2010. Seasonal Energetics and Condition of Bonefish from Different Subtropical Tidal Creeks in Eleuthera, the Bahamas. Mar. Coast. Fish. 2, 249–262. doi:10.1577/C09-042.1

O'Reilly, P.T., Hamilton, L.C., McConnell, S.K., Wright, J.M., 1996. Rapid analysis of genetic variation in Atlantic salmon (Salmo salar) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Can. J. Fish. Aquat. Sci. 53, 2292–2298. doi:10.1139/f96-192

Panserat, S., Perrin, A., Kaushik, S., 2002. High dietary lipids induce liver glucose-6-phosphatase expression in rainbow trout (Oncorhynchus mykiss). J. Nutr. 132, 137–141.

Parker, G.A., 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. J. Theor. Biol. 96, 281–294.

Paterson, S., Piertney, S.B., Knox, D., Gilbey, J., Verspoor, E., 2004. Characterization and PCR multiplexing of novel highly variable tetranucleotide Atlantic salmon (Salmo salar L.) microsatellites. Mol. Ecol. Notes 4, 160–162. doi:10.1111/j.1471-8286.2004.00598.x

Pritchard, A.L., 1937. Variation in the Time of Run, Sex Proportions, Size and Egg Content of Adult Pink Salmon (Oncorhynchus gorbuscha) at McClinton Creek, Masset Inlet, B.C. J. Biol. Board Canada 3, 403–416. doi:10.1139/f37-023

Rincón, P.A. and Lobón-Cerviá, J. 1997. Temporal patterns in macroinvertebrate drift in a northern Spanish stream. Marine & Freshwater Research, 48(5), 455-464.

Rousset, F., 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Resour. 8, 103–6. doi:10.1111/j.1471-8286.2007.01931.x

Salem, M., Kenney, P.B., Rexroad, C.E., Yao, J., 2006. Molecular characterization of muscle atrophy and proteolysis associated with spawning in rainbow trout. Comp. Biochem. Physiol. Part D Genomics Proteomics 1, 227–237.

Sargent, J., Tocher, D., Bell, J., 2002. Fish Nutrition, in: Fish Nutrition. pp. 181–257.

Schroder, S.L., 1981. The role of sexual selection in determining the overall mating patterns and mate choice in chum salmon. University of Washington, Seattle.

Schulte-Hostedde, A.I., Millar, J.S., Hickling, G.J., 2001. Evaluating body condition in small mammals. Can. J. Zool. 79, 1021–1029.

Seiliez, I., Gabillard, J.-C., Riflade, M., Sadoul, B., Dias, K., Avérous, J., Tesseraud, S., Skiba, S., Panserat, S., 2012. Amino acids downregulate the expression of several autophagy-related genes in rainbow trout myoblasts. Autophagy 8, 364–75. doi:10.4161/auto.18863

Silbergeld, E.K., 1974. Blood glucose: A sensitive indicator of environmental stress in fish - 10.1007/BF01685023.pdf. Bull. Environ. Contam. Toxicol. 11.

Skiba-Cassy, S., Panserat, S., Larquier, M., Dias, K., Surget, A., Plagnes-Juan, E., Kaushik, S., Seiliez, I., 2013. Apparent low ability of liver and muscle to adapt to variation of dietary carbohydrate:protein ratio in rainbow trout (Oncorhynchus mykiss). Br. J. Nutr. 109, 1359–72. doi:10.1017/S0007114512003352

Slettan, A., Olsaker, I., Lie, Ø., 1995. Atlantic salmon, Salmo salar , microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. Anim. Genet. 26, 281–282. doi:10.1111/j.1365-2052.1995.tb03262.x

Stearns, S., 1992. The evolution of life histories (POD).

Stevenson, R.D., Woods, W.A., 2006. Condition indices for conservation: new uses for evolving tools. Integr. Comp. Biol. 46, 1169–90. doi:10.1093/icb/icl052

Stone, D.A.J., 2003. Dietary Carbohydrate Utilization by Fish. Rev. Fish. Sci. 11, 337–369. doi:10.1080/10641260390260884

Takeuchi, H., Takhi, K., Satou, M., Matsushima, T., Okumoto, N., Ueda, K., 1987. Visual Cues as Key Stimuli for Courtship Behaviour in Male Hime Salmon (Landlocked Red Salmon, *Oncorhynchus nerka*). Anim. Behav.35, 936–939. doi:10.1016/S0003-3472(87)80137-9.

Tappel, P.D., Bjornn, T.C., 1983. A New Method of Relating Size of Spawning Gravel to Salmonid Embryo Survival. North Am. J. Fish. Manag. 3, 123–135. doi:10.1577/1548-8659(1983)3<123:ANMORS>2.0.CO;2

Tentelier, C., Larrieu, M., Aymes, J.-C., Labonne, J., 2011. Male antagonistic behaviour after spawning suggests paternal care in brown trout, Salmo trutta. Ecol. Freshw. Fish 20, 580–587. doi:10.1111/j.1600-0633.2011.00507.x

Toonen, R.J., Hughes, S., 2001. Increased throughput for fragment analysis on an ABI Prism 377 automated sequencer using a 1 membrane comb and STRand software. Biotechniques 31, 1320–1324.

Tyler, C.R., 1990. The dynamics of oocyte growth during vitellogenesis in the rainbow trout (Oncorhynchus mykiss). Biol. Reprod. 43, 202–209. doi:10.1095/biolreprod43.2.202

Vézina, F., Williams, T.D., 2005. The metabolic cost of egg production is repeatable. J. Exp. Biol. 208, 2533–8. doi:10.1242/jeb.01675

Williams, G.C., 1966. Natural selection, the costs of reproduction, and a Refinement of Lack's principle. Am. Nat. 100, 687–690.

Wilson, R.P., 1994. Utilization of dietary carbohydrate by fish. Aquaculture 124, 67–80.

Figures and tables Legends

Fig. 1. Principal component analysis biplot of axes A1 and A2. Arrows represent scores of variables on first and second axes (bottom and left scales respectively) and male and female symbols represent individual scores on first and second axes (top and right scales respectively). The upper right panel presents the eigenvalues ranked by axis number.

Fig. 2. Predictions of the number of offspring produced made from the negative binomial regression model. Predictions are calculated over a [-1.5;1.5] range on the A2 PCA score, as well as for two initial weight (126g, 301g) and two relative variation of weight (5.6%, 14%), representing each time the 25% and 75% quantiles of their observed distribution in our data.

Table 1. Differences between males and females in initial and relative variation of weight and concentrations of metabolites.

Table 2. Analysis of deviance table for the negative binomial regression model. A1 and A2 are the scores on the first and second axes of the principal component analysis respectively, IW is the initial weight and RVW is the relative variation of weight.

Fig. 1

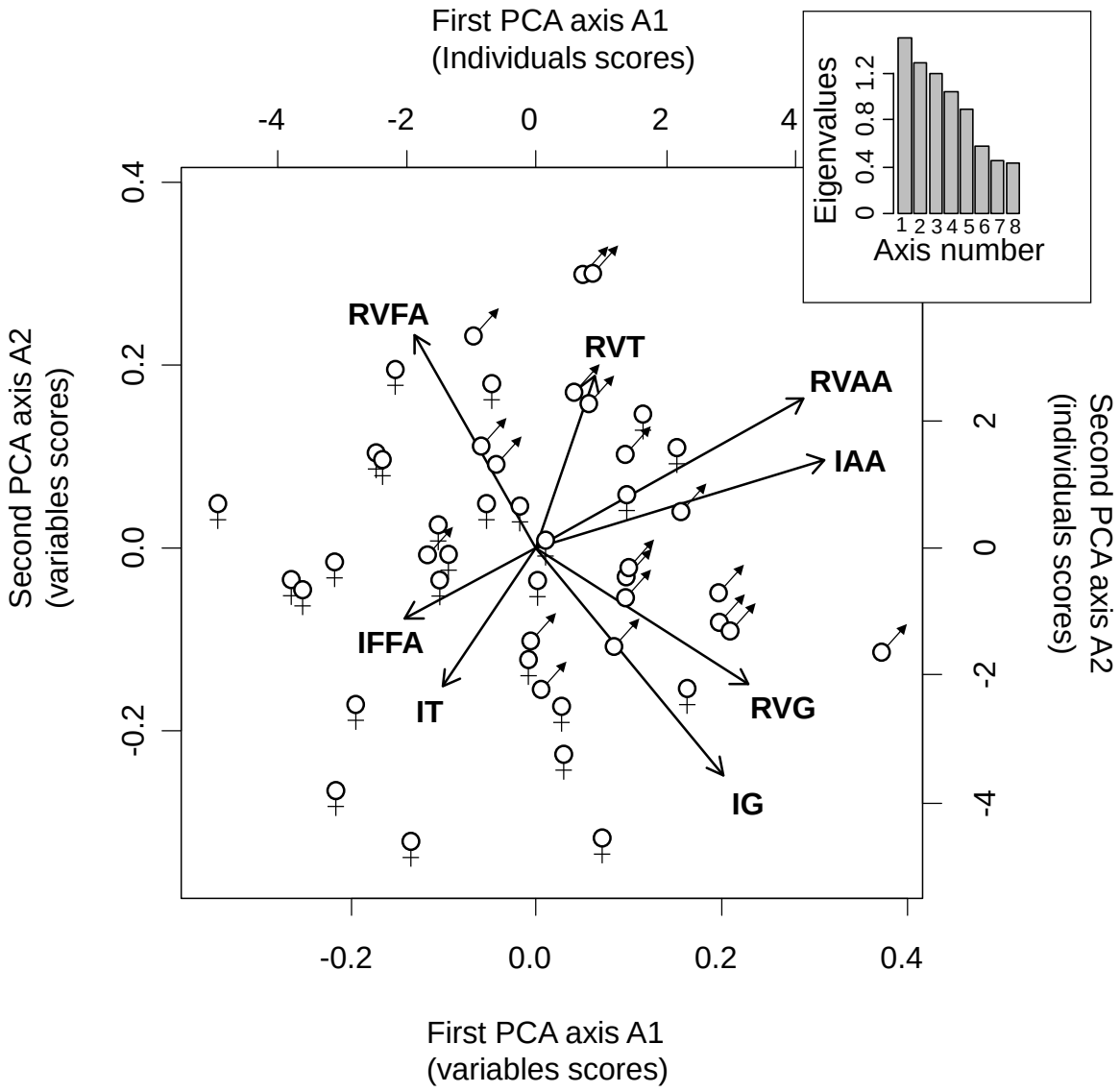

First PCA axis A1
(Individuals scores)
-4
-2
0
2
4
Eigenvalues
0
0.4
0.8
1.2
1 2 3 4 5 6 7 8
Axis number
RVFA
RVT
RVAA
IAA
IFFA
IT
RVG
IG
Second PCA axis A2
(variables scores)
0.4
0.2
0.0
-0.2
Second PCA axis A2
(individuals scores)
2
0
-2
-4
-0.2
0.0
0.2
0.4
First PCA axis A1
(variables scores)

Fig. 2

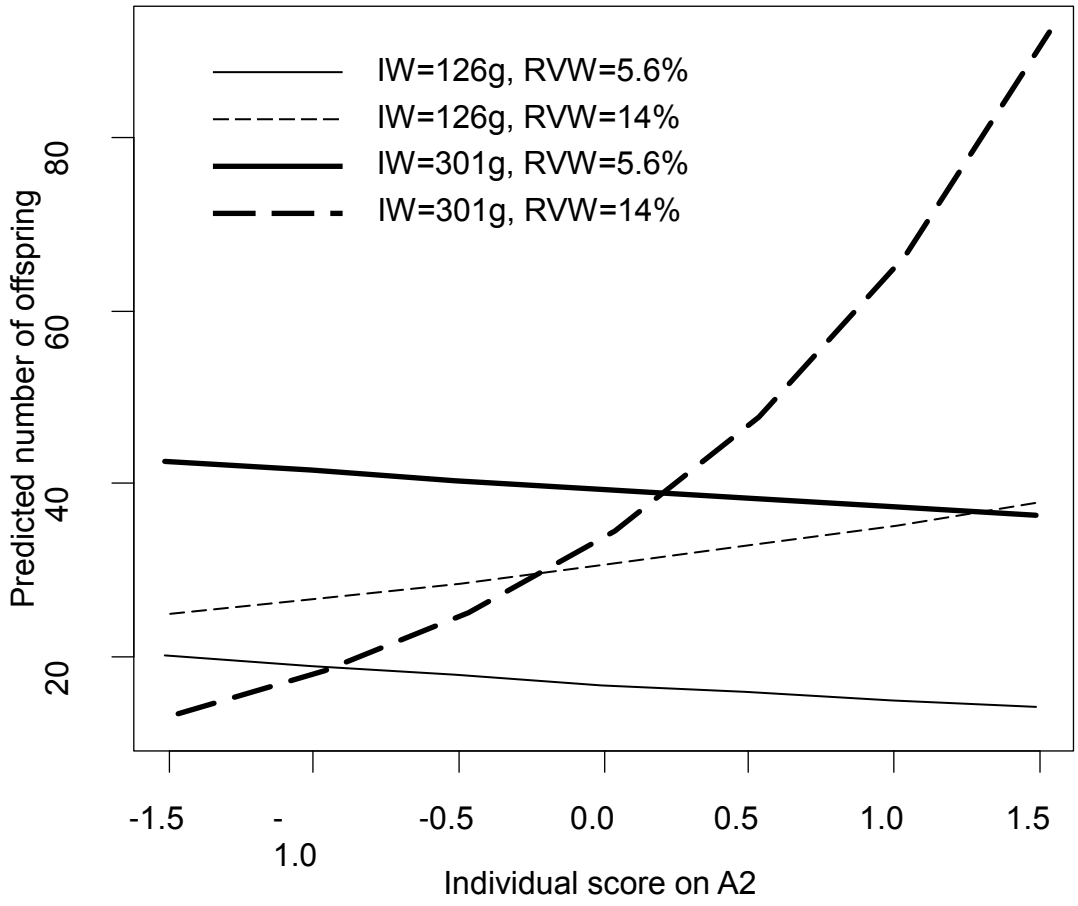

IW=126g, RVW=5.6%
IW=126g, RVW=14%
IW=301g, RVW=5.6%
IW=301g, RVW=14%
Predicted number of offspring
20
40
60
80
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Individual score on A2

Table 1

	Males (mean ± sd)	Females (mean ± sd)	p (Ho: males=females)
Initial level of:			
Weight (g)	273.05 (± 121.49)	194.18 (± 123.91)	**0.005**
Glucose (g/L)	1.02 (± 0.19)	0.96 (±0.25)	0.14
Triglycerides (g/L)	1.41 (± 1.32)	1.89 (± 0.99)	**0.02**
Free fatty acids (g/L)	0.11 (± 0.03)	0.14 (± 0.05)	**0.002**
Amino acids (g/L)	0.36 (± 0.08)	0.27 (±0.07)	**0.0003**
Final level of:			
Weight (g)	252.97 (±106.38)	163.70 (±102.82)	**0.0007**
Glucose (g/L)	0.90 (±0.17)	0.91 (±0.17)	0.81
Triglycerides (g/L)	0.56 (±0.48)	1.08 (±0.77)	**0.06**
Free fatty acids (g/L)	0.04 (±0.02)	0.05 (±0.02)	0.09
Amino acids (g/L)	0.31 (±0.08)	0.28 (±0.04)	0.1
Relative variation of:			
Weight	0.06 (± 0.04)	0.12 (± 0.09)	**0.002**
Glucose	0.15 (± 0.28)	0.06 (±0.27)	0.40
Triglycerides	0.55 (± 0.23)	0.42 (± 0.32)	0.11
Free fatty acids	0.62 (± 0.23)	0.62 (± 0.14)	0.86
Amino acids	0.11 (± 0.24)	-0.13 (±0.37)	**0.02**

Degree of freedom is equal to 1 for each test. Bold values indicate a significant difference between males and females.

Table 2

Variable	Degrees of freedom	Explained deviance	P-value (α=0.05)
A1	1	3.0379	0.08134
A2	1	21.2474	**4.036e-06 *** **
IW	1	1.0305	0.31004
RVW	1	4.4390	**0.03513 ***
A1× IW	1	3.0533	0.08057
A2× IW	1	9.4411	**0.00212 ** **
A1×RVW	1	0.5168	0.47220
A2×RVW	1	3.0275	0.08186
IW×RVW	1	2.8449	0.09166
A1×IW×RVW	1	1.5815	0.20855
A2×IW×RVW	1	0.7746	0.37880
Residuals	34	51.263	-

Appendix legends

Appendix A. Fish recognition. Pictures of two fish before and after reproduction .The number and the color of spots vary from one individual to another one but stay constant for one individual. The white arrows and open circles show specific spots in specific areas determinant for fish recognition.

Appendix B. Genetic analysis protocol.

Appendix C. Raw data of reproductive success (RS), initial plasma concentration of triglycerides, free fatty acids, amino acids and glucose (respectively I.T; I.FFA; I.AA and I.G), final plasma concentration of triglycerides, free fatty acids, amino acids and glucose (respectively F.T; F.FFA; F.AA and F.G) and relative variation of triglycerides, free fatty acids, amino acids and glucose (respectively T.V; FFA.V; AA.V and G.V) for the 29 individuals of the experiment.

Appendix D. Matrix of number of offspring resulted from parentage assignment between each pair of genitors (29 females and 20 males).

Appendix A

	Before reproduction	*After reproduction*
Fish 1		
Fish 2		

Appendix B

DNA extraction

DNA was extracted with a modified NaCl / chloroform based protocol (Müllenbach, Lagoda &Welter 1989) to use 96 wells plates allowing extracting high quality DNA from 192 samples per day at low cost:0.5 cm² of fin clipwas lysedwith 200 µL of buffer (NaCl 75 mM, EDTA 25 mM, Sulfate Dodecyl Sodium 1%, pH 8) containing 10 µL of proteinase K at 20mg/µL in a 1.2 ml microtube. Samples were then incubated at 55°C overnight. 100 µL of NaCl 5M was added, tubes were gently shaken, and 300 µL of chloroform were added. After gently mixing for 10 minutes, samples were centrifuged at 2000 rpm for 10minutes, the upper phase removed to a new microtube. DNA was precipitated with250 µL of isopropanol, and after 5 min of mixing samples were centrifugate for 5 minutes at 4100 rpm. The supernatant was removed and the DNA pellet washed with 500 µL of 70% ethanol for one hour. After a centrifugation step of 5 minutes at 4100 rpm, ethanol was discarded, the DNA was dried at ambient temperature and pellet was finally resuspended in 100 µL of TE 1X buffer.

Microsatellite multiplex PCR

Amplification of the eight microsatellites was carried out in a 5 µL final volume using Qiagen Type-it Microsatellite kits. Each reaction contained 1X PCR Master Mix, 0.2 µM of each unlabeled reverse (Eurofins MWG Operon) and labeled forward primer (6-FAM: Ssa85, Str73INRA, Ssa410Uos, HEX: Str60INRA, Ssosl417, Ssa408Uos (Eurofins MWG Operon) or NED: SsoSL438, Sssp2216 (Life Technologies)) and approximately 25 ng of template DNA. The amplification reaction was carried out using a Applied Biosystem 2720 thermal cycler (Life Technologies) and consisted first in an initial denaturation at 95 °C for5 min, followed by 30 cycles of denaturing at 95 °C for 30 s, annealing at 57°C for 3 min, extension at 72 °C for 30 s and a final extension step at 60 °C for 30 min.

Reference:

Müllenbach R, Lagoda PJL, Welter C, 1989. An efficient salt-chloroform extraction DNA from blood and tissues. Trends in Genetics, 5, 391.

Appendix C

Sex	RS	I.W	F.W	W.V	I.T	F.T	T.V	I.FFA	F.FFA	FFFA.V	I.AA	F.AA	AA.V	I.G	F.G	G.V
Female	2	96	89	0.069	4.1753	1.4732	0.6472	0.159	0.099	0.375	0.28	0.27	0.062	0.9452	0.6357	0.487
Female	43	207	151	0.269	0.4788	0.2626	0.4515	0.036	0.013	0.6466	0.36	0.26	0.27	0.8072	0.6645	0.2148
Female	41	383	300	0.217	0.5437	0.0465	0.9145	0.102	0.008	0.9174	0.27	0.32	-0.175	0.8752	0.7509	0.1655
Female	46	123	121	0.019	2.4351	1.3759	0.435	0.199	0.063	0.6852	0.32	0.41	-0.275	0.9117	0.9077	0.0044
Female	67	169	139	0.177	0.7382	0.5545	0.2489	0.132	0.033	0.7507	0.21	0.23	-0.086	0.7646	0.9646	-0.2073
Female	80	142	122	0.141	2.781	1.3111	0.5286	0.245	0.103	0.5812	0.27	0.21	0.244	1.0822	0.8159	0.3264
Female	13	210	NA	NA	1.1922	NA	NA	0.101	NA	NA	0.31	NA	NA	1.2159	NA	NA
Female	29	NA	100	NA	NA	0.2735	NA	NA	0.022	NA	NA	0.25	NA	NA	0.8863	NA
Female	4	86	86	-0.001	1.992	1.3543	0.3201	0.118	0.065	0.4482	0.29	0.28	0.047	0.9177	0.9171	0.0007
Female	52	200	153	0.238	2.8134	0.6409	0.7722	0.188	0.046	0.755	0.32	0.29	0.102	0.8863	0.6819	0.2997
Female	4	190	166	0.126	1.257	3.0945	NA	0.099	0.043	0.5603	0.32	0.25	0.211	0.9094	0.771	0.1795
Female	20	114	103	0.101	1.6353	1.0517	0.3569	0.157	0.038	0.759	0.15	0.25	-0.656	0.5928	1.0209	-0.4194
Female	138	522	443	0.152	1.538	0.3707	0.759	0.137	0.023	0.8335	0.21	0.2	0.029	0.6926	0.7824	-0.1148
Female	1	519	438	0.155	1.3219	1.2895	0.0245	0.128	0.052	0.5923	0.15	0.3	-0.983	1.0903	0.8668	0.2578
Female	0	338	322	0.049	0.7382	0.2951	0.6003	0.144	0.057	0.6068	0.42	0.25	0.421	0.9191	0.7442	0.235
Female	6	111	91	0.181	1.7218	0.7598	0.5587	0.173	0.082	0.5247	0.41	0.25	0.398	0.9472	0.9177	0.0321
Female	2	300	253	0.158	3.0026	3.4403	-0.1458	0.096	0.049	0.4875	0.19	0.31	-0.666	1.1931	0.9914	0.2034
Female	15	102	93	0.085	3.3971	1.538	0.5472	0.255	0.061	0.76	0.26	0.3	-0.158	0.8976	1.1127	-0.1933
Female	13	76	68	0.105	1.6569	0.9868	0.4044	0.118	0.041	0.6553	0.16	0.27	-0.734	0.8501	1.0826	-0.2148
Female	163	306	250	0.184	4.3645	0.1762	0.9596	0.105	0.02	0.8077	0.22	0.26	-0.222	0.9881	0.8367	0.181
Female	3	136	109	0.199	1.7434	1.5921	0.0868	0.135	0.084	0.3799	0.25	0.26	-0.023	1.2069	0.9606	0.2563
Female	96	262	203	0.226	2.2298	1.3435	0.3975	0.18	0.094	0.4745	0.36	0.3	0.164	0.7074	0.8943	-0.209
Female	11	103	108	-0.045	1.9488	0.5761	0.7044	0.125	0.043	0.6569	0.31	0.27	0.128	1.7921	1.4725	0.217
Female	36	85	76	0.109	1.6461	1.0517	0.3611	0.171	0.07	0.5912	0.26	0.36	-0.383	1.4983	0.8507	0.7612
Female	61	206	180	0.129	2.0028	0.8139	0.5936	0.162	0.042	0.7413	0.24	0.27	-0.107	0.7388	0.893	-0.1726
Female	1	127	113	0.113	0.922	1.3543	-0.4689	0.187	0.059	0.6824	0.23	0.31	-0.383	1.0075	1.0685	-0.0571
Female	1	109	120	-0.104	1.6786	0.9003	0.4636	0.167	0.054	0.6789	0.19	0.33	-0.734	0.8574	0.9673	-0.1136
Female	20	121	108	0.106	1.3543	0.9976	0.2634	0.103	0.057	0.452	0.28	0.33	-0.187	0.7496	1.112	-0.326
Female	15	94	81	0.135	1.5489	1.2138	0.2163	0.117	0.066	0.4364	0.31	0.24	0.234	0.7871	0.8461	-0.0697
Male	51	346	321	0.072	0.922	0.4032	0.5627	0.205	0.018	0.9112	0.5	0.49	0.02	1.0993	0.9151	0.2014
Male	152	308	271	0.12	2.8243	0.6518	0.7692	0.103	0.029	0.716	0.29	0.29	-0.007	0.7837	0.7911	-0.0093
Male	6	173	167	0.037	1.1165	0.6518	0.4163	0.086	0.05	0.4204	0.51	0.31	0.39	1.3405	0.7737	0.7326
Male	3	248	233	0.062	1.0841	0.5761	0.4686	0.114	0.02	0.8275	0.32	0.35	-0.093	0.9847	1.0571	-0.0685
Male	16	147	145	0.014	1.4083	0.2951	0.7905	0.141	0.043	0.6927	0.31	0.34	-0.075	1.0893	0.631	0.7263
Male	212	396	367	0.074	1.2678	0.0681	0.9463	0.133	0.005	0.9622	0.32	0.35	-0.089	0.7817	0.7811	0.0009
Male	13	288	270	0.064	0.6409	0.3707	0.4216	0.063	0.04	0.3705	0.38	0.26	0.313	0.9077	0.8266	0.0981
Male	41	337	312	0.074	0.241	0.1546	0.3587	0.052	0.014	0.7208	0.28	0.17	0.401	0.8521	0.909	-0.0627
Male	8	138	131	0.054	2.219	1.1057	0.5017	0.149	0.063	0.5803	0.42	0.35	0.18	1.0641	0.9218	0.1545
Male	0	341	327	0.043	0.414	0.2368	0.4281	0.108	0.011	0.901	0.46	0.26	0.437	0.7342	0.8628	-0.1491
Male	1	536	477	0.11	0.9652	0.4248	0.5599	0.075	0.025	0.6622	0.34	0.28	0.173	1.3445	0.9033	0.4884
Male	5	298	272	0.087	0.8355	0.3923	0.5304	0.117	0.051	0.5685	0.26	0.37	-0.431	1.0605	0.7462	0.4211
Male	258	555	498	0.103	0.2843	0.0739	0.7402	0.076	0.009	0.8841	0.37	0.25	0.316	0.7831	0.834	-0.0611
Male	3	185	170	0.079	0.5869	0.468	0.2026	0.112	0.063	0.4406	0.36	0.39	-0.064	1.2692	1.3338	-0.0485
Male	1	249	237	0.05	0.522	0.5112	0.0207	0.095	0.073	0.2338	0.4	0.21	0.487	1.0752	1.0122	0.0622
Male	2	130	137	-0.055	3.5214	2.0352	0.422	0.105	0.047	0.5497	0.26	0.28	-0.093	0.7546	0.8896	-0.1518
Male	62	184	181	0.017	5.5966	1.3003	0.7677	0.113	0.065	0.4229	0.37	0.43	-0.174	1.1643	1.2775	-0.0886
Male	15	212	197	0.069	2.2514	0.9652	0.5713	0.098	0.064	0.3495	0.44	0.32	0.26	1.1991	0.9553	0.2553
Male	11	155	146	0.059	1.2138	0.4896	0.5966	0.112	0.074	0.3375	0.34	0.28	0.169	1.0223	0.9868	0.036
Male	123	235	202	0.14	0.3815	0.0141	0.9632	0.114	0.011	0.9033	0.27	0.23	0.146	0.9948	0.6873	0.4475

Appendix D

		Females																											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**
Males	**1**	0	0	0	1	0	0	0	0	0	0	0	8	0	0	0	0	0	1	0	1	0	1	0	0	16	0	0	18	5
	2	0	0	25	42	0	0	0	0	0	0	0	0	56	0	0	4	1	0	6	0	1	6	4	0	0	0	0	0	7
	3	1	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	14	0	0	0	0	0	0	0	0	1
	6	0	35	16	0	4	0	0	0	0	22	1	0	2	0	0	1	0	0	0	128	0	0	1	0	0	0	0	0	2
	7	0	1	0	0	0	0	1	8	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	29	0	9	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	12	0	3	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	13	0	0	0	3	0	80	0	0	0	0	2	1	78	0	0	0	0	0	2	0	1	81	6	0	3	1	0	0	0
	14	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	16	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	17	0	0	0	0	9	0	0	2	0	0	0	0	0	0	0	0	0	14	1	0	0	1	0	35	0	0	0	0	0
	18	0	0	0	0	0	0	10	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	19	1	0	0	0	0	0	2	1	0	1	0	0	0	0	0	0	0	0	1	1	0	4	0	0	0	0	0	0	0
	20	0	4	0	0	50	0	0	0	4	0	0	1	1	0	0	0	0	0	0	19	0	0	0	0	42	0	1	1	0

Supplementary Information 2:

A draft for a publication around the model presented in chapter IV.IV.

The draft is here provided in its current shape.

Both male and female contribute to mating success: improvements in quantifying sexual selection.

Zoé Gauthey[a,c], Laura Royer[a], Arturo Elosegi[d], Cédric Tentelier[a,c] & Jacques Labonne[a,c].

[a] INRA, UMR 1224, Ecologie Comportementale et Biologie des Populations de Poissons, Aquapôle, quartier Ibarron, 64310, Saint-Pée sur Nivelle, France.

[b] Faculty of Science and Technology, University of the Basque Country UPV/EHU, 48080 Bilbao, Spain.

[c] Univ Pau & Pays Adour, UMR 1224, Ecologie Comportementale et Biologie des Populations de Poissons, UFR Sciences et Techniques de la Côte Basque, Allée du parc Montaury, 64600, Anglet, France.

Keywords: reproductive success, fitness, encounter rate, Bateman gradient.

Corresponding author:

zoe.gauthey@googlemail.com

Introduction

Sexual reproduction requires to find a sexual partner in order to produce offspring to the next generation. The reproductive output is therefore always attributable to both partners for a given episode of reproduction and can be conditioned by environment (Kokko & Jennions 2008). Mating success depends on availability of sexual partners whereas the number of offspring will be affected by mate quality through direct (*e.g.*: parental care) and indirect benefits (*e.g.*: good genes) relative to the breeding environment (Andersson 1994; Hanna Kokko et al. 2003).

For iteroparous species, the reproductive success RS_i of an individual i over a reproductive season is often used as a proxy of fitness within this reproductive season. The distribution of RS_i in a population is generally summarized by an array of number of offspring produced between all possible pairs of males and females for each mating episode. Then, the sum of overall mating episodes may be reduced to a matrix of offspring produced by each pair, the so-called *parental table* (Arnold & Duvall 1994). An estimate of such matrix is typically generated by parentage analysis based on genetic markers (Bateman 1948, Garant et al. 2001; Avise et al. 2002, Jones & Ardren 2003; Jones et al, 2003; Serbezov et al.2010) possibly complemented by direct observations of mating behavior (Pemberton et al. 1992; Coltman et al. 1999, Collet et al. 2014).

Classical methods in sexual selection use these parental tables to study adaptive value of traits in populations by measuring different indices of sexual selection in males and females such as opportunity for selection, selection gradients and selection differentials (Bateman 1948; Crow, 1958; Wade 1979; Wade and Arnold 1980). To do so, they further reduce the matrix to its margins, individual reproductive success being the sum of offspring on the individual's row or column, and mating success being the number of non-null cells on the individual's row or column, *i.e.* the number of different individuals with which at least one offspring was produced. Sexual selection is predicted to operate provided there is variance in reproductive success and in mating success, and a strong link between these two.

This approach presents two important caveats: first, the definition and/or the estimation method of mating success, second, the lack of consideration for the fundamental dependency between the mating and reproductive success of an individual and the mating and reproductive success of its mate(s).

An illustration of the first caveat is the wealth of definitions for individual mating success during one reproductive period (Bateman 1948, Arnold & Duvall 1994, Jones2009, Gowaty et al., 2012, Parker & Tang-Martinez 2005, Uller & Olsson 2008). Mating success can be viewed as the number of copulations, the number of individuals with which the focal individual has copulated, the number of copulations that yield progeny or the number of individuals with which progeny is produced. While the two latter definitions inform precisely on the benefits, the first and second definitions also integrate potential costs, whether it is time, energy, predation risk, or disease transmission. Because benefits and costs are both essential to understand the evolution of sexual selection, it should be of interest to study both points of view in a single framework to estimate sexual selection indices, especially for iteroparous species that may regulate reproductive investment between reproductive seasons depending on their age or on environmental variation (Jones 2009, Pélissié et al. 2014).

It is noteworthy that the definition of mating success is to a great extent constrained by methodological possibilities. Standard methodological approaches using parental tables obtained from genetic assignations can only target the fourth definition and generally produce biased estimates of it (Snyder & Gowaty 2007, Collet et al. 2014). These approaches deduce individual mating success by counting the number of non-zero elements on the individual line of the parental table. In this case, a zero value for a given pair can be the outcome of pre-copulatory, post-copulatory or sampling processes: no copulation, no gamete fertilization, offspring dying before sampling, offspring failing to be sampled. Similarly, a non-zero value can also carry more information than just the total reproductive success between a pair of individuals, since it can be the outcome of a variable number of matings, which is of importance to measure reproductive investment. In this perspective, matrices of copulation success as obtained by direct observations of mating behavior obviously contain data that are complementary to parentage assignation methods. We therefore need statistical models integrating both behavioral and molecular data to provide estimates of the various definitions of mating success, by disentangling pre-copulatory and post-copulatory components as already suggested by several authors (Arnold & Wade, 1984; Pischedda, 2012; Pélissié et al. 2013).

The second caveat is less evoked in the literature although intuitively simple: in sexual reproduction, reproductive success between two individuals should be attributable to both, and yet we usually analyze reproductive success as an individual characteristic, with no regard for the effect of the sexual partner. Classical studies only focus on the marginal

sums of the parental table, and therefore cannot control for sexual partner trait or mating success variation. Selection indices are estimated by regressing the margins of the parental table against the vector of values of phenotypic traits, independently for males and females. A direct consequence is that we might detect a significant correlation between a trait and mating success or reproductive success for a sex, and interpret it as evidence of direct selection, whereas indirect selection could for instance be at work by mean of non-random association between sexual partners' traits. Moreover, the environment in which individuals encounter each other may also vary and play a role in the mating success and reproductive success of a pair at each mating episode. We therefore need an approach in which the mating and reproductive success of a pair of individuals accounts for the phenotype of both individuals and the features of the environment where individuals encounter.

To solve both matters, we propose a model that 1) combines molecular data (parental table) and behavioral data (encounter and mating matrix) to estimate the different components of reproductive success (here encounter rate, copulation rate, number of offspring produced) for each mating episode within the reproductive season, and 2) infers the joint effects of both male and female phenotypes and characteristics of mating episode on each component of the reproductive success (Figure 1).

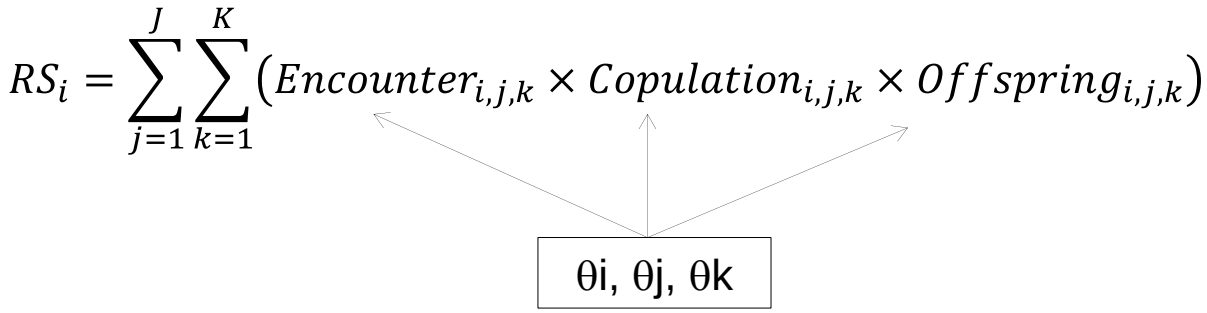


Figure 1. *Decomposition of individual reproductive success (RS_i) as modelled in this study. Individual reproductive success of individual i is the sum across all j partners and all mating k episodes of the product of encounter, copulation and number of offspring produced between i and j at the k^{th} episode. Each component of the reproductive success at a given occasion may be affected by individual phenotypes (θi, θj = body size,*

secondary sexual characters...) and environmental features (θk = operational sex ratio, wind speed...).

The conditional structure linking the successive components of pairwise reproductive success is the key to extract information from both behavioral and molecular data: presence of offspring for a pair of parents implies encounter and copulation, even if these are absent from behavioral data, whereas observation of copulation allows distinguishing between zero-value due pre-copulatory and post-copulatory mechanisms. We illustrate the model using *Salmo trutta* as a case study, with body size as an example of phenotypic covariate as it is known to be involved in sexual selection in salmonids (Tappel & Bjornn 1983, Labonne et al. 2009) and could therefore have an effect on each of these components of sexual selection.

Methods

Samples and experimental procedure

The study was conducted from November 2012 to the end of March 2013 (reproductive period for brown trout) in an experimental channel beside Lapitxuri stream, a tributary to the Nivelle River in south-western France (+43° 16' 59", -1° 28' 54") (Gaudemar, Bonzom, & Beall, 2000). Three linear and communicating sections were used during the experiment, each measuring 10 meter long and 2.80 meters wide. The central section was fit out for spawning, with the appropriate gravel size (1 to 4 cm diameter), water depth (20 cm) and current speed (220 $m.h^{-1}$). In the two extreme sections, a more complex environment was installed with bigger substrate size, visual obstacles (woods, bricks) and pools that provided hiding and resting areas. Brown trout adults (19 males and 33 females) were electrofished in two rivers: River Bastan (+43° 16' 2.51", -1° 22' 32.46") and River Urumea (+43° 14' 31.81", -1° 55' 28.98"). Each trout was anesthetized (0.3 $mg.l^{-1}$ benzocaïne), measured, weighed, and photographed to allow individual identification on subsequent video recordings. On waking, fish were released in the three section of the semi-natural river, where they were free to move until the end of the experiment.

Videos recordings were performed during these few weeks in order to acquire behavioral data. To do so, individuals were observed each day from the river side. When reproductive behaviors indicating that a female and one/or several male(s) were close to

spawning (digging female, chases between males), subaquatic and aerial digital camera videos were placed in the river or from the bank (Aymes et al. 2010, Tentelier et al. 2011).

Behavioral data

For each observed mating episode (one female lays her eggs and at least one male releases sperm), 3 hours of videos were analyzed, 1h30min before gamete release and 1h30 thereafter in order to identify individuals that participated to the encounter process followed by copulation process. To do so, a zone of one meter around the female's nest construction was defined. Individuals were considered as present when they entered on the zone. They were considered as absent when they were outside of the zone. The pair of individuals present during an event of reproduction was noted as an encounter. A female and a male were considered to have encountered each other on a given mating episode if they were both present on the zone at least once during the three-hour period. The total number of encounters observed during the experiment was stored in a males x females matrix. Copulations were also observed as the gamete release of both male and female. The total number of observed copulations over the experiment was also stored in a males x females matrix. Individual recognition was performed by comparing pictures took before the experiment to the image on the video. As black and red spot density and position vary consistently between individuals and do not change during the reproduction period, they were accurate tools for individual discrimination (§ II.II.3). However, in some occasions, some individuals, despite being present in the spawning zone, were too far from the camera to be unambiguously identified. These individuals were therefore not taken into account for the encounter observations.

Molecular data

Juveniles stemming from the reproduction in the experimental channel were electrofished at emergence (800 degree.days: about two months after the last spawning event). They were anesthetized and killed under a lethal dose of 2-phenoxyethanol and placed individually in a tube of absolute ethanol (90°) upon molecular analysis. A small piece of pelvic fin was also taken on adults and stored in 90% ethanol upon molecular analysis. DNA extraction, PCR amplification and genotyping at eight microsatellite loci fed parentage analyses on Cervus software, as described in Gauthey et al. (submitted).

Statistical model

The model used both behavioral data and molecular data that can generally come in three different arrays confronting pairs of individual of each sex: 1) a three dimensional array of observed pairwise encounters for each of the K_{obs} mating episode that were video recorded: $OE_{i,j,kobs}$, 2) a three dimensional array of pairwise copulations observed for each of the *Kobs* mating episodes that were video recorded: $OC_{i,j,kobs}$ and 3) a matrix of the total number of offspring produced by each pair, estimated from genetic assignment $N_{i,j}$.

The general philosophy is to estimate the real data variation between all male-female pairs at each *k* mating episode: the encounter (a binomial variable indicating if male *i* met female *j* at mating event *k*), the copulation (a binomial variable indicating if male *i* mated with female *j* at mating event *k*), and the number of offspring produced (a discrete quantitative non negative variable describing the number of offspring produced by male *i* and female *j* at mating episode *k*).

A first problem is the unfolding of the reproductive success matrix $N_{i,j}$ in *K* sub matrices, with *K* the total number of mating episode that occurred in the mating season. This problem arises because usually offspring are sampled at the end of the reproductive season and all clutches are therefore pooled. We here assume that:

$$N_{i,j} = \sum^{K} N_{i,j,k}$$

Because behavioral data are generally incomplete ($K_{obs} \leq K$), a second problem lies in the probability to actually observe individuals within the k^{th} mating event: as stated previously, some individuals may not be identified, or may participate while staying out of the video camera's reach. This is solved by stating that:

$$OE_{i,j,k} = E_{i,j,k} \times O_{i,j,k}$$

Where $E_{i,j,k}$ and $O_{i,j,k}$ are both binomial variables sampled in Bernoulli distributions of mean *pe* and *po*, respectively the probability that the encounter for the pair *i* and *j* at mating episode *k* happened and the probability that it was observed (both individuals I and j were correctly identified). When $O_{i,j,k}$ is zero, we have no observed behavioural data for the pair *i* and *j* at mating episode *k*, so encounter rate and copulation rate cannot be directly estimated.

A second problem lies in the probability to actually observe all K mating episodes. In general, this is not so, and we observe K_{obs} mating episodes. If $K_{obs} < K$, then no behavioural data are available for some mating episodes. Additionally, the actual number of mating episode K may not be known. In such case, we propose to simply simulate expected behavioural data using the posterior densities from estimated parameters for the K_{obs} mating episodes where behavioural data are known.

The effect of male and female body size (BS_i and BS_j) on encounter rate ($E_{i,jk}$), copulation rate ($C_{i,j,k}$) and offspring number ($N_{i,j,k}$) were modeled as follows:

$$\text{logit}(E_{i,j,k}) = a_1 + b_1 \times BS_i + c_1 \times BS_j$$

$$\text{logit}(C_{i,j,k}) = a_2 + b_2 \times BS_i + c_2 \times BS_j$$

$$\log(N_{i,j,k}) = a_3 + b_3 \times BS_i + c_3 \times BS_j$$

Statistical inference was conducted in the Bayesian framework. The joint posterior distribution of all unknown quantities of the model was approximated by MCMC sampling as implemented by the OpenBUGS (version 3.21) software. A MCMC sample of 11320 draws with a thinning of 100 was used, after checking its convergence by applying the Gelman-Rubin test (Gelman & Rubin, 1992). We used non informative Gaussian and independent priors distributions (mean=0, precision=0.001) for hyperparameters: a_1, a_2, a_3, b_1, b_2, b_3, c_1, c_2, c_3, a Beta prior distribution $\beta(1,1)$ for po, and a uniform distribution [0,100] for K. The full code in OpenBugs language is provided in Supplementary file 5.

Selection indices from raw data and from the model output

In order to compare different measures of sexual selection between the raw data and data simulated from the model combining behavioral and molecular data, we computed different quantitative measurements of sexual selection for each sex. Opportunity for selection (I) and sexual selection (Is) were computed as the ratio of variance on squared mean of reproductive success and mating success, respectively (Wade & Arnold, 1980). Bateman gradient (β_{ss}) was measured using a simple linear regression between

reproductive success and number of mates. To compute these indices from the raw molecular data, we conformed to the classical view: individual reproductive success was considered as the number of offspring produced (sum of the individual's line in the parental table) and individual mating success was considered as the number of individuals with which the focal individual produced offspring (number of non-null cells in the individual's line in the parental table). From the raw behavioral data, we computed opportunity for selection on the number of partners encountered and opportunity for selection on number of mates with which copulation occurred. For the latter, we only considered individuals for which at least one encounter was recorded. To combine behavioral and molecular data, we ran the model, then simulated behavioral and molecular data using parameter values drawn from the joint posterior distributions, and finally computed the indices of selection on these simulated data. Here, individual reproductive success was again computed as the number of offspring produced, but mating success was decomposed in encounter success, *i.e.* number of individuals of the other sex encountered by the focal individual, copulation success, *i.e.* number of individuals of the other sex with which the focal individual emitted its gametes, and mating success *sensu* Bateman, *i.e.* number of individuals with which the focal individual produced offspring. Opportunity for sexual selection and Bateman gradient were computed using each definition of mating success.

Results

Behavioral and molecular data

Three individuals were removed from the data set because of they escaped from the experimental channel (2 males and 1 female). This event happened during the two first week of the experiment when reproductive period just started and these individuals were not observed as sexually active on the videos. These three individuals were therefore discarded from the different analyzes.

In total, 22 spawning acts were video recorded (K_{obs} mating episodes) during the reproductive season. Within these K_{obs} mating episodes, 14 females out of 32 and 12 males out of 17 were observed, totalizing 75 pairwise encounters. Thirteen females and 7 males were observed releasing their gametes, totalizing 22 pairwise copulations (no multiple mating was observed). Stripping at recapture showed that almost all individuals

(especially females) had released their gametes by the end of the experiment (only two females did not lay their eggs), and redds were detected in places where we did not place our cameras so we must have missed a proportion of mating episodes.

A total of 555 juveniles and 49 parents were genotyped. Among those individuals, 551 juveniles were assigned to 41 pairs of parents (10 males and 22 females) at a confidence level of 95%. Number of offspring varied from 0 to 201 in males (mean ± sd= 32 ± 64) and between 0 and 86 for females (mean ± sd= 17 ± 24). Only 12 pairs were both seen copulating and assigned offspring.

Estimates of selection indices

Although only 22 pairwise copulations were recorded on video and 41 families (=productive pairwise copulations) were detected by molecular analysis, joint posterior distribution of model parameters indicated that 41 (sd = 9) mating episodes occurred, involving 56 (sd= 14) pairwise copulations. The opportunity for selection and sexual selection, Bateman gradient and the maximum standardized sexual selection gradient, computed from both raw molecular data and output of the null model, are given in Table 1. Opportunity for selection on number of partners encountered was much lower when computed from the model output than from raw behavioral data, whereas opportunity for selection on number of copulation partners was the same from both methods. Opportunity for selection on number of mates and number of offspring as estimated from molecular data was approximately the same from both methods for males but was much lower for females when estimated from the model output.

The data simulated from model output allowed computing sexual selection indices on behavioral dimensions of mating success, which are also given in table 1. Selection gradient on number of individuals encountered and number of copulation partners was significantly positive for males but did not differ from zero for females. Finally, the selection gradient on number of mates estimated from molecular data, Bateman gradient, was also positive for males and null for females.

Table 1. *Opportunity for selection ($I_{offspring}$), opportunity for sexual selection and Bateman gradient computed for males and females, on raw molecular data and on data simulated from the output of the null. Opportunity for sexual selection and Bateman gradient was*

computed for different definitions of mating success: number of mates estimated from molecular data ($I_{molecular\ mates}$, $\beta_{molecular\ mates}$), number of other-sex individuals encountered ($I_{encounters}$, $\beta_{encounters}$) and number of other-sex individuals with which copulation occurred ($I_{copulations}$, $\beta_{copulations}$).

	$I_{encounters}$	$I_{copulations}$	$I_{molecular\ mates}$	$I_{offspring}$	$\beta_{encounters}$	$\beta_{copulations}$	$\beta_{molecular\ mates}$
From molecular data							
Males	-	-	2.15	3.93	-	-	17.45***
Females	-	-	0.80	2.07	-	-	13.19***
From behavioural data							
Males	1.05	1.36	-	-	-	-	-
Females	1.82	0.34	-	-	-	-	-
From model output							
Males	0.05	1.46	1.48	3.38	7.78*	8.84***	8.84***
Females	0.06	0.40	0.40	0.61	-0.01	-0.08	-0.09

Effect of individual phenotypes

Using the molecular data only, male body size increased mating success (t = 3.938 on 15 df, p = 0.001, Fig. 2.a) whereas body size had no effect on female mating success (t = 0.661 on 30 df, p = 0.514, Fig. 2.b). Likewise body size had a positive effect on reproductive success for males (t = 0.2579 on 15 df, p = 0.003, Fig. 2.c) but not for females (t = 0.1779 on 30 df, p = 0.867, Fig. 2.d). Using the behavioral data only, body size only affected the number of females with which males copulated: larger males copulated with more females (slope = 0.016, t = 2.22 on 10 df, p = 0.05). Male body size did not affect number of females encountered, and female body size affected neither the number of males encountered nor the number of males copulated (all ps > 0.2).

The model including the effect of male and female body size on the probability of encounter, the probability of copulation and the average number of offspring converged and updated posterior distributions for the parameters associated to the effects of body size (Fig. 3). Body size had a positive effect on all components of male mating and reproductive success. Larger males had a greater probability to encounter females, had a greater probability to copulate with the females they encountered, and produced a larger number of offspring once mated. The effect of body size was quite different for females, as it did affect neither the probability of encounter (although a positive trend could be noticed) nor the probability of copulation upon encounter. However, and quite counter-intuitively, bigger females produced less offspring once mated.

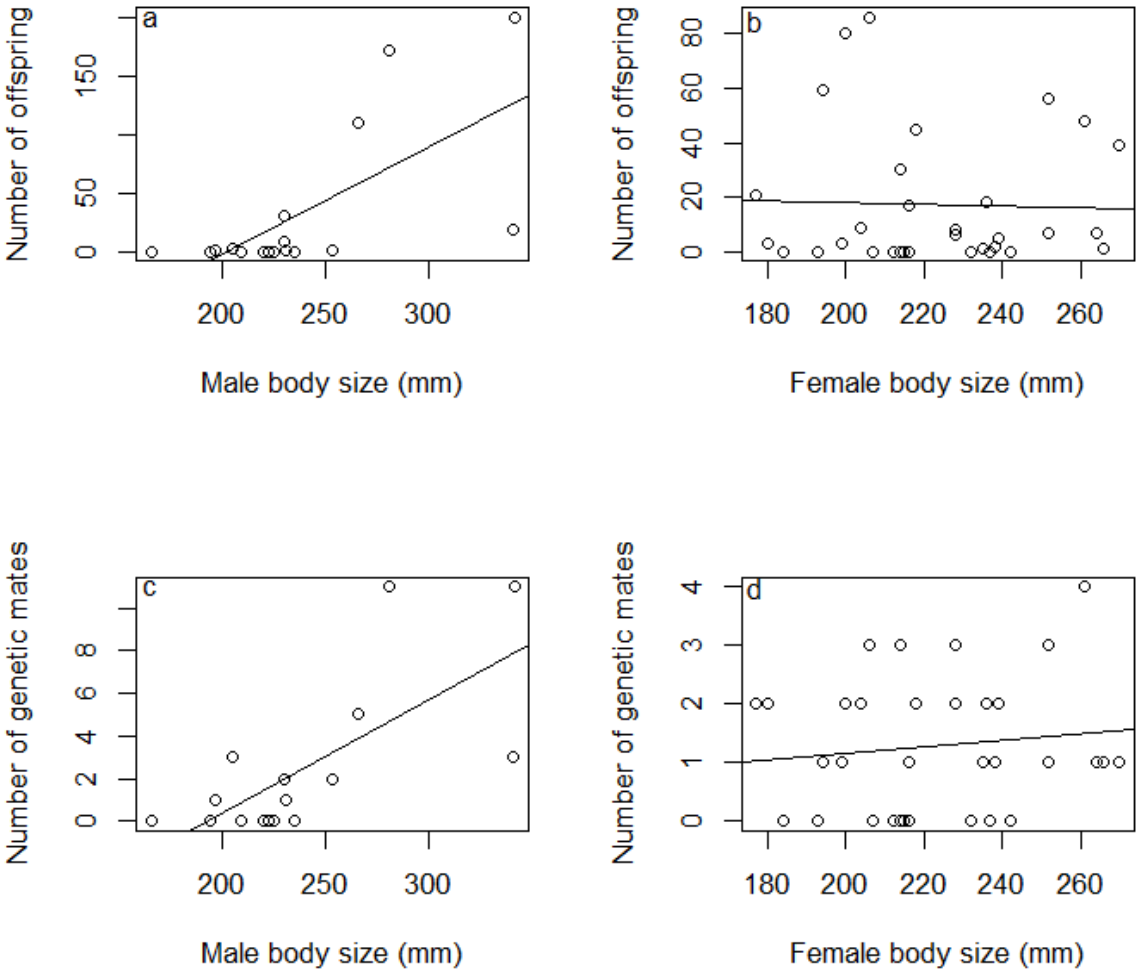


Figure 2. *Effect of body size on male (a, c) and female (b, d) reproductive success (a, b) and mating success (c, d), computed as the number offspring of the focal individual and the number of individuals with which the focal individual produced offspring, according to molecular analysis. The slope of the regressions are significantly positive for males but not for females.*

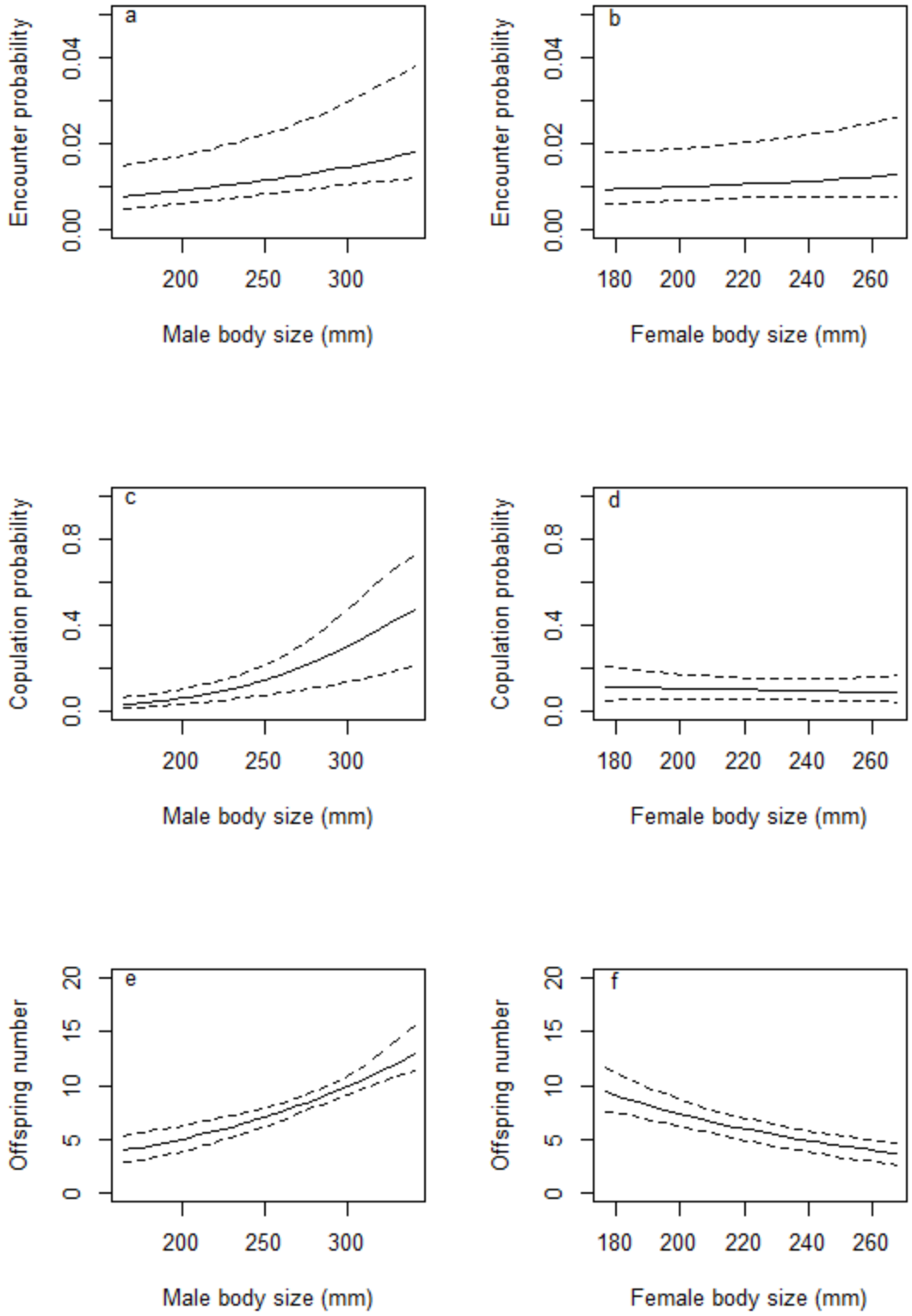


Figure 3. *Model predictions on the effect of male and female body size on the probability of encounter (a, b), the probability of copulation upon encounter (c, d) and the average*

number of offspring produced upon copulation (e, f). Predictions are computed after the joint posterior distributions of hyper-parameters of the model including effects of body size and random individual effects (model 2), for each iteration once the MCMC chains have converged (=11320 iterations). Solid lines represent the median, and dashed lines represent 5% and 95% quantiles. When predicting the effect of one sex's body size, body size of the other sex is set at its median (217mm for females and 230mm for males).

Discussion

In this study, we used two approaches to quantify sexual selection and estimate the effect of a phenotypic trait (here body size) on different components of reproductive success in brown trout. Both approaches lay on behavioral observation of mating and genetic assignation of offspring. On the one hand, we applied classical analyses on data obtained from the margins of each male × female matrix: observed encounter, observed gamete release and offspring number inferred from genetic assignation. On the other hand, we developed a statistical framework combining all these data, thereby enabling information to be shared through the successive processes of encounter, gamete release and offspring production. This new approach accounted for the three-dimensional structure of the data: males, females and mating episodes. This allowed a better definition of mating success and untangling the joint effects of male and female phenotypes on the different components of reproductive success.

What is mating success?

The multiple definitions of mating success have been shaped by a dichotomy of approach, which our model aimed at overcoming. On the one hand, because the classical approach based on the parental table obtained from molecular data is oblivious to both ineffective matings and successive copulations between the same pair of individuals, it has constrained the definition of mating success to the number of individuals with which the focal individual produces offspring that are alive at sampling (Arnold & Duvall, 1994). On the other hand, the not less classical approach based on the sole observation of copulatory behavior, unable to access the reproductive output, focused the definition of mating success on the number of copulations or number of sexual partners. By combining behavioral and molecular data in a common framework, our analysis embraces multiple aspects of mating success. At the scale of the reproductive group, our behavioral

observations showed 75 male * female encounters and 22 mating episodes (all leading to a copulation), while the parental table based on genetic assignation indicated that 41 families (pairwise copulations leading to offspring production) were produced. The model, accounting for the possibility that 1) a brood was the result of several copulations, 2) some copulations led to no offspring production, and 3) some copulations could not be observed, estimated that 41 mating episodes occurred involving 56 pairwise copulations.

At the individual level, the reconstruction of the mating process from behavioral and molecular data allowed a more accurate estimate of mean, variance and covariance of the different aspects of mating success and reproductive success, which are at the basis of selection indices such as the opportunity for selection and selection gradients. Our results indicated that the opportunity for selection on number of partners encountered was not as high as it was estimated using behavioral observation alone, certainly because estimating the probability of observation (po = 0.53) removed some sampling variance in behavioral observation. Estimates of opportunity for selection on number of copulation partners did not change between raw behavioral data and data simulated from the model, mainly because when estimating it from the raw data, we only accounted for individuals which were observed, assigning missing data rather than zero-value to those which were not observed. In our model, the probability of observation was inferred thanks to information from the molecular data, which contains reproductive success for pairs that were not seen copulating. In this way, our approach is different from the ones adopted by Collet et al. (2012) or Pelissié et al. (2014), which rely on complete knowledge of copulation events in the mating group to disentangle the contribution of pre-copulatory and post-copulatory components of reproductive success. Such complete behavioral data may be available in experimental setups involving few individuals in a restrained habitat, but is far less accessible in real populations (but see (Krause et al., 2013)).

Moreover, because it gathers behavioral observations and genetically-inferred reproductive success, our approach allows computing gradient of selection on behavioral components such as the probability of encounter and probability of copulation upon encounter. Opportunity for selection on number of offspring were much lower for females than for males, probably because most variation in pairwise reproductive success was attributed to males. As a consequence, selection gradients on number of partners encountered, number of copulation partners and number of mates estimated from molecular data were all significantly positive for males and null for females.

Combined effects of male and female phenotype on the components of reproductive success

Sexual selection on phenotypic traits is classically quantified for each sex separately, by regressing the number of mates against phenotypic trait in a separate model for each sex. Here, the statistical unit is the individual, and individual mating success and reproductive success are assumed independent. However, both male and female traits contribute to pairwise mating success and reproductive success. Our approach was therefore to consider the mating episode as the statistical unit, and infer the effect of traits borne by individuals involved in that episode on its outcome. This approach departs from selection theory, to which regression models fit well (Lande & Arnold, 1983; Moorad & Wade, 2013; Price, 1970), but it allows insight on the mechanisms by which traits affect individual fitness.

Applying our model to body size, we showed that male size affected positively the three components of reproductive success considered: probability of encounter with females, probability of copulation upon encounter and number of offspring produced with mates. Model predicting reproductive success of males with an average female showed that the effect was the strongest on probability of copulation upon encounter, which reached 0.4 for the largest male while it was nearly null for the smallest one. Once mated, the effect of body size was such that the largest male would get three times as many offspring than the smallest one. The results agreed with the classical approach, which revealed a positive correlation between body size and number of mates estimated from molecular data. For females, results were less straightforward. From the molecular data, number of mates was not related to female body size. The predictions of our model indicated that female body size had a very weak positive effect on probability of encounter but no effect of probability of copulation upon encounter. In other words, bigger females tended to meet slightly more males than smaller ones but did not mate more with the males they met than smaller females. Surprisingly, female size had a negative effect on the number of offspring produced by a pair on a given mating occasion. This contradicts the regression-based analysis which showed no relationship between female body size and offspring number. This was probably due to bigger females mating with bigger males, and the positive effect of male size balancing the negative effect of female size. This discrepancy between the results of the two methods points at the benefits of our methods when data are not independent.

The ability to disentangle male and female effects on pairwise reproductive success is crucial when the parental table is sparse, which is likely in most reproductive groups of realistic size. In our data, 41 full-sib families were detected by the parentage analysis, among the 544 theoretically possible crosses (32 females times 17 males). In such cases, assortative or disassortative encounter and mating, be it the result of mate choice, intrasexual competition or chance, leads to an unbalanced design, where the many zeros hinder the independent computation of selection gradients for males and females.

Further applications of the model

The experimental design and the quantity of data we used to illustrate our model indubitably constrained the analyses we carried out, and one can wonder how the model can be transposed to other systems, with other types of data on either the components of reproductive success or traits affecting them. For instance, because we sampled all offspring at the end of the experiment, the molecular data did not inform much on the number of offspring produced at each copulation. However, in other systems where clutches are well separated in time or space, even within a reproductive season, the parental table would also be three-dimensional (male × female × episode) and inference about environmental effects on reproductive success would be more accurate. Also, depending on the system studied, reproductive success may be further decomposed, and inference might be done on individual or environmental features affecting the additional components. For example, one may disentangle copulation from gamete fertilization by combining behavioral data and sampling of zygotes just after copulation. Here, an additional three-dimension matrix containing gamete fertilization of each male-female pair at each occasion would be built, and fertilization success would be included in the model, conditioned by copulation success, and conditioning the number of offspring. This would disentangle fertilization success from zygote survival, something we were not able to do in our case study on trout.

Regarding traits affecting components of reproductive success, we did not include interactions between individual phenotypes and environmental features of the mating occasion but it could be done provided one has enough data. For brown trout, male size and OSR might interact to affect copulation probability (by male intrasexual competition) or number of offspring (by sperm competition). Transposed to another system, tree position in a forest stand and wind speed on each day of the reproductive season may have an interactive effect on pairwise reproductive success through the probability of

encounter between gametophytes. Additionally, dynamic traits could also be included in our model, traits which value may change at each mating episode. In our case study, individual body size did not change across mating occasions. However, one could consider testing the effect of experience, or level of energetic reserves on each component of reproductive success. For example, sperm depletion may lead to reduced number of offspring sired by a male on late mating occasions without affecting probability of copulation.

References

See general references.

Supplementary Information 3:

A draft for a publication around the effects of environmental stochasticity on reproductive investment presented in chapter V.II.

The draft is here provided in its current shape.

Experimental evidence of population differences in reproductive investment conditional on environmental stochasticity.

Zoé Gauthey[a,d], Alexandre Herman[b], Stéphane Panserat[b], Arturo Elosegi[c], Cédric Tentelier[a,d] & Jacques Labonne[a,d*]

[a] INRA, UMR 1224, Ecologie Comportementale et Biologie des Populations de Poissons, Aquapôle, quartier Ibarron, 64310, Saint-Pée sur Nivelle, France.

[b] INRA, UR 107, Nutrition Metabolism Aquaculture, Aquapôle, 64310, Saint Pée sur Nivelle, France.

[c] Faculty of Science and Technology, University of the Basque Country UPV/EHU, 48080 Bilbao, Spain.

[d] Univ Pau & Pays Adour, UMR 1224, Ecologie Comportementale et Biologie des Populations de Poissons, UFR Sciences et Techniques de la Côte Basque, Allée du parc Montaury, 64600, Anglet, France.

Keywords: reproductive investment, climate change, environmental stochasticity, ecological contrast, salmonids

Corresponding author:

zoe.gauthey@googlemail.com

Introduction

The principle of energy allocation (Fisher, 1930, Williams 1966) predict that reproductive investment should result from a trade-off between current benefits of reproduction, and future opportunities of reproduction, increased by higher survival. This concept has been widely applied and demonstrated, with a special regard to age dependent condition (Clutton-Brock and Guiness, 1982). Some authors also indicated that environmental variation should directly affect the allocation followed made by individuals (McNamara and Houston 1996). While trans-generational selection is expected to shape such evolutionary optima, it has also been shown that plasticity is possible, and that individuals may adapt to within lifetime changing environment (Bardsen et al 2010, Bardsen et al 2014). The use of environmental cues allows for the allocation process to be regulated in an adaptive way: different populations in different environments do not display the same reproductive effort patterns.

The fact that long term evolutionary processes (selection) and short term adaptive response (plasticity through perception of environmental cues) both contribute to the tuning of reproductive investment is of major interest for forecasting future evolutionary patterns regarding reproductive investment. This question is especially pregnant in the context of rapid climate change (IPCC) which predicts – and verifies (Milly et al. 2002) – increased stochastic climatic events in temperate areas, with increasing occurrence of extreme rainfalls and droughts. The magnitude of this stochasticity may not be unheard of at the scale of evolution. However, understanding by which evolutionary path and measuring how quickly organisms can answer such environmental change remains a challenge.

In the present paper, we investigate this question with a special focus on aquatic systems that are the most vulnerable to this increased climatic stochasticity: extreme rainfall will translate in sudden and major floods, and intermittent droughts are susceptible to greatly affect life histories. Gauthey et al. (see Supplementary File 1) recently demonstrated how dynamics of metabolic status throughout reproductive period could efficiently complement weight variation based measures of reproductive investment in an aquatic vertebrate, the brown trout (Salmo *trutta* L). In particular, weight variation is a good proxy of gametic investment, whereas variation of metabolic status would translate investment in behavioural activity related to reproduction. Here, we propose an experimental manipulation that investigates how different populations adjust their reproductive investment within a single reproductive season, in reaction to an ecological contrast in river flow regime: a constant environment versus a stochastic environment.

Methods

Population sampling and measures

Genitors of brown trout were sampled in two different rivers: River Bastan (France, +43° 16' 2.51", -1° 22' 32.46") and River Urumea (Spain, +43° 14' 31.81", -1° 55' 28.98"). The two rivers present a comparable annual mean discharge (about $6m^3.s^{-1}$). However, the river Bastan presents more predictable flow conditions than the River Urumea, with mainly less numerous high and low pulse events per year, a lower coefficient of variation of annual discharge, as well as lower coefficients of dispersion for monthly discharge (estimation on daily discharge time series over 31 and 17 years respectively, IHA Software, standard parameterization, see § II.II.7). Genitors were sampled by electrofishing in their natural river and brought back to the laboratory where

they were acclimatized in separated tanks - corresponding to origins - during 48 hours without food. Maturity of fish was diagnosed by palpation through the presence of sperm for males and eggs for females. Only mature fish were selected for the experiment. After the acclimatization period fish were individually anesthetized (0.3mL/L of 2-phenoxyethanol), measured, weighted and photographed. In complement to weight and body size, photography enabled us to recognize fish at the beginning and at the end of the experiment through position and identification of different red and black points that do not change over the reproductive season (Supplementary Information S2). This method allowed avoiding the use of visual tag that may affect mating behavior and individual condition.

Experiences

An experiment of semi-natural reproduction was conducted from November 2012 to February 2013 in an experimental channel beside Lapitxuri Stream, a tributary to the Nivelle River in south-western France (+43° 16' 59", -1° 28' 54"). This experimental design closely matches Gauthey et al. (Supplementary File 1) first experimental design, whose results will serve as a control in the present study.

Two separated reaches of 30 meters each were constituted to form two distinct environments controlled by different water flow during the entire experiment: constant water flow (experiment B1, constant environment) and stochastic water flow (experiment B2, variable environment). In experiment B1, water flow was maintained at an intermediate value around 210 $m^3.h^{-1}$. In experiment B2, rapid water flow variations were executed and followed three modalities: low water flow (180 $m^3.h^{-1}$), intermediate water flow (210 $m^3.h^{-1}$) and high water flow (360 $m^3.h^{-1}$). Within each environment, three communicating linear sections were used, each measuring 10 meters long and 2.80 meters

wide. In the middle section (10 meters), cover bed was set up with specific size of substrate and dedicated to reproduction whereas the remainder 20 meters was arranged with bigger particle size and visual obstacles, in order to provide hiding and resting areas for fish. Within each environment, fish were free to move between the spawning ground and the resting area. Nets were placed downstream of the two environments reaches to catch drifting individuals.

We started releasing fish on November 21th to December 13th. Fish of both populations were attributed quasi-randomly to an environmental section: we made sure that the distribution of body size between the two different environments were the same. Fish were removed using electrofishing from the artificial river on February 13th 2013, two weeks after the last reproduction observed. At this period, we did not see any more reproductive behaviors, as characterized by females digging or male chasing. Fish were anesthetized, measured and weighted, a blood sample was taken. Fish were stripped to assess if there was any remaining eggs or sperm. Each fish was identified thank to pictures taken before the reproduction, kept in a tank and released 48 hours later in their original river.

In Gauthey et al. (Supplementary File 1) initial experiment (from November 2010 to February 2011), a single reach of 50m long was used, composed of 5 adjacent sections, and a nesting site was arranged in each section. Discharge was maintained at 210$m^3.h^{-1}$, and only fish originating from the Bastan population were used (experiment A).

Measurement of metabolites in plasma

Blood samples were centrifuged for 5 min at 3500 rpm, 300 µl of plasma were removed and placed in a new tube, and immediately frozen at -20°C and then at -80°C. As previously measured in other studies (Panserat et al., 2002; Kamalam et al., 2012), the

concentration of plasma triglycerides (Sobioda kit, bioMérieux) and free fatty acids (NEFA HR kit, Wako Chemicals, Neuss, Germany) were determined using commercial kits adapted to a microplate format. The metabolite concentration for each adult was measured in $g.l^{-1}$ before and after the reproduction.

Statistical analyses

The relative variation of triglycerides and free fatty were calculated as the difference of the plasma concentration before the reproduction to plasma concentration after the reproduction, divided by the plasma concentration before the reproduction. These variations generally ranged between -1 and 1, hence, when the variation was positive, the level of plasma metabolites decreased during the reproductive season whereas it increased when the variation was negative. The relative variation of weight was calculated in the same way. Weight, triglycerides and free fatty acids relative variations during the reproduction between experiments and sex for each population were studied using a Kruskal-Wallis rank sum test (KW test). Because we sometimes used multiple comparisons in our tests (two multiple tests), the Sydak adjustment method was used to adjust the classical alpha threshold (alpha = 0.02532 instead of 0.05 in that case).

Results

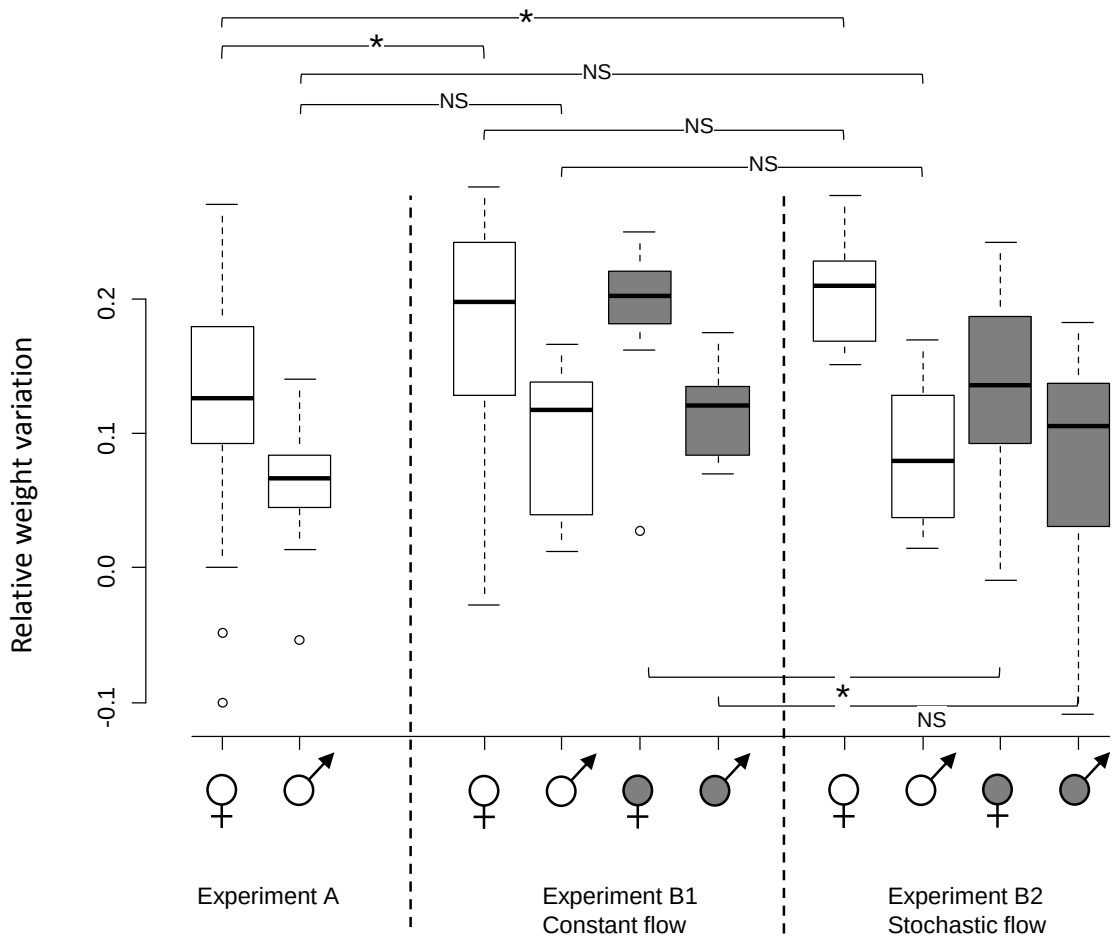


*Figure 1: box plots of relative weight variation for experiments A, B1 and B2, for females and males of the Bastan population (open symbols) and the Urumea population (grey symbols). NS = non significant difference between distributions, * = significant difference between distributions.*

Weight variations (Figure 1)

Weight variations for Bastan females were slightly more important in experiment B1 than in experiment A (KW test 1 df, $p = 0.01234$), and much higher in experiment B2 compared to experiment A (KW test 1 df, $p = 0.000314$). However, weight variations in

Bastan females were not different between experiments B1 and B2 (KW test 1 df, p = 0.8689).

For Bastan males, there was no difference in weight variation between experiments A and B1 (KW test 1 df, p = 0.2421) or between experiments A and B2 (KW test 1 df, p = 0.5428). There was no difference either between experiments B1 and B2 (KW test 1 df, p = 0.6985).

For Urumea fish, weight variation for females were higher in experiment B1 compared to experiment B2 (KW test 1 df, p = 0.0293), but no difference was observed in males between experiments B1 and B2 (KW test 1 df, p = 0.5732).

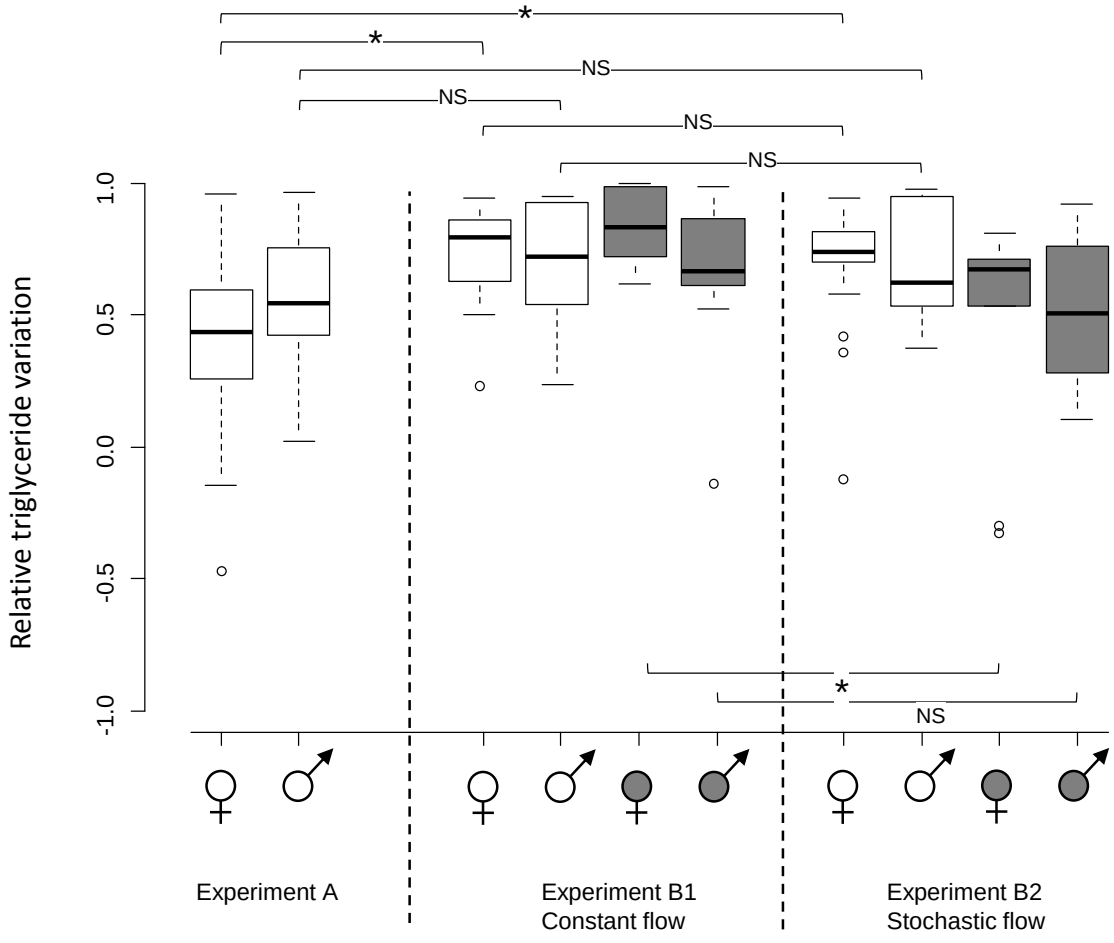


*Figure 2: box plots of relative triglycerides concentration variation for experiments A, B1 and B2, for females and males of the Bastan population (open symbols) and the Urumea population (grey symbols). NS = non-significant difference between distributions, * = significant difference between distributions.*

Triglycerides variations (Figure 2)

Triglycerides variations for Bastan females were more important in experiment B1 than in previous experiment A (KW test 1 df, $p = 0.00045$), and also higher in experiment B2 compared to experiment A (KW test 1 df, $p = 0.0018$). However, triglycerides variation in Bastan females were not different between experiments B1 and B2 (KW test 1 df, $p = 0.7962$).

For Bastan males, there was no difference in triglycerides variations between experiments A and B1 (KW test 1 df, $p = 0.1271$) or between experiments A and B2 (KW test 1 df, $p = 0.2476$). There was no difference either between experiments B1 and B2 (KW test 1 df, $p = 0.8973$).

For Urumea fish, triglycerides variations for females were higher in experiment B1 compared to experiment B2 (KW test 1 df, $p = 0.001331$), and no difference was observed in males between experiments B1 and B2 (KW test 1 df, $p = 0.21$).

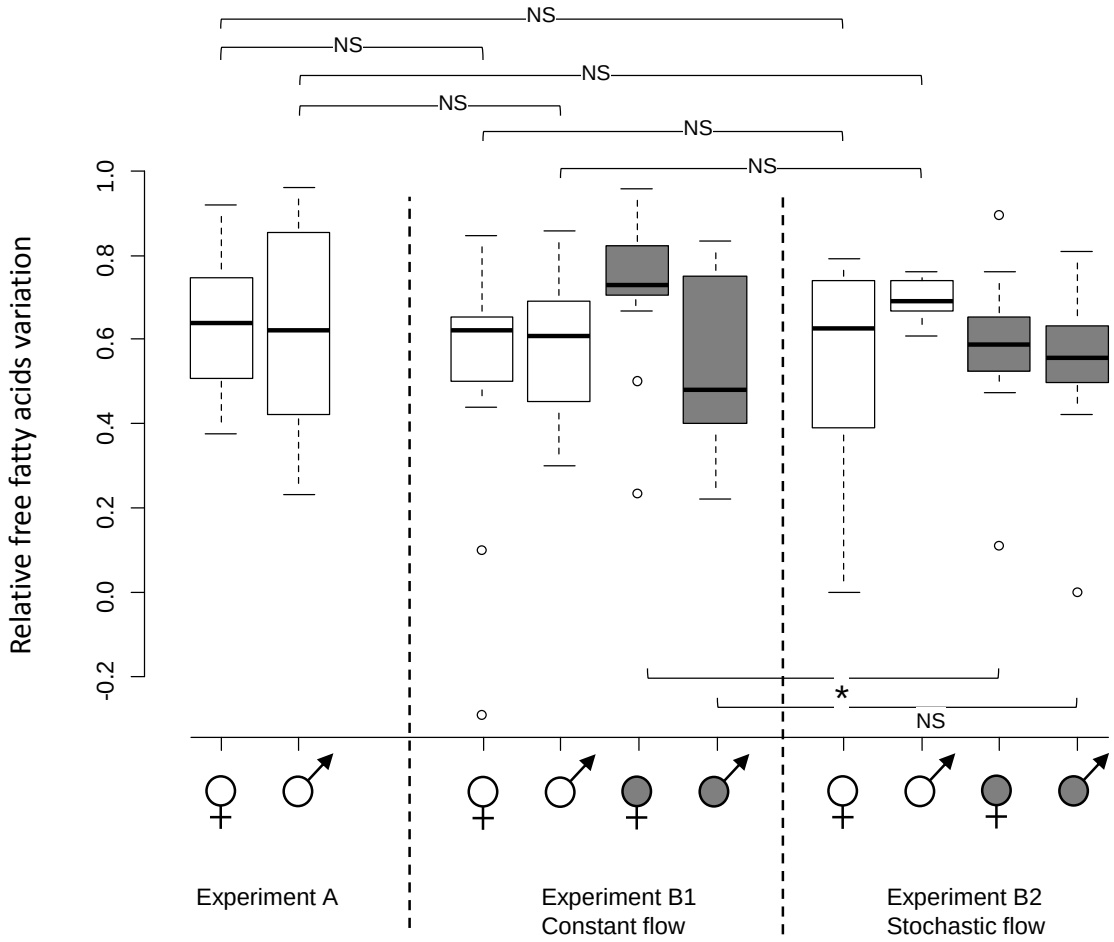


*Figure 3: box plots of relative free fatty acids concentration variation for experiments A, B1 and B2, for females and males of the Bastan population (open symbols) and the Urumea population (grey symbols). NS = non-significant difference between distributions, * = significant difference between distributions.*

Fatty acids variation (Figure 3)

Free fatty acids variations for Bastan females were not different between experiments A and B1 (KW test 1 df, $p = 0.3662$), or between experiments B1 and A (KW test 1 df, $p = 0.3988$), and between experiments B1 and B2 (KW test 1 df, $p = 0.9868$).

For Bastan males, there was no difference in free fatty acids variations between experiments A and B1 (KW test 1 df, $p = 0.5248$), between experiments A and B2 (KW test 1 df, $p = 0.5029$) and between experiments B1 and B2 (KW test 1 df, $p = 0.2144$).

For Urumea fish, free fatty acids variations for females were higher in experiment B1 compared to experiment B2 (KW test 1 df, $p = 0.02029$), and no difference was observed in males between B1 and B2 experiments (KW test 1 df, $p = 0.622$).

Discussion

The present results indicate that weight and metabolites variation throughout reproductive period tend to show replicable results to some extent for a given population: the Bastan population show the same range of variation for free fatty acids for females between experiments A and B1, and some differences in weight and triglycerides variations between the same two experiments.

These two experimental setups (A and B1) differ in their spatial organization of favorable particle size for reproduction, as well as for the length of channel used: in experiment A, favorable sites were distributed among the five sections, whereas in experiment B1, all the favorable sites were located on the same section, and the total length of river was shorter (30 m versus 50m). Additionally, in experiment B1, although density and sex ratio were in the same range than for experiment B1, fish were placed in sympatry with the Urumea population. All these factors may have modified the decision of females from Bastan to invest relatively more energy in reproduction. For the Bastan males, these differences did not seem to have modified their reproductive investment at all.

Overall, the range of variation observed here in experiment B1 confirm the previous results from Gauthey et al. (in review): both weight and metabolites concentration vary negatively during reproductive season, in relatively similar proportions respectively (about 60%).

When considering weight and metabolites variations for the Urumea population, a clear difference appears between experiments B1 and B2. This difference is expressed only in females, not males. Females tend to lose less weight, less triglycerides, and less free fatty acids in the stochastic environment. The fact that the pattern is replicated for the three indicators of reproductive investment strengthens the feeling that these females really condition their reproductive investment to environmental factors – here the level of water flow stochasticity-, whereas males from Urumea, as well as both sexes from the Bastan population, do not.

The possible causes and consequences of such reduced investment are multiple. First, these females may invest less for a maintained benefit in terms of offspring production: this would indeed points at a strong adaptation to environmental stochasticity, showing that in this condition, Urumea females achieve a better tradeoff than Bastan females. Second, they may invest less, for a proportional benefit: in that case, it would indicate that the tradeoff between reproductive investment and offspring production remains the same, but these females may choose to allocate their energy differently, for future reproductive seasons. Third, they invest less, and they gain a lower proportional benefit, indicating that the trade-off for these females has changed negatively, thereby lowering their adaptive value. In an adjacent study, we show that these females somehow manage to ensure a significant reproductive success (Gauthey et al., Supplementary File 4), favoring the second hypothesis as the most probable: the tradeoff between investment and offspring

production has not changed, but these females are able display an adaptive strategy in terms of energy allocation within and potentially between reproductive periods.

Because individuals from genetically different populations (Gauthey et al., Supplementary File 4) show differences in energy allocation strategies, where some may change their energy allocation tactics, whereas some other cannot, all populations will not react similarly to increased environmental variation. It is possible that these differences will trigger contrasted evolutionary mechanisms in response to increased stochasticity. For the Bastan population, if increased water flow stochasticity has an impact on offspring survival for instance (see § V.II), a strong selection may shape the future strategy, by selecting negatively individuals investing too much energy at the wrong moment. The outcome of such process can also lead to extinction (Tuljapurkar, 1990). For the Urumea population on the contrary, because females are already able to perceive and adapt their investment to environmental variation, increased stochasticity will therefore directly modify the energy allocation pattern between reproductive seasons, thereby affecting the life history, with a possible increase of lifespan and a decrease of reproductive output per reproductive season.

As a conclusion, using an experimental manipulation of water flow variation, we showed that there were population differences in reproductive investment allocation with respect to this environmental factor. Some populations may be better prepared to face increasing environmental stochasticity of water discharge than others. Additionally, the evolutionary mechanism triggered by this environmental variation may differ between populations, which implies that different populations of a same species cannot be considered as replicates when forecasting the effects of future climate change on life history evolution.

References

See general references.

Supplementary Information 4:

A draft for a publication around the effects of environmental stochasticity on reproductive isolation presented in chapter V.III.

The draft is here provided in its current shape.

TITTLE:

Context-dependent assortative mating between conspecific salmonid populations.

AUTHORS:

Zoé Gauthey[a,d], Andrew P. Hendry[b], Arturo Elosegi[c], Cédric Tentelier[a,d] & Jacques Labonne[a,d*]

[a] INRA, UMR 1224, Ecologie Comportementale et Biologie des Populations de Poissons, Aquapôle, quartier Ibarron, 64310, Saint-Pée sur Nivelle, France.

[b] Redpath Museum and Department of Biology, McGill University, 859 Sherbrooke Street West, Montreal, QuebecH3A 2K6, Canada.

[c] Faculty of Science and Technology, University of the Basque Country UPV/EHU, 48080 Bilbao, Spain.

[d] Univ Pau & Pays Adour, UMR 1224, Ecologie Comportementale et Biologie des Populations de Poissons, UFR Sciences et Techniques de la Côte Basque, Allée du parc Montaury, 64600, Anglet, France.

Keywords: reproductive isolation, climate change, environmental stochasticity, ecological contrast, salmonids

Corresponding author: zoe.gauthey@googlemail.com

INTRODUCTION

Ecological speciation is now considered to be one of the main mechanisms of speciation, and thus the evolution of biological diversity (Schluter 2000; Rundle and Nosil 2005). The first step in this process occurs when divergent selection between environments leads to adaptive divergence between populations. The second step occurs when this adaptive divergence leads to a reduction of gene flow. In some cases, these two steps can reciprocally reinforce each other (adaptive divergence reduces gene flow, which increases adaptive divergence and so on) to the point that conspecific populations embark on independent evolutionary trajectories. That is, they become permanently sundered into separate biological species. Of course, many adaptively-divergent populations never progress that far and instead remain stuck in an intermediate state of partial reproductive isolation (Hendry et al., 2009; Hendry, 2009; Nosil et al., 2009).

Most studies of ecological speciation focus on its very early stages: i.e., adaptive divergence and partial reproductive barriers between conspecific populations (Schluter 2000; Rundle and Nosil 2005). These studies can be roughly divided into two sets. One set focuses on genetic differences between populations inhabiting different environments (theory: Thibert-Plante and Hendry 2010; reviews: Orsini et al. 2013; Shafer and Wolf 2013; Sexton et al. 2014). The other set focuses on specific reproductive barriers between those populations (reviews: Nosil et al. 2005; Funk et al. 2006). Particularly common in this latter set are studies focusing on the evolution of assortative mating, a reproductive barrier expected to be critical in generating strong reproductive isolation (Bolnick & Kirkpatrick, 2012, Langerhans & Makowicz, 2013; Maan et al., 2010; Servedio & Kopp, 2012). Our study falls into this final category.

Studies of assortative mating between conspecific populations are frequently used for inferring ecological speciation, but these studies typically suffer from several limitations . First, many studies are conducted in the laboratory with controlled pairings (Nosil, 2002; Schmid et al., 2013; Schwartz et al. 2010), in which case the estimated assortative mating might not reflect patterns evident in more natural contexts. Second, nearly all studies assess assortative mating in only one environmental context (one lab setting or one environment in nature), and so the context-dependency of the barrier is not known. Our study eliminates both of these limitations and thus provides new insights into the strength

and type of assortative mating that can evolves between conspecific populations. Of course, our study has its own limitations, which will be discussed later.

Salmonid fishes are appropriate for studying the early stages of ecological speciation because they show clear evidence of the two steps discussed above. First, they are renowned for their tendency to show strong and repeatable adaptive divergence between populations in different environments (reviews: Taylor 1991; Quinn 2005; Fraser et al. 2011). Second, adaptively divergent populations often show evidence of reproductive barriers and restricted gene flow (Hendry et al., 2000; Pearse et al., 2009; Peterson et al., 2014). All of this work has this far employed the first type of study design – testing for genetic differences between populations in different environments. Our study here implement the second type of study design – testing for specific reproductive barriers between populations – while also removing the above-described limitations. Specifically, we allow free interactions between populations in semi-natural stream channels representing two different ecologically-relevant environmental conditions (constant flow and variable flow). In each experimental environment, we use the genetic assignment of offspring among putative parents to quantify assortative mating based on within- and between-population (1) male-female pairings, (2) total offspring produced, and (3) total offspring produced conditional on observed male-female pairings.

Our study focused on two populations of brown trout (*Salmo trutta*) from rivers with ecologically contrasted environmental stochasticity: the River Bastan has relatively low and predictable variations in water flow. Whereas the River Urumea has higher and less predictable variations in water flow. Given that divergent water flow conditions are known to impose divergent selection, promote adaptive divergence, and reduce gene flow for fish in general (Cureton & Broughton, 2014), and in salmonids in particular (Beechie et al., 2006; Hendry et al., 2000; Imre et al., 2002), we would expect the same here. However, because we do not have replication of populations from these two types of environmental conditions, we cannot be certain that our findings are the result of adaptation to different flow conditions. Our experiment thus tests for assortative mating between populations from contrasting environments, but cannot with surety attribute the observed patterns to any specific environmental difference – although we will certainly speculate as to the possibilities.

II MATERIALS AND METHODS

Population sampling and measures

Our study populations originate from the River Bastan (France, +43° 16' 2.51", -1° 22' 32.46") and the River Urumea (Spain, +43° 14' 31.81", -1° 55' 28.98"). The two rivers present a comparable annual mean discharge (about 6 m^3s^{-1}) where fish were sampled. However, the River Bastan has more predictable flow conditions than does the River Urumea, with fewer high and low pulse events per year, a lower coefficient of variation for annual discharge, and a lower coefficient of dispersion for monthly discharge (based on daily discharge time series over 31 and 17 years, respectively: IHA Software, standard parameterization, see § II.II.7). We will hereafter refer to populations originating from River Bastan and River Urumea as Pop A and Pop B respectively. Adults were sampled from the rivers by electrofishing and were brought back to the laboratory where they were acclimatized in separated tanks for 48 h without food.

After the acclimatization period, the fish were individually anesthetized (0.3 $ml.l^{-1}$ of 2-phenoxyethanol), measured, weighted and photographed. The photographs allowed us to identify individual fish at the beginning and end of the experiment through the position and shape of red and black spots (§ II.II.3). This method allowed us to avoid the use of visual tags that might affect mating behavior and individual condition. Sexual maturity was assessed by palpation to reveal the presence of sperm for males and eggs for females. Only mature fish were selected for the experiment.

The experiment

The experiment was conducted from November 2012 to April 2013 in a controlled channel beside the Lapitxuri Stream, a tributary to the Nivelle River in south-western France (+43° 16' 59", -1° 28' 54"). This experimental channel is fed with natural river water and presents a 2% slope. Because the experimental channel is derived from a natural river, food is readily available by drift from incoming water. This channel has been used in a number of experiments of reproductive behavioral in salmonid fishes (Hendry & Beall 2004, Gauthey et al. Supplementary Information 1)

Two separate reaches of 30 meters each were used to generate two distinct environments controlled by different water flows: constant flow (Constant environment) and variable flow (Variable environment). In the Constant environment, water flow was maintained around 210 $m^3.h^{-1}$ (+/- 5 $m^3.h^{-1)}$. In the Variable environment, rapid discharge variations were implemented in three consecutive modalities: high (360 $m^3.h^{-1}$) intermediate (210 $m^3.h^{-1}$) and low (180 $m^3.h^{-1}$). The duration of each modality was drawn randomly from a discrete uniform distribution [1-4 days]. This magnitude of discharge variation was low compared to natural conditions so as to avoid nest scouring at high discharge and nest drying at low discharge. However, the rate of water level change was much faster (about 1 to 3 minutes) than in natural environments. Within each environment, the channel was divided into three sequential sections, each measuring 10 m long and 2.80 m wide. The middle section was optimized for spawning, with 5-20 mm substrate sizes and 10-20 cm water depths at intermediate discharge. The upstream and downstream sections were optimized for hiding and resting, with 40-80 mm gravel, up to 60 cm water depth, and visual obstacles. Fish were free to move between the three sections in each environment but could not move between environments. Fish were released into the channels as they matured between November 21th to December 13th. Fish were quasi-randomly assigned to the two environmental sections, while making sure the distribution of body sizes were similar.

Fish were removed from the channels by electrofishing on February 13th 2013, two weeks after the last observed reproductive activity (digging, antagonistic behaviours). All fish were anesthetized, measured, weighed, and stripped to assess any remaining eggs or sperm. In addition, a small piece of caudal fin was sampled in order to perform genetic analysis. Each fish was identified based on the photographs taken before the reproduction, kept in a tank, and released 48 hours later into their original river.

After removal of the adults, net traps at the ends of the channels were checked every day to capture emergent juveniles. Approximately 75 days after last reproduction (about 800 degree days), all remaining juveniles were captured by electrofishing. A subsample of the juveniles was kept for genetic analysis: up to 20 juveniles were taken randomly from the traps each day per environment (irrespective of the total number of juveniles trapped) and 20% of the electrofished juveniles were kept at random. Juveniles were killed with a lethal dose of 2-phenoxyethanol and placed individually in a tube of absolute ethanol to later conduct genetic parentage analysis.

Parentage analysis

DNA was extracted using NaCl/chloroform (see detailed protocol in the supporting information) and eight microsatellites previously developed for salmonids were amplified: *Str60INRA*, *Str73INRA* (Estoup et al., 1993), *SsoSL438* (Slettan 1995), *Ssa85* (O'Reilly et al., 1996), *SsoSL417* (Slettan et al., 1995), *SSsp2216* (Paterson et al., 2004), and *Ssa410Uos* and *Ssa408Uos* (Cairney et al., 2000). We used a multiplex protocol allowing amplification of the eight loci in one polymerase chain reaction (multiplex PCR) following (Lerceteau-Köhler & Weiss, 2006). Fragments were sized on a ABI 3100-Avant (Life Technologies) using a GeneScan 500 LIZ internal size standard (Life Technologies), scored using STRand software (Toonen & Hughes, 2001), and raw allele sizes were binned into discrete allele classes using MSatAllele package (Alberto, 2009) for R version 2.13.0 (R Development Core Team 2011).

We used the "parents pair analysis, sexes known" option in Cervus (version 3.0.3, Kalinowski 2002) to assign parents to each sampled offspring based on allele frequencies computed from genotypes of the candidate parents. The following simulation parameters were used: 10000 cycles, 33 candidate mothers and 19 candidate fathers in the Constant environment, and 31 candidate mothers and 19 candidate fathers in Variable environment, a mistyping error rate of 1%, and a genotyping error rate of 1% to assign juveniles to parents. All juveniles with more than one locus missing were removed from the analysis. We accepted parentage assignment at a confidence level of 95% and only when the juvenile was assigned to both parents.

Hardy Weinberg equilibrium and linkage disequilibrium between loci were tested using Genepop 4.2 (Rousset, 2008) with Bonferroni correction for multiple comparisons. Genetic distance between the populations was calculated using (Weir & Cockerham, 1984) θ on adult genotypes and a bootstrap method was used to assess if this distance was significantly different from a random value based on observed genotypes distribution.

Reproductive isolation calculation

Reproductive success data calculated from the parentage assignment resulted in a matrix of non-negative integers quantifying the number of offspring assigned to all possible pairs of males and females in each environment (see Appendix 1). From this matrix, two reproductive isolation indexes were calculated for each experimental environment based

on Sobel & Chen (2014). The first index estimated reproductive isolation based on mating success (at least one offspring detected for a given male-female pair) as follows:

$$\mathrm{RI_{ms}} = 1 - 2 \times \frac{(M_{between})}{(M_{between} + M_{within})}$$

where $M_{between}$ represents the number of matings between populations, and M_{within} represents the number of matings within populations. The second index estimated reproductive isolation based on total reproductive success conditional on having mated (number of offspring detected per successful pair):

$$\mathrm{RI_{rs}} = 1 - 2 \times \frac{(O_{between})}{(O_{between} + O_{withn})}$$

where $O_{between}$ represents the total number of offspring produced by pairs from different populations and O_{withn} the total of juveniles produced by pairs from the same population.

To determine if RI_{ms} and RI_{rs} in each environment were greater or lesser than expected by chance, we calculated expected values under a panmictic scenario using a bootstrap approach. For RI_{ms}, we generated 10000 new matrices of mating success by randomizing pairs of males and females and attributing an observed mating success to each new pair. That is, the observed matings (number of offspring per pair) were assigned to new pairs of parents chosen at random from all possible pairs in a given environment. For each simulated matrix, RI_{ms} was calculated and the observed RI_{ms} was compared to this randomized distribution. For RI_{rs}, we used the same randomized distributions but this time took into account the number of offspring. Finally, we calculated expected random reproductive success conditioned by the observed matings: pairs of individuals that actually mated were randomly associated to observed reproductive success. This alternative approach allowed us to check if RI_{rs} values were solely driven by pre-zygotic isolation (i.e., RI_{ms}) or if there was additional post-zygotic isolation.

III RESULTS

Parentage assignment and genetic distance

Adults from the two populations were strongly genetically differentiated (θ = 0.147, $p<0.00001$), which suggests the potential for reproductive barriers to have evolved and

for our methods to reveal them. A total of 1305 juveniles were sampled and only 18 were excluded owing to missing data at more than one locus (Constant environment= 13; Variable environment = 5). In the Constant environment, 555 juveniles were successfully genotyped and 552 could be assigned to both parents (95% confidence level). In the Variable environment, 732 juveniles were successfully genotyped and 731 juveniles could be assigned to both parents. In the constant environment, three Pop A adults (two males and one female from Pop A) escaped at the beginning of the experiment and one Pop A female died. Only one offspring was assigned to each of the females (none to the males) and so these four individuals (and the two juveniles sired) were excluded from further analyses.

Mating success and reproductive success

Parentage analysis revealed at least 40 successful mating pairs in the Constant environment and 55 successful mating pairs in the Variable environment (Table 1). Out of the 63 females, only four (Constant environment: one from population B; Variable environment: two from population A and one from population B) were still ovigerous at the end of the reproduction, indicating that they did not lay their eggs during the experiment. The total number of offspring inferred per male (remembering that juveniles were subsampled) varied between 0 and 201 in the Constant environment and between 0 and 270 in the Variable environment. The total number of offspring inferred per female varied between 0 and 86 in Constant environment and between 0 and 112 for Variable environment.

Table 1: Number of inferred mating pairs between ($M_{between}$) and within (M_{within}) populations, the number of offspring inferred between ($O_{between}$) and within (O_{within}) populations, and the associated reproductive isolation indexes (RI_{ms} and RI_{rs} respectively).

	$M_{between}$	M_{within}	RI_{ms}	$O_{between}$	O_{within}	RI_{rs}
Constant environment	17	23	0.15	246	302	0.11
Variable environment	17	38	0.38	210	521	0.43

Reproductive isolation

In the Constant environment, reproductive isolation based on mating pairs ($RI_{ms,}$ Figure 1) was low and not significantly different from zero (RI_{ms}=0.15, *P* 0.87), implying random mating between individuals from the two populations (Figure 1). In the Variable environment, however, RI_{ms} was more than twice as high and significantly different from zero (RI_{ms}=0.38, *P*=0.002), implying positive assortative mating. The reason for this assortment was that Pop A males achieved higher than expected mating success with females from Pop A but not with females from Pop B. By contrast, males from Pop B had lower than expected mating success with females from both populations (Figure 2).

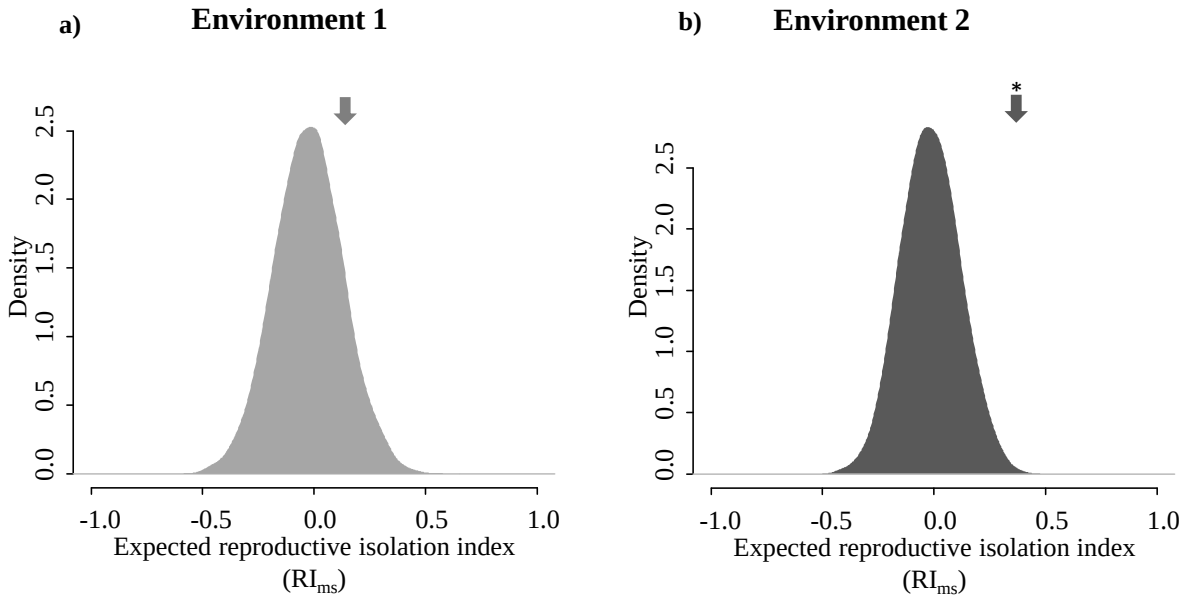


Figure 1: Density of expected reproductive isolation index calculated under a pancmictic scenario in a) Environment 1 (constant water flow) and b) Environment 2 (variable water flow). Arrows show the observed Ri_{ms} and stars represents significant difference between expected and oberved distribution.

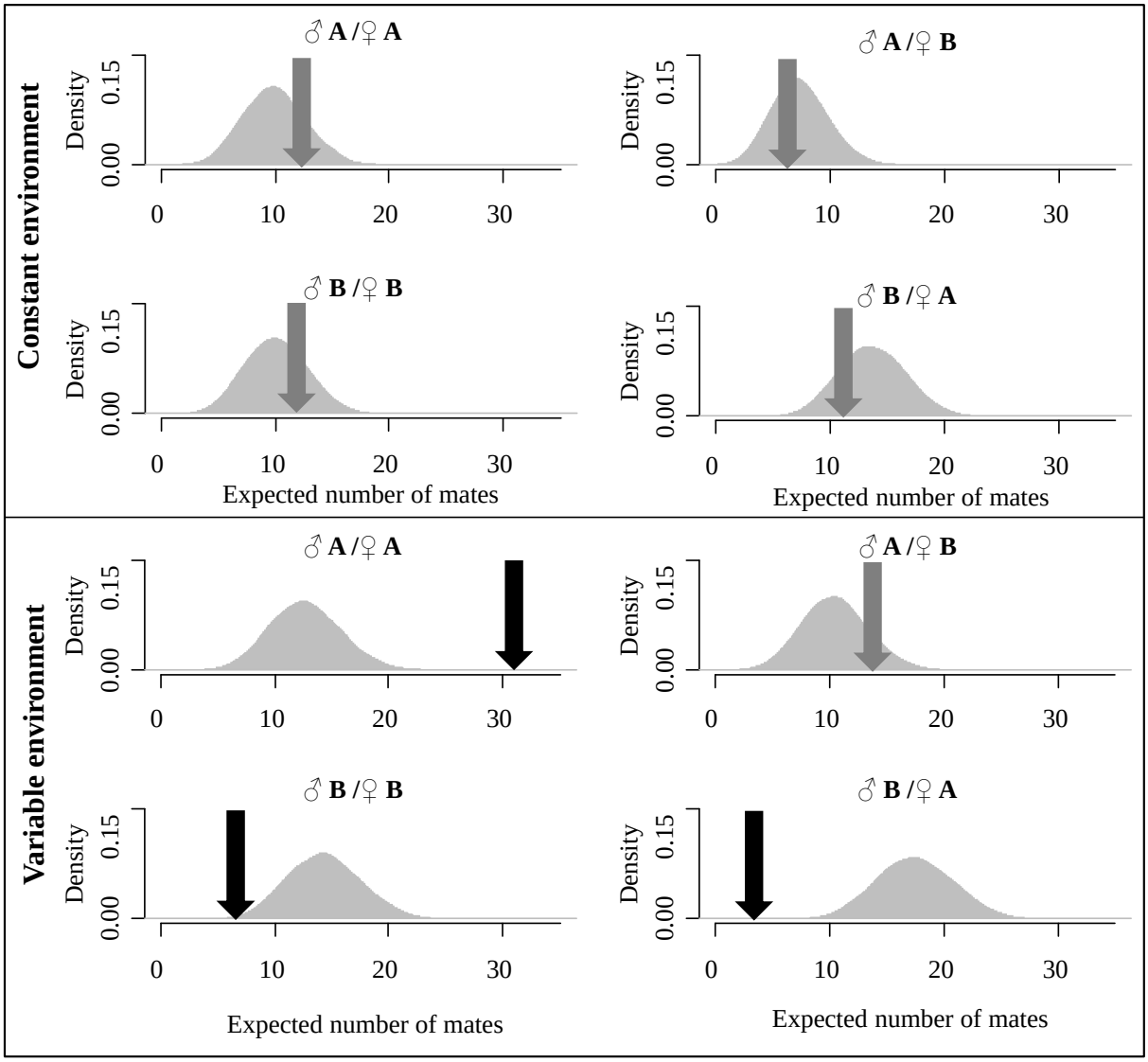


Figure 2: Observed number of mates (arrows), and expected number of mates (density probability) calculated under a panmictic scenario between populations in a) Constant environment and b) Variable environment. Grey arrows indicate that the observed number of mates falls within expected values for a panmictic scenario, black arrows indicate a significant difference between observed and expected number of mates).

Similar results were obtained when accounting for relative numbers of offspring produced per mating pair. In the Constant environment, RI_{rs} = 0.11 (P >0.05) implying random mating between the populations (Figure 1). In the Variable environment, RI_{rs}=0.43 (P =0.046), implying positive assortative mating. After accounting for realized matings (offspring produced conditional on a mating having occurred), RI_{rs} was not significantly in either environment (Constant: P=0.3189; Variable P=0.168). This result shows that

variation in RI_{rs} was fully driven by variation in RI_{ms} (whether or not a pair mated) as opposed to differential offspring reduced per mating pair.

DISCUSSION

Our experiment revealed environment-dependent reproductive isolation by means of mate choice. In the Constant experimental environment, no reproductive isolation was evident between individuals from the two populations. In the Variable experimental environment, however, positive assortative mating (measured as both male-female pairings and total offspring production) was evident between individuals from the two populations. This later result was driven primarily by male mating success: males originating from the River Urumea had a low mating success with females from both populations, whereas males originating from the River Bastan had much higher mating success with females from its own population. Additionally, positive assortment with respect to offspring production was driven by positive assortment with respect to male-female pairing, with no further contribution of differential offspring production depending on the type of pairing.

What is the cause of assortative mating? Positive assortment in the Variable environment might have been driven by inter- or intra-sexual selection. Inter-sexual selection is perhaps less important because females are not very discriminatory in salmonids in general (Garner et al. 2010) and brown trout in particular (Petersson et al 1999, Blanchfield and Ridgway 2001, Labonne et al. 2009). Two aspects of our experiment further support this inference. First, any female preference in brown trout is usually associated with trait variation, such as body size (Labonne et al. 2009), but we designed the experiments so these traits would not be contrasted between our populations and environments. Second, once accounting for observed mating pairs, we found no differential production of offspring, suggesting that females did not bias paternity against males from different populations. By contrast, intra-sexual competition driven by male-male aggression is very common in salmonids in general and brown trout in particular (Petersson and Jarvi, 1999). In our experiment, it seems that River Bastan males were more efficient at monopolizing females than were River Urumea males and that, through this ability, they directed this effort toward females from the same population. Our experiment thus adds to the growing body of literature suggesting that, although female

choice is often an important driver of assortative mating, so too can be male-male competition.

What is the cause of context dependence? The ecological contrast we implemented in the testing environments was a constant discharge versus a randomly timed variable discharge. Given that we did not replicate these treatments, we cannot be sure that this specific contrast was the cause of the differential outcomes: no assortative mating in the Constant environment but assortative mating in the Variable environment. It thus safest to state that context dependence is present in the form of different outcomes in different experiments. However, the flow contrast is certainly the most obvious candidate for a causal driver and so it is at least valuable to discuss its potential influence. The magnitude of discharge variation was not very large in the Variable environment compared to natural environments, whereas the speed of water level change was much faster (1 to 3 minutes) than in nature. We therefore here center our speculation on this latter contrast. Sudden changes in water level might be use by individuals as a cue for strong environmental unpredictability, which might then influence reproductive decisions. In our case, River Urumea males (but not River Bastan males) had reduced mating success in the Variable environment, suggesting they were less able or less willing to breed under environmental uncertainty. Interestingly, the River Urumea is much more prone to environmental fluctuations in nature than is the River Bastan, making it tempting to suggest they have evolved risk averse strategies to environmental variation in response to selection from past environmental variation.

Although our study avoided some of the limitations that attend many studies of assortative mating (laboratory settings, no test for context-dependency), it had its own set of limitations. First, as noted above, we used only two populations and only two stream channels, so we cannot confidently attribute the observed isolation to any specific environmental difference, most temptingly the different flow regimes in nature and the experiment. Second, we used wild-caught individuals, and so we cannot determine the extent to which assortative mating reflects genetic differences between the populations – it could instead reflect plasticity or prior experience. Importantly, however, the use of wild-caught individuals is the most relevant context to infer reproduction isolation in nature because any plasticity would be active there too. Moreover, it is worth noting that many previous studies similarly used wild-caught individuals in such experiments (e.g.,McKinnon et al., 2004; Nosil, 2002; Rundle, 2000).

Conclusions and implications

Despite the above limitations, we can certainly say with confidence that the two populations showed positive assortative mating in one replicate/context but not another. This finding has important implications for ecological speciation and how it is studied. First, it shows that assortative mating can arise between closely-related and geographically-proximate conspecific populations of salmonids. This result complements previous salmonid studies using genetic markers to reveal reproductive isolation between closely-related and geographically-proximate conspecific populations (Hendry et al. 2000). Unlike those previous studies, however, we have revealed a specific reproductive barrier: assortative mating. Second, the context-dependence we observed means that the strength of reproductive barriers depends critically on the specific current conditions. Although this should be considered a surprising outcome, it highlights the need for experimental studies of assortative mating to employ multiple, ecologically-relevant environments. It also shed some light on the debate between the feedback between reproductive barriers and gene flow: despite a strong genetic isolation of these two populations, some environments provide conditions for a complete gene flow, whereas other environments strongly prevent this gene flow.

Context dependence of reproductive isolation could have important implications in the context of climate change and other environmental disturbances. If, for example, flow regimes are the reason for context dependence in our experiment, we should consider the expected increase of stochastic events in river flow regimes as predicted by the coupling of climatic and hydraulic models (Milly et al. 2002, IPCC 2013). Our results suggest that these changing flow regimes could change the nature of reproductive isolation between populations. Depending on the specific populations involved, reproductive isolation might increase or decrease. In the former case, interbreeding might decrease between populations and thereby limit the potential for adaptation to changing conditions – an effect suggested in several models and experiments (Bell & Gonzalez, 2011). Decreasing gene flow might also increase the potential for inbreeding effects to depress population fitness. Alternatively, an increase in inbreeding might reduce local adaptation and thus compromise mean population fitness and contribute to range limits (Kirkpatrick & Barton, 1997). The specific effects are uncertain but the key point is that we should be not only assessing the effects of environmental change on evolution within populations but

also interactions between them, specifically the degree of interbreeding and therefore gene flow.

REFERENCES

See general references.

		CONSTANT ENVIRONMENT																														
		Female ID																														
		BF104	BF105	BF106	BF108	BF53	BF55	BF56	BF60	BF61	BF63	BF64	BF65	BF68	BF71	BF74	BF75	BF77	UF01	UF03	UF05	UF07	UF09	UF12	UF15	UF17	UF18	UF20	UF22	UF25	UF27	UF28
Male ID	BM81	0	0	14	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM87	2	0	1	0	0	0	2	0	0	8	0	0	7	0	0	33	0	0	27	0	0	0	0	0	0	39	0	0	2	79	0
	BM90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	3	0	0	0
	BM94	3	0	0	0	0	0	0	0	0	0	7	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM34	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM36	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	7	0	0	59	0	40	0	0	0	0
	UM37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM39	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
	UM41	0	44	15	0	0	3	0	0	0	0	0	0	0	17	0	5	0	0	28	0	1	0	0	1	0	0	40	18	0	1	0
	UM43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM44	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM49	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 1: Matrices of reproductive success between pairs of mates for Constant (above) and stochastic (below) environments.

		STOCHASTIC ENVIRONMENT																														
		Female ID																														
		BF101	BF107	BF51	BF52	BF54	BF57	BF58	BF59	BF62	BF66	BF67	BF69	BF72	BF76	BF78	BF80	UF02	UF04	UF06	UF08	UF10	UF11	UF13	UF14	UF16	UF19	UF21	UF23	UF24	UF26
Male ID	BM82	0	0	0	9	0	0	1	1	8	4	0	0	0	0	0	5	25	0	0	0	0	0	0	0	0	0	0	1	0	0
	BM83	0	0	0	10	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
	BM86	0	0	2	42	0	5	1	1	23	6	24	0	0	0	21	3	3	0	112	0	11	4	12	0	0	0	0	0	0	0
	BM88	0	0	0	3	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	3	0	0	0	0	0	0	0	0
	BM89	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM91	0	0	0	0	0	0	73	0	0	0	12	9	0	75	0	37	0	0	0	0	0	0	0	4	0	18	2	0	0	1
	BM93	0	0	0	1	0	0	2	0	0	0	0	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	12	0	0	0	1	6	0	0	0	0	4
	UM32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	1	0	0	0	0	0	0
	UM42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37	0	0	0	0	0	0	0
	UM47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM50	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary Information 5:

OpenBugs code, inits, and data for the fitness model presented in § IV.IV.3.

MODEL:
EFFECT OF BODY SIZE ON ENCOUNTER RATE, COPULATION RATE AND OFFSPRING NUMBER

 # likelihood

```
model {
          # Encounter rate
for (i in 1:I) {
      for (j in 1:J) {
         for (k in 1:Kobs) {
            # actual meeting process
            E[i,j,k] ~dbern(pe[i,j])          #pe=encounter probability
            # noise process (observed meeting?)
            O[i,j,k]~dbern(po)               #po= probability to observe the I and j pair during mating episode k
         }
         Ecumul[i,j]<-sum(E[i,j,])          # Sum of encounters
         logit(pe[i,j]) <- a[1]+b[1]*BSM[i]/1000+c[1]*BSF[j]/1000   # inference of male and female body size
on pe
   }
   # Splitting the observed data matrix in k occasions.
   for (i in 1:I) {
      for (j in 1:J) {
         for (k in 1:Kobs) {
            OEinter[i,j,k]<-O[i,j,k]*E[i,j,k]
         }
         OES[i,j]<-sum(OEinter[i,j,])
         OE[i,j]~dnorm(OES[i,j],100)    #relation with observed data on encounter
      }
   }
          # Copulation rate
   for (i in 1:I) {
      for (j in 1:J) {
         for (k in 1:Kobs) {
         #actual gamete release process
         G[i,j,k]<-step(E[i,j,k]-0.1)*GE[i,j,k]     # if no encounter occurred, then no gamete release occurred.
         GE[i,j,k]~dbern(pg[i,j])                   #gamete release probability pg
         }
       logit(pg[i,j]) <- a[2]+b[2]*BSM[i]/1000+c[2]*BSF[j]/1000  # inference of male and female body size on
pe
       Gsum[i,j]<-sum(G[i,j,])
       Gcumul[i,j]~dnorm(Gsum[i,j],100)       # relation with observed data on copulation
      }
    }
```

```
# generation of K matrices of encounter and copulation. We assume NME<Kmax to limit possible
#numerical problems.
   counterVar~dpois(NME)                        # NME is the number of mating episode to estimate.
    for (i in 1:I) {
       for (j in 1:J) {
          for (k in 1:Kmax) {
           counter[i,j,k]<-step(counterVar-k)   # no need for inference If NME is reached and inferior to Kmax.
             Etheo[i,j,k] ~ dbern(pe[i,j])      # actual probability of encounter for a given pair in any mating
episode
             Ereal[i,j,k] <- counter[i,j,k]* Etheo[i,j,k]
             Ginter[i,j,k]<-step(Ereal[i,j,k]-0.1)*Greal[i,j,k]  # no copulation if no encounter.
              Greal[i,j,k] ~ dbern(pg[i,j]) # actual probability of copulation for a given pair in any mating
episode
              SRreal[i,j,k]<- Ginter[i,j,k]*Nreal[i,j,k]   # no offspring if no copulation.
              Nreal[i,j,k]~dpois(pn[i,j])  # expectancy of offspring number.
           }
             # Offspring number
             log(pn[i,j])<- a[3]+b[3]*BSM[i]/1000+c[3]*BSFj]/1000 # inference on number of offspring
produced
          SR[i,j]~dnorm(mu[i,j],1000)  # relationship with data.
          mu[i,j]<-sum(SRreal[i,j,])
          Erealcumul[i,j]<-sum(Ereal[i,j,])
       }
    }
    # non informative priors
for(i in 1:3) {
    a[i]~dnorm(0,0.001)
    b[i]~dnorm(0,0.001)
    c[i]~dnorm(0,0.001)
}
    po~dbeta(1,1)
   NME~dunif(0,100)         # this prior is informative. Based on biological knowledge.
}
```

 # Some initialization values for hyper-parameters

```
list(a = c(0.2459,30.87,4.508), b = c(4.322,-11.53,-7.405), c = c(-5.245,-7.346,2.56), po = 0.4845, NME=55)
```

data

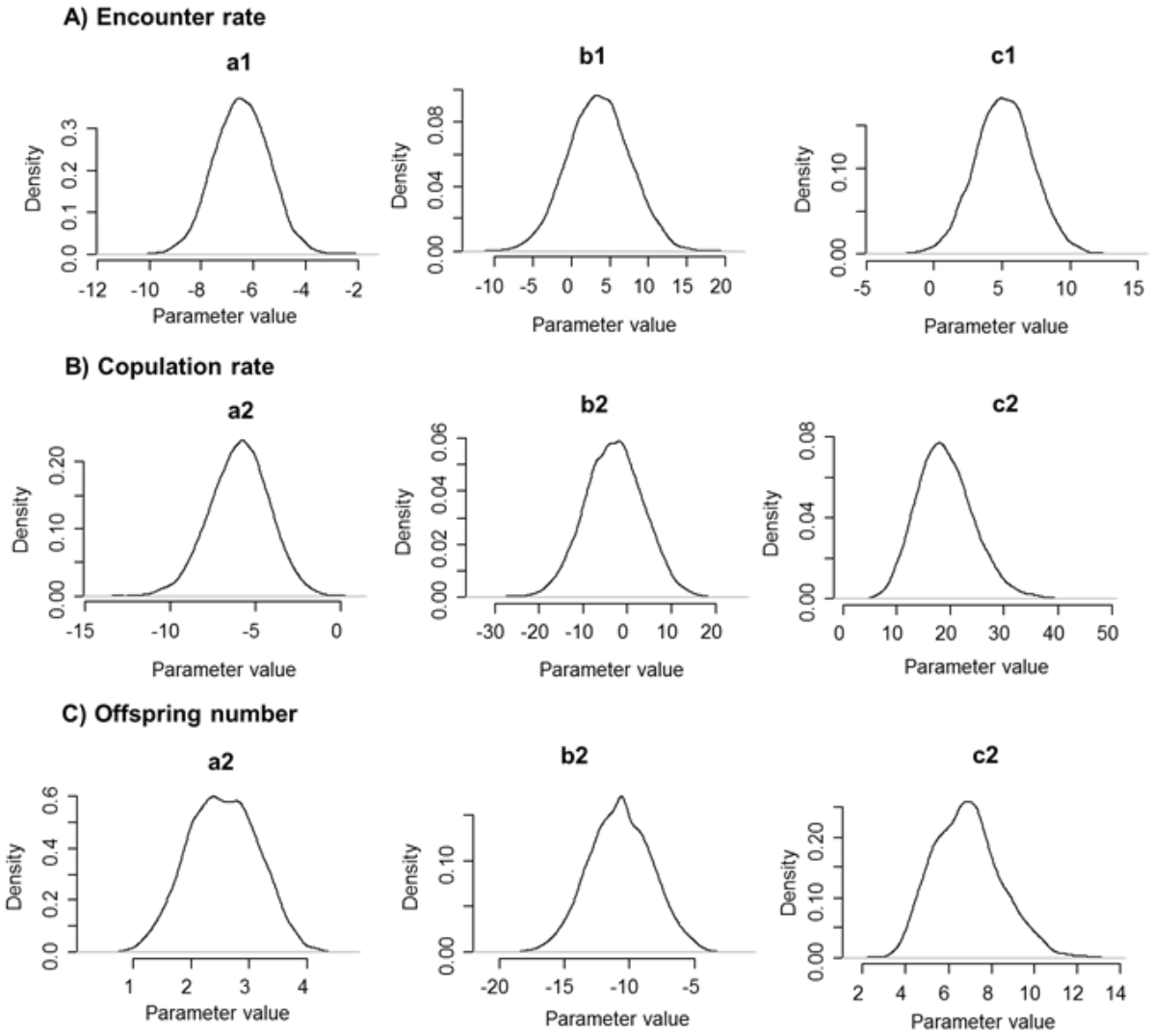
```
list(


BSF=c(228,218,214,232,236,199,238,216,237,204,228,242,252,266,216,215,261,184,207,252,193,239,264
,214,235,194,270,206,177,180,200,212),


BSM=c(230,225,342,230,341,235,165,194,196,266,209,205,281,222,231,220,253),


SR=structure(.Data = c(0,0,14,0,17,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
```

```
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
2,0,1,0,0,0,2,0,0,8,0,0,7,1,0,0,33,0,0,27,0,0,0,0,0,0,39,0,0,2,79,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,6,3,0,0,0,
3,0,0,0,0,0,0,0,0,0,7,0,0,0,0,0,9,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,4,7,0,0,59,0,40,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,0,1,0,0,
0,44,15,0,0,3,0,0,0,0,0,0,0,0,17,0,5,0,0,28,0,1,0,0,1,0,0,40,18,0,1,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0),.Dim=c(17,32)),

OE=structure(.Data = c(0,0,1,0,0,0,0,0,0,0,0,0,0,0,2,0,0,0,0,1,0,0,0,0,0,2,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,0,0,1,0,0,
0,0,0,0,0,0,0,0,0,1,0,0,0,0,1,0,0,0,0,1,0,0,0,0,0,0,3,0,0,0,3,0,
0,0,0,0,0,0,0,0,0,0,1,0,0,0,1,0,1,0,0,0,0,0,0,0,0,1,3,0,1,1,3,0,
0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,1,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,0,0,0,0,
0,0,0,0,0,0,0,1,0,0,0,0,0,0,1,0,1,0,0,0,0,0,1,0,0,2,2,0,0,0,3,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,2,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,1,0,0,0,1,0,1,0,0,1,0,0,0,0,0,1,2,0,1,1,2,0,
0,0,0,0,0,0,0,0,0,0,1,0,0,0,2,0,1,0,0,1,0,0,0,0,0,0,3,1,2,0,2,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
```

```
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,1,0),.Dim=c(17,32)),

Gcumul=structure(.Data = c(0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,2,0,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0,3,0,0,0,1,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,0,0,2,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,0,0,0,1,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,2,0,1,0,0,0,0,0,0,0,0,0,0,0,2,0,1,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,0,0),.Dim=c(17,32)),
I=17,
J=32,
Kobs=22,
Kmax=100
)
```

Supplementary Information 6:

Posterior densities for the hyper-parameters of interest for the fitness model presented in § IV.IV.3.



a[i] parameters are intercepts, b[i] parameters stand for the effect of male body size, c[i] parameters stand for the effect of female body size, on each *i* process (1=encounter rate, 2= copulation rate, 3=offspring number).

Supplementary Information 7:

Parental tables as obtained from genetic assignation for experiments A, B1 and B2.

Experiment A

		Females																											
	ID	A	AA	BB	D	E	F	F265	G	GG	HH	II	J	K	KK	M	MM	NN	OO	P	PP	R	S	T	TT	U	UU	VV	Y	Z
Males	B	0	0	0	1	0	0	0	0	0	0	0	8	0	0	0	0	0	1	0	1	0	1	0	0	16	0	0	18	5
	C	0	0	25	42	0	0	0	0	0	0	0	0	56	0	0	4	1	0	6	0	1	6	4	0	0	0	0	0	7
	CC	1	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	DD	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	EE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	14	0	0	0	0	0	0	0	0	1
	FF	0	35	16	0	4	0	0	0	0	22	1	0	2	0	0	1	0	0	0	128	0	0	1	0	0	0	0	0	2
	H	0	1	0	0	0	0	1	8	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	29	0	9	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
	JJ	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	LL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	N	0	3	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	O	0	0	0	3	0	80	0	0	0	0	2	1	78	0	0	0	0	0	2	0	1	81	6	0	3	1	0	0	0
	Q	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	QQ	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	RR	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	SS	0	0	0	0	9	0	0	2	0	0	0	0	0	0	0	0	0	14	1	0	0	1	0	35	0	0	0	0	0
	V	0	0	0	0	0	0	10	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	W	1	0	0	0	0	0	2	1	0	1	0	0	0	0	0	0	0	0	1	1	0	4	0	0	0	0	0	0	0
	X	0	4	0	0	50	0	0	0	4	0	0	1	1	0	0	0	0	0	0	19	0	0	0	0	42	0	1	1	0

Experiment B1

		EXPERIMENT B1																														
		Females Bastan																			Females Urumea												
	ID	BF104	BF105	BF106	BF108	BF53	BF55	BF56	BF60	BF61	BF63	BF64	BF65	BF68	BF70	BF71	BF74	BF75	BF77	UF01	UF03	UF05	UF07	UF09	UF12	UF15	UF17	UF18	UF20	UF22	UF25	UF27	UF28
Males Bastan	BM81	0	0	14	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM87	2	0	1	0	0	0	2	0	0	8	0	0	7	1	0	0	33	0	0	27	0	0	0	0	0	0	39	0	0	2	79	0
	BM90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	3	0	0	0
	BM94	3	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Males Urumea	UM102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM34	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM36	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	7	0	0	59	0	40	0	0	0	0
	UM37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM39	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
	UM41	0	44	15	0	0	3	0	0	0	0	0	0	0	0	17	0	5	0	0	28	0	1	0	0	1	0	0	40	18	0	1	0
	UM43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM44	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM49	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Experiment B2

EXPERIMENT B2

		Females ID from Bastan																	Females ID from Urumea													
	ID	BF101	BF107	BF51	BF52	BF54	BF57	BF58	BF59	BF62	BF66	BF67	BF69	BF72	BF73	BF76	BF78	BF80	UF02	UF04	UF06	UF08	UF10	UF11	UF13	UF14	UF16	UF19	UF21	UF23	UF24	UF26
Males Bastan	BM82	0	0	0	9	0	0	1	1	8	4	0	0	0	72	0	0	5	25	0	0	0	0	0	0	0	0	0	0	1	0	0
	BM83	0	0	0	10	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
	BM86	0	0	2	42	0	5	1	1	23	6	24	0	0	0	0	21	3	3	0	112	0	11	4	12	0	0	0	0	0	0	0
	BM88	0	0	0	3	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	3	0	0	0	0	0	0	0	0
	BM89	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM91	0	0	0	0	0	0	73	0	0	0	12	9	0	0	75	0	37	0	0	0	0	0	0	0	4	0	18	2	0	0	1
	BM93	0	0	0	1	0	0	2	0	0	0	0	0	4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Males Urumea	UM103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	12	0	0	0	1	6	0	0	0	0	4
	UM32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	1	0	0	0	0	0	0
	UM42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37	0	0	0	0	0	0	0
	UM47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary Information 8:

Behavioural interactions matrices between pairs of individuals for Experiment B1 (summed over the Kobs=22 mating episodes recorded).

Encounter matrix

EXPERIMENT B1

		Females Bastan																		Females Urumea														
	ID	BF104	BF105	BF106	BF108	BF53	BF55	BF56	BF60	BF61	BF63	BF64	BF65	BF68	BF70	BF71	BF74	BF75	BF77	UF01	UF03	UF05	UF07	UF09	UF12	UF15	UF17	UF18	UF20	UF22	UF25	UF27	UF28
Males Bastan	BM81	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0
	BM84	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
	BM87	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	3	0	0	0	3	0
	BM90	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	3	0	1	1	3	0
	BM94	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Males Urumea	UM102	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	UM33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	UM36	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	2	2	0	0	0	3	0
	UM37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
	UM39	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0	1	2	0	1	1	2	0
	UM41	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	1	0	0	1	0	0	0	0	0	0	3	1	2	0	2	0
	UM43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM49	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0

Copulation matrix

EXPERIMENT B1

		Females Bastan																		Females Urumea														
	ID	BF104	BF105	BF106	BF108	BF53	BF55	BF56	BF60	BF61	BF63	BF64	BF65	BF68	BF70	BF71	BF74	BF75	BF77	UF01	UF03	UF05	UF07	UF09	UF12	UF15	UF17	UF18	UF20	UF22	UF25	UF27	UF28
Males Bastan	BM81	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM87	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3	0	0	0	1	0
	BM90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM94	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	BM96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Males Urumea	UM102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0
	UM37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM39	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
	UM41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0
	UM43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	UM49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0