Year : 2021

The influences of maternal effect and inbreeding on phenotypic variation in Alpine whitefish

De Guttry Christian

De Guttry Christian, 2021, The influences of maternal effect and inbreeding on phenotypic variation in Alpine whitefish

Originally published at : Thesis, University of Lausanne

Posted at the University of Lausanne Open Archive http://serval.unil.ch
Document URN : urn:nbn:ch:serval-BIB_DC670348EAA37

UNIL | Université de Lausanne
Faculté de biologie et de médecine

Département d'écologie et évolution

The influences of maternal effect and inbreeding on phenotypic variation in Alpine whitefish

Thèse de doctorat ès sciences de la vie (PhD)

présentée à la

Faculté de biologie et de médecine
de l'Université de Lausanne

par

Christian de Guttry

Master of science in marine biology, University of Pisa (Italy)

Jury

Prof. Grégoire Millet, Président
Prof. Claus Wedekind, directeur de thèse
Prof. Nicolas Salamin, expert
Prof. Henrik Jensen, expert

Lausanne
(2021)

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole Doctorale
Doctorat ès sciences de la vie

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Président·e	Monsieur	Prof.	Grégoire	**Millet**
Directeur·trice de thèse	Monsieur	Prof.	Claus	**Wedekind**
Expert·e·s	Monsieur	Prof.	Nicolas	**Salamin**
	Monsieur	Prof.	Henrik	**Jensen**

le Conseil de Faculté autorise l'impression de la thèse de

Monsieur Christian de Guttry

Master in Marine Biology, Università di Pisa, Italie

intitulée

The influences of maternal effect and inbreeding on phenotypic variation in Alpine whitefish

Date de l'examen : 20 mai 2021

Date d'émission de l'imprimatur : Lausanne, le 3 juin 2021

pour le Doyen
de la Faculté de biologie et de médecine

Prof. Niko GELDNER
Directeur de l'Ecole Doctorale

Acknowledgement

Thank you Claus! Very often you have heard these words come from me and each time they have not been written or said simply out of politeness. I thank you because you believed in me and gave me this opportunity. When I first arrived I spoke almost no English and did not know much about evolution. Through your knowledge and the means you provided, I was able to learn everything that today you can read in this thesis. I also wanted to thank you because during my PhD you let me attend conferences and workshops all over the world and meet extraordinary people that decided, as me, that science is a good idea after all. Sometimes we take all of this for granted but I think it is not. In summary, thank you for everything you have taught me in science and about life.

I also want to thank Henrik because he is the first person to be interested in me when I was looking for a place to do my PhD. Thank you also for agreeing to be a member of my thesis committee and I hope to finally collaborate in science actively in the future. Your work has been a source of inspiration during these years. Thanks also to Nicolas for agreeing to be the internal expert of my thesis. You may not know but I have benefited greatly from the experience of some members of your research group. Thanks also to Gregoire for devoting his time as president of the thesis commission. A special thanks also go to Yann who supervised my project on whitefish sex determination. Sorry for being late but we finally got it done.

This thesis is a thanks for all the people who gravitated around me over these years at the DEE and outside. I am certain that without their knowledge and patience I would never have made it this far.

Genetics has always been my passion, but when I arrived I knew what microsatellites were and little more. I was lucky enough to meet a lab technician who wasted a lot of his time explaining all his secrets to me. Thank you for the advice you continue to give me also today. Later I was lucky enough to meet a post doc who, despite his passion for frogs and sex chromosomes, taught me how to bring out the best from fish data. Thanks mate for the evenings spent outside talking about science and life over a (some) beer. In the department, I happened to be teaching assistant in population genetics, one of the most difficult courses students can have in the bachelor's degree at UNIL. Fortunately, the course professor decided to spend some time to make me understand the importance of inbreeding and given the content of the thesis, a big thank you is due. Thanks also to the members of your group for the endless discussions on genomics. With one I have shared the joys and sorrows of a PhD since the beginning, and with another I continue to share the pains of trail running and evenings of fun.

During one of my trips to the University of Rennes, I met an expert bioinformatician who later decided to come and do his PhD in Lausanne. Thanks to him I was able to switch to hardcore (for me) bioinformatics. Finding a person and a scientist like you is rare. Thanks for everything!

Being one of the marine biology teaching assistants in Roscoff's class allowed me to see the ocean over the years away from him. I am grateful for the trust that Nicolas and Tanja had in me because there I met a lot of friends who are still part of my life. Thanks also to the fireball, your smile and your presence alone manages to bring a good mood like few other things in the world.

There aren't many people in Lausanne who work on salmonids, but I want to thank who have shared or is sharing this journey with me in Claus' group. One in particular, passionate of salmonids semen, has befriending me since I came back to Switzerland. Thank you for the days spent running, swimming, riding motorcycles, talking, travelling and dreaming about the startup.

Thanks to all the colleagues I've had in the office to whom I also apologize for my never-ending desire to talk. One in particular has become a constant in my life. From advice on data analysis to advice on how to successfully repair my heart. Looking in the mirror means understanding who we are, but when the mirror can also speak back, this happens faster. Thanks also to you, that since we met in the Evolution workshop in Arolla, you always tried to make me feel at home. In a discreet way you have always been there, and this has led to the beautiful friendship we have today.

Thanks to my family because when I talk about what I do in their eyes it seems that I'm changing the world. And thanks also to my second family in Vevey, I'm really lucky to have you there for me.

The biggest thanks go to you, the only person in the world with whom I do not have to pretend, Eléonore. Your love brought me through to the end of this wonderful experience.

Summary

Phenotypic variation within a population is the result of specific genotype-environment combinations. Maternal effects have thus a major impact on the observed phenotypic variation since changes in maternal phenotypes and genotypes might result in causal changes on offspring phenotypes. The complexity of these dynamics is increased by considering that the maternal genome is itself influenced by other population processes such as inbreeding, a concern for many populations nowadays. In this thesis we tried to understand how maternal effects, inbreeding and their interaction influence population characteristics and offspring performances in whitefish, a salmonid.

We estimated the inbreeding coefficient, using next generation sequencing data, to study how inbreeding influences female reproductive traits, including secondary sexual ornamentations, and its intergenerational effect in a species without parental care. We investigated which, among maternal effects, has the greater influence on the offspring phenotypes. We then sought to understand the selective pressures exerted on eggs of different sizes during incubation by exogenous chemical and biological stress factors. We studied the mechanism of sex determination in whitefish to obtain a sex-linked marker that would be essential for aquaculture and conservation purposes. We finally investigated the heritable nature of inbreeding coefficient in a population endemic of a lake that is still recovering from a eutrophication crisis.

We found that maternal inbreeding directly influences females reproductive traits and indirectly influences embryos growth, and that inbreeding might have driven the evolution of female sexual ornamentations. We show that maternal age and egg size are the main determinants of offspring size and that larger eggs suffer stronger selective pressures during incubation than smaller eggs. We discovered that the mechanism of sex determination in whitefish relies on the presence of multiple complete copy of the SDY gene in males and in multiple truncated copies in females. We finally found that the inbreeding coefficient can be heritable under specific circumstances. The results of this thesis highlight the importance of maternal effects and inbreeding on population dynamics and phenotypic variability of adults and hatchlings. Not only they have distinct effects, but they interact influencing the characteristics of the next generations.

Résumé

La variation phénotypique au sein d'une population est le résultat d'interactions génotype-environnement spécifiques. Les effets maternels ont donc un impact majeur sur la variation phénotypique observée puisque des modifications dans les phénotypes et génotypes maternels peuvent causer des changements importants sur les phénotypes des descendants. La complexité de cette dynamique est encore accrue si l'on considère que le génome maternel est lui-même influencé par divers processus au sein même de la population, tels que la consanguinité, un problème concernant de nombreuses populations de nos jours.

Au cours cette thèse, nous avons essayé de comprendre comment les effets maternels, la consanguinité ainsi que leur interaction influencent les caractéristiques de la population et les performances de la descendance chez les Corégones des Alpes, un salmonidé présent dans de nombreux lacs de Suisse. Afin de comprendre comment la consanguinité influence les traits liés à la reproduction et les ornementations sexuelles secondaires chez les femelles, ainsi que son effet intergénérationnel chez cette espèce sans soins parentaux, nous avons utilisé des données de séquençage de nouvelle génération couplées à de nombreuses mesures phénotypiques. Parmi les différents effets maternels, nous avons investigué lequel a la plus forte influence sur les phénotypes de la descendance. Nous avons ensuite étudié l'effet des pressions sélectives exercées par des facteurs de stress chimiques et biologiques exogènes sur des œufs de différentes tailles pendant l'incubation. Par la suite, nous avons caractérisé le mécanisme de détermination du sexe chez les Corégones afin d'identifier un marqueur génétique lié au sexe, ce qui pourrait être avantageux pour l'aquaculture et la conservation. Finalement, nous avons étudié la nature héréditaire du coefficient de consanguinité dans une population endémique d'un lac qui se remet encore actuellement d'une crise d'eutrophisation.

Nous avons trouvé, non seulement, que la consanguinité maternelle influence directement les traits reproductifs des femelles et indirectement la croissance des embryons, mais aussi que la consanguinité pourrait avoir conduit à l'évolution des ornementations sexuelles chez les femelles. Nous avons également montré que l'âge de la mère et la taille de l'œuf sont les principaux déterminants de la taille de la progéniture et que les œufs les plus gros subissent des pressions sélectives plus fortes pendant l'incubation par rapport aux œufs plus petits. Nous avons découvert que le mécanisme de détermination du sexe chez les Corégones repose sur la présence de multiples copies complètes du gène SDY chez les mâles et de multiples copies tronquées chez les femelles. Nous avons enfin démontré que le coefficient de consanguinité peut être héritable dans certaines circonstances spécifiques.

Les résultats de cette thèse soulignent l'importance des effets maternels et de la consanguinité sur la dynamique des populations et la variabilité phénotypique des adultes et des juvéniles. Ces derniers ont non seulement des effets distincts, mais interagissent aussi entre eux en influençant les caractéristiques des générations suivantes.

.

Table of content

General introduction

Natural diversity

Since the earliest studies on the evolution of species, scientists have been fascinated by the astonishing diversity of living beings. Different evolutionary trajectories have led to the formation of kingdoms, phylum and classes that differ greatly at the phenotypic level. Yet diversity is also observed among individuals belonging to the same species, population, and even family. Therefore, evolutionary biologists have tried to understand which were its bases, how it is maintained and, how it can be preserved to allow species to persist and evolve in a constantly changing environment. Together with the evolution of the species, the understanding of evolutionary mechanisms has also changed and continues to do so at an unprecedented rate. Nowadays, their understanding becomes increasingly urgent given the enormous loss of biodiversity that our planet has been suffering in recent years (Teixeira and Huber 2021a).

One of the first evolutionary biologists who attempted to explain how diversity within a species and a family can be generated and maintained across generations was Darwin with his theory on natural selection (Darwin 1859). He reported that organisms belonging to the same population exhibit a high level of variation in phenotypes and behavior and that those traits had a heritable basis. After his observations, he presented the species as a dynamic entity that evolves gradually in time thanks to the variability of individuals present within it. In parallel, Gregory Mendel, studied how traits were inherited from one generation to the next. He theorized the presence of 'heritable elements' introducing the idea that genes were largely responsible for the observed diversity. The combination of Darwin and Mendel theories gave birth to the modern synthesis that combined genetics and natural selection. The view that alleles with higher fitness increase their frequency across generations became commonly accepted, turning in one of the cornerstones of quantitative genetics. Modern synthesis is based on the study of the gene pool of a population and does not investigate other possible mechanisms determining the observed variation. Thanks to the technological advancement in the field of genetics, epigenetics and molecular biology, we know now that genes are not the only source of variation. Certainly, genetic diversity is the foundation for evolution. Neutral processes (as opposed to selective processes) such as genetic drift and mutation can also contribute to phenotypic diversity. However, phenotypic diversity is also determined by the environment, more specifically, by the interaction between genotype and environment (Freeman and Herron 2007). The ability of an organism to express different traits according to the surrounding environment is called phenotypic plasticity and its key role in rapid adaptation was immediately recognized (Pigliucci 2005). The relative importance of an individual's genome in determining its phenotype led to a new framework: the extended synthesis (Pigliucci 2007;

Wilkins 2008; Pigliucci and Muller 2010). A new role was therefore given to epigenetics effects. Epigenetics effects can be divided into transgenerational epigenetic effects and transgenerational epigenetic inheritance (Ho 2014). The former are the role of parental effects on offspring phenotypes and the latter involve the inheritance of epigenetic markers that eventually influence offspring phenotypes in the next generations (Richards 2006; Gavery, et al. 2018). This form of heritability encompasses all factors that have an influence on phenotypic determination other than the DNA sequences (Bonduriansky and Day 2009). Thanks to this renewed view of evolution, a new importance has been given to the role of parental effects in determining phenotypic diversity. Among these, one of the most important forms of non-genetic inheritance that largely influences the phenotype of the offspring are the maternal effects.

Maternal effects

The phenotype of an individual may be influenced by the genotype of a conspecific or of a group of unrelated individuals in a process called "indirect genetic effect" (Moore, et al. 1997). In many organisms, the strongest indirect genetic effects on offspring phenotype are parental effects (Uller 2008). Among them, most transgenerational interactions are caused by maternal effects (Mousseau and Fox 1998). Maternal effects are the influence of maternal phenotypic traits and of the environment experienced by the mother on the phenotype of the offspring (Falconer 1989). In addition, the mother also provides the environment in which the embryo develops until maturity. This can be the maternal body, as is the case in mammals, or an egg as in the case of birds, reptiles, fish and insects. Given the intimate relationship between the mother and embryo, maternal effects might have a strong influence on offspring phenotypes in early life-stages but also later in life (Goos, et al. 2019). Given their ubiquity, maternal effects are not limited to influencing phenotypic characteristics, but they play a role in population dynamics (Ginzburg 1998; Proulx, et al. 2019), evolutionary processes (Mousseau and Fox 1998) and also in the evolutionary response to selective pressures (Wolf and Wade 2016). Indeed maternal effects can become adaptive when the mother modify the phenotype of the offspring in response to environmental stimuli (Wade 1998). Females can adapt egg size, growth rate, sex and resistance to pathogens of their progeny based on information processed from their surrounding environment (Reznick 1991; Rossiter, et al. 1993; Bernardo 1996). Wade (1998) divided adaptive maternal effects into two categories: (i) prezygotic maternal effect, where the mother modifies gametic size or due to physiological or morphological constrains and (ii) postzygotic maternal effect depending on offspring nutrition and maternal care. If adaptive, maternal effects may then play a major role on the evolutionary response to selection. Indeed, changes arising from maternal effects occur faster and

at a higher rate compared to processes which involve DNA mutation (Kirkpatrick and Lande 1989; Crean and Marshall 2009). Among maternal effects, egg size is one of the most investigated in oviparous organisms (Bernardo 1996).

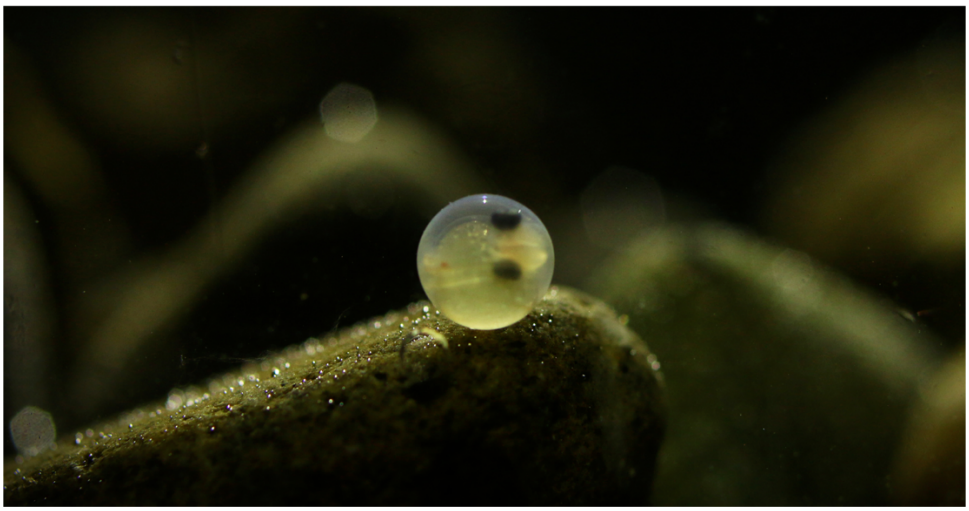


Figure 1. Incubating European grayling embryo. Photo by David Nusbaumer

This is a key trait that embodies ecological and evolutionary processes acting on the mother and on her offspring. Therefore, the size of the eggs can also be seen as the connection between the fitness of the mother and the fitness of the offspring (Bernardo 1996). Early optimality theories on egg size evolution suggested that the environmental stimuli perceived by the mother are the main determinant of egg size (Smith and Fretwell 1974). Here, ecology and evolution interact via the so called environmentally induced maternal effect making egg size adaptive (Lacey 1998). In this context, a specific egg size is expected for specific environmental conditions. Despite this, large variations in egg size within a population are frequently reported. In an attempt to explain these observations, later optimality models on egg size evolution tried to explain this variation using maternal phenotypic characteristics such as length and age (Parker and Begon 1986; Hendry, et al. 2001; Sakai and Harada 2001). Researchers were able to explain only part of the variance in egg size in a population including these two traits in optimality models but the mechanisms by which such variation is maintained are still unclear.

The size and the age of the female are two maternal traits that can greatly influence the investment in reproduction and therefore the production of eggs (Pianka and Parker 1975; Sakai and Harada 2001; Kindsvater, et al. 2011; Barneche, et al. 2018). One of the current challenges for researchers is to isolate the effect of each maternal trait and study its consequences on offspring, since most of them are strongly correlated. For conservation purposes, the identification of the maternal phenotypic traits that allow the production of high-quality offspring is crucial. For instance, in

threatened fish populations, restocking practices are implemented and larger females are commonly used for reproduction. Despite this, it has been demonstrated that in some species the oldest females and not the largest produce offspring of a higher quality (Marshall, et al. 2010).

A large body of literature demonstrated an isometric relationship between egg size and the size of the offspring after hatching (i.e. larger eggs result in larger individuals) (Einum and Fleming 1999; Leblanc, et al. 2011; Self, et al. 2018). These larger individuals have been shown to be more competitive than small individuals in swimming performance (Leblanc, et al. 2011), predator avoidance (Van den Berghe 1984) and ability to forage for food (Auer, et al. 2018). Some authors have also suggested that egg size may underlie the choice of specific life history strategies that the offspring will adopt at maturity (Hutchings and Myers 1994; Cogliati, et al. 2018). Despite all this, the evolutionary trajectories of egg size are not towards the production of larger eggs. As mentioned before, maternal traits can influence egg production and it must be considered that females have a limited amount of energy and space available for egg production, which leads to the commonly observed trade-off egg size / fecundity (Roff 1992; Einum and Fleming 1999). In addition, a crucial but overlooked life stage that could help explain egg size evolution is the incubation period. The egg provides the developmental environment for embryos during a relatively long stage of life which is characterized by a high mortality rate (Brousseau and Baglivo 1988; Iverson 1991). In aquatic environment, while incubating, selection exerted by predation (Rijnsdorp and Jaworski 1990), increasing temperatures (Regnier, et al. 2013) or anoxic conditions (Einum, et al. 2002) can act on egg-specific phenotypic traits such as size. However, these are the only selective pressures that have been tested in the literature notwithstanding the presence of multiple sources of stress. Investigating whether pollutants, pathogens, or other physical parameters have different effects depending on egg size may help us understand the observed within population/variation. In addition to egg size, egg content could also influence offspring characteristics. During oogenesis, the female provides nutrients, lipids, proteins, mRNA and other products inside the egg. The combination of these will drive development at the embryonic level and in the first post-hatching stages when organisms develop yolk sacs (Abrams and Mullins 2009). If the mother is characterized by a low genetic quality, this will be reflected in the development of the individual.

Inbreeding

Another factor that might influence not only phenotypes but also populations growth is inbreeding. Inbreeding is a term commonly used to describe the consequences of mating between relatives (Keller and Waller 2002). The study of the effects of inbreeding are returning to the spotlight due to the

worldwide decrease in genetic diversity in terrestrial and aquatic populations we are currently witnessing (Leigh, et al. 2019; Manel, et al. 2020; Teixeira and Huber 2021b). This decrease is frequently caused by climate changes (Pauls, et al. 2013), anthropogenic disturbance (Schaberg, et al. 2008), speciation reversal due to habitat-loss (Vonlanthen, et al. 2012) and overexploitation of natural populations (Pinsky and Palumbi 2014). All these phenomena can result in an increased proportion of inbreeding within a population. It happens to vary degrees naturally in almost all natural populations (Hamilton 2011; Agrawal and Whitlock 2012) but its negative consequences are usually subjected to genetic purging by natural and/or sexual selection when occurring at low frequency (Hedrick and Garcia-Dorado 2016; Caballero, et al. 2017; Noel, et al. 2019). However, in presence of an important reduction in population size, the proportion of mating between relatives increases. Under this scenario, inbreeding can have dramatic consequences on population dynamics and eventually leads the population to extinction (Losdat, et al. 2018).

When consanguineous mating happens, it increases the probability of two alleles being identical-by-descent (IBD) in the progeny leading to an increase of genome-wide homozygosity. The deleterious effects of consanguinity have their genetic basis on the accumulation of deleterious recessive mutations and/or loss of overdominance at some loci (Charlesworth and Willis 2009). As a result, inbreeding eventually changes the frequencies of the genotypes in the population in favor of homozygous genotypes (Keller and Waller 2002). In the most extreme scenarios, the increase of deleterious recessive mutations can result in inbreeding depression (Charlesworth and Charlesworth 1987). This can be defined as the reduction in the mean fitness values of inbred individuals (Szulkin, et al. 2010). The effects of inbreeding depression are more likely to be detected on life-history traits such as survival, individual growth, fitness and reproduction given their polygenic nature. Sexually selected traits are also strong determinant of reproductive success but surprisingly they have been less investigated in the context of inbreeding (Marsh, et al. 2017; Sakaluk, et al. 2019). Nonetheless, it has been demonstrated that inbreeding could also affect phenotypic traits (DeRose and Roff 1999; Wright, et al. 2008) and behavior (Ala-Honkola, et al. 2009). Depending on the trait analyzed, the effect of inbreeding depression can also be sex-specific (Ebel and Phillips 2016). For instance, it has been demonstrated that inbreeding depression act with a stronger magnitude on females when reproductive traits are considered (Su, et al. 1996; Keller 1998; Gallardo, et al. 2004; Hayes, et al. 2005; Billing, et al. 2012; Ebel and Phillips 2016). Such stronger effect is expected to be sex-dependent considering the higher investments females make in reproduction (Hayes, et al. 2005).

To quantify the magnitude of inbreeding, the pedigree-based inbreeding coefficient F_P has been traditionally used (Wright 1922). F_P estimates the proportion of the genome that is identical-by-descent (IBD) using genealogical trees and thus requires deep knowledge of the population genealogy (strong

observational component). Thus, it has always been difficult to make correct inferences in terrestrial wild populations and it is virtually impossible with aquatic organisms. Since this method does not consider Mendelian segregation and recombination stochasticity, its estimator is also called "expected *F"*. In addition, the assumption of this method such as the non-relatedness between the founders of the pedigree and between each new member entering the genealogical tree are rarely met (Huisman, et al. 2016). Nowadays with the advent of "broad sense" genomics estimates of *F* can be performed using thousands of SNPs distributed genome-wide (Garner, et al. 2016). This increased the accuracy of the estimates and outperformed the old molecular and observational techniques as it was demonstrated with simulations and empirical data (Yanez, et al. 2014; Kardos, et al. 2015; Kardos, et al. 2018; Wells, et al. 2018; McLennan, et al. 2019; Galla, et al. 2020). Among the most recent methods to estimate inbreeding, the relatedness matrices (F_{beta}) (Goudet, et al. 2018) and runs of homozygosity (F_{roh}) (McQuillan, et al. 2008) are widely used. Genomic based estimators succeed to capture the variance due to the segregation and the recombination and for this reason they are also defined 'observed *F* '(Hill and Weir 2011).

Salmonids

Historically, members of the salmonid family have always had great cultural value. In recent years, with the growth of commercial fishing and aquaculture, their economic importance has also begun to increase. As a result, they have been intensively studied in the field of applied biology. In addition, their complexity at genetic, phenotypic, behavioral and life cycle levels led them to become one of the main subjects for the development of evolutionary theories (Stearns and Hendry 2004). Salmonids are divided in 3 subfamilies: Salmoninae, Coregoninae and Thymallinae. They are ubiquitous all around the world and differ in rearing and spawning habitat. Some species complete their entire life cycle in freshwater while others make transitions between fresh and saltwater. Salmonids also differ according to their reproductive cycle. Semelparous species, such as the Atlantic salmon (*Salmo salar*), reproduce only once in a lifetime while iteroparous species, such as whitefish (*Coregonus spp*) go through more than one spawning season. This makes the latter interesting subjects to understand how aging affects life history traits. Salmonids are external fertilizers characterized by the production of thousands of gametes per reproductive bout. During the first life-stages they suffer a very high mortality (Bradford 1995) allowing the study of the related causal selective pressures as well as the identification of the affected traits. A common feature to all members of the family is the presence of sexual selection. Consequently, they have been subjects for the study of the evolution of sexual ornamentation (Andersson 1994) and mate choice (Auld, et al. 2019). Their genome is large and complex as a result

of two whole genome duplications (Macqueen and Johnston 2014). Nowadays, it can be considered diploid although the diploidization process is not yet finished and therefore paralogues are still present (Glasauer and Neuhauss 2014).

Salmonids in Switzerland

In Switzerland, salmonids are endemic in many lakes and rivers (Kottelat and Freyhof 2007). The most represented species are *Salmo trutta spp*, *Salvelinus umbla*, *Coregonus spp* and *Thymallus thymallus* that complete their entire life cycle in freshwater. Despite this, species such as lake trout (*Salmo trutta lacustris*) still migrate from the lake to the river during the spawning period (Büttiker and Matthey 1986). Most of these populations have suffered a sharp decline during the 50s to 70s due to a eutrophication crisis that has affected water bodies in the whole country. This, along with pressures from commercial fisheries in the following years, led government agencies to start ecosystem restoration and wild population management.

The lake Halwill whitefish *Coregonus suidteri*

Whitefish are found in 25 Swiss lakes where up to five ecotypes can be observed (Kottelat and Freyhof 2007). Their speciation began after the last ice age about 15,000 years ago and today they are suffering a loss of diversity that is leading to speciation reversal (Vonlanthen, et al. 2012). The lake Hallwil is a typical example of post-glacial lake of the Swiss plateau. It has a surface of about 10km^2, maximum depth of 46 and a residence time of the water of nearly 3.8 years (Liechti 1994). It has been severely affected by eutrophication and among the various measures undertaken to restore the ecosystem, a process of aeration of the deepest layers has been carried out (Müller and Stadelmann 2004). Nowadays, the trophic status of the lake improved but a large number of larvae have to be released yearly via artificial propagation to sustain the wild population and commercial fisheries. These characteristics makes the species inhabiting the lake excellent for studying the effects of inbreeding.

The endemic whitefish species, *Coregonus suidteri*, is a fast growing whitefish descending from *Coregonus lavaretus*, the common ancestor of all European whitefish (Kottelat and Freyhof 2007).

Figure 2. The lake Hallwil whitefish *Coregonus suidteri*. Photo by Christian de Guttry

During the breeding season, which takes place between December and January, individuals of both sexes gather on spawning grounds where reproduction occurs through the release of gametes into the water column. Sexual selection is thought to act at this time although the behavioral mechanism is not yet clear (Rudolfsen, et al. 2008). The occurrence of sexual selection is suggested by the presence of breeding tubercles on the side of the bodies.

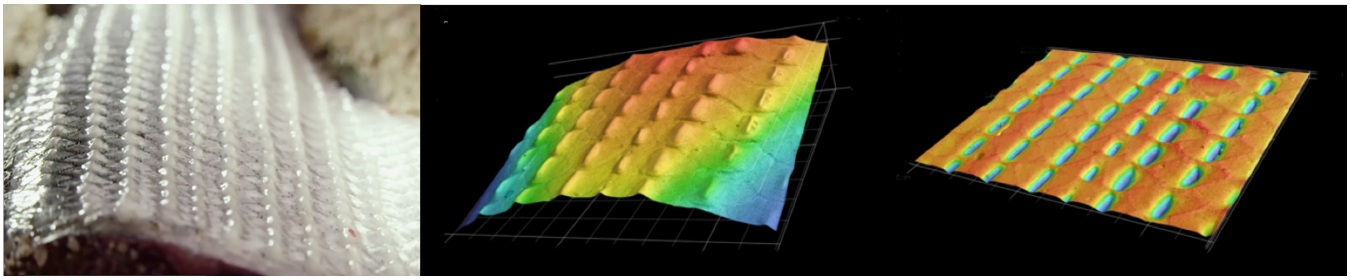


Figure 3. A whitefish body section caught during the spawning season where breeding tubercles can be observed (© arte tv; reproduced with permission). The images produced by the 3D scanner for measuring breeding tubercle characteristics (image by Christian de Guttry).

This sexual ornamentation is produced shortly before the breeding season begins and is lost during or immediately after. The peculiarity of this species is that both sexes present these tubercles even if those of females are smaller than those of males (Wedekind, et al. 2008). During the spawning season, the large number of eggs produced are left to incubate on the bottom of the lake where they do not benefit from parental care. The aforementioned characteristics make whitefish an excellent study organism for investigating the influence of maternal effects on offspring. Indeed, in the context of quantitative genetics, maternal effects are usually quantified via the proportion of variance of an offspring trait, which is explained by the identity or characteristics of the mother. To obtain accurate estimates, at least indirectly, full factorial breeding experiments (North Carolina II design) including several parents and offspring are needed (Lynch and Walsh 1998) and are possible in this species because of the large number of gametes produced by both sexes.

Figure 4. The fertilization process following a full factorial breeding design and the embryos left to incubate singly in the climate chamber. Photos by Christian de Guttry.

Another feature of Coregoninae is their sexual determination that differ from all the other members of the family. The majority of salmonids have a male heterogametic sex determination system where males differ from females due to the presence of the SDY (sexually dimorphic on the y chromosome) gene (Yano, et al. 2013). In whitefish, the SDY gene is present in both sexes and the mechanism of sex determination is still unknown.

Thesis outline

The main objective of my thesis is to identify the determinants of the phenotypic variation observed within a population in early life stages and in sexually mature individuals. I mainly looked at the influence of maternal effects, inbreeding and their interaction. Their effects were extensively hypothesized in theoretical settings but still too many inconsistencies emerge in empirical studies. I approached maternal effects by trying to understand whether egg size underlies phenotypic differences observed in early life stages and how it may act as a buffer under stressful environmental conditions. I also investigated which maternal characteristics can determine the size and quality of eggs. I then focused on the effects of inbreeding depression on adult traits and how this can interact with maternal effects on shaping population dynamics. I took advantage of the opportunity to estimate inbreeding coefficients in a wild population using genomic data. The complexity of the organism on which my thesis focused also allowed me to investigate aspects of sexual selection and sex determination, clarifying previous misconceptions. To summarize, I have divided my research into 7 main chapters:

Chapter 1: In this chapter, we focused on understanding the role of female sexual ornamentation in the lek-breeding whitefish. We had the rare opportunity to work with a species with

mutual sexual ornamentation. We started by investigating the connection between inbreeding, reproductive-related traits, and sexual ornaments to better understand what type of genetic quality is signalled by female sexual ornaments. Additionally, we looked for a possible indirect effect of maternal inbreeding coefficient on offspring performances. To accomplish this, we used genomic data (ddRADseq) to estimate the inbreeding coefficient of 31 adult whitefish. Those individuals were used in a full factorial breeding design to generate 5600 embryos raised in controlled condition. We measured the size of sexual ornaments with unprecedented precision using a 3D scanner.

Chapter 2: Phenotypic variability among eggs/offspring within the same brood has been frequently explained as the results of environmental factors (Marshall, et al. 2008). We investigated which maternal characteristics could influence the production of different sized eggs with a particular focus on inbreeding using whitefish. We have also further explored the indirect effect of maternal inbreeding coefficient on offspring performance via the size (quality) of the eggs produced. To accomplish this, we used genomic data (ddRADseq) to estimate the inbreeding coefficient of 90 adult whitefish. Those individuals were used in a full factorial breeding design to generate 7200 embryos raised in controlled condition.

Chapter 3: Two of the most frequently studied maternal effects are size and age of the mother. Understanding which of those traits have an effect on offspring has always been difficult because of their tight correlation (Marshall, et al. 2010). In this chapter, we have tried to disentangle their effect. Using fishing nets, we collected females of similar size but belonging to different age classes. This allowed us to study if size or age have an influence on egg production and on the phenotypes of offspring. To accomplish this, we collected 40 females from the whitefish population of Lake Hallwil. Using in vitro fertilization, we produced 12000 embryos divided in several experimental blocks. We monitored offspring development up to 21 days after hatching.

Chapter 4: Freshwater acidification is an underestimated process that is affecting the dynamics of these ecosystems worldwide (Ou, et al. 2015). In this chapter, we investigated the potential of the whitefish population to rapidly adapt to acidification by standing genetic variation and the possible contribution of maternal environmental effects. We took advantage of our experimental design to also investigate egg size evolution in aquatic environment. Females produce an optimal size of eggs as a result of the perception of environmental stimuli in order to maximize their fitness (Smith and Fretwell 1974). However, it is not yet clear whether small or large eggs are advantaged in stressful conditions in the incubation environment. To accomplish this, we collected 90 individuals from the whitefish population of Lake Hallwil. We used *in vitro* fertilization to produce 7200 embryos that differed in size on a continuous scale, expose them to three different pH levels and followed their development from incubation until hatching.

Chapter 5: Continuing in the same line of thought as in Chapter 4, we exposed whitefish embryos during incubation to a common salmonid pathogen *Pseudomonas fluorescence.* We wanted to test if the effect of exposure varies with egg size to determine the presence of selective pressures on different-sized eggs. To date, only the effects of hypoxia (Einum, et al. 2002) and temperature (Regnier, et al. 2013) during incubation had been tested on eggs of different sizes. In this chapter, we also looked at whether egg size determines the larval size post hatching as in other salmonids. To accomplish that we used more than 12000 whitefish embryos and exposed half of them to the pathogen. We followed their development until 21 days after hatching.

Chapter 6: Most of the salmonids are characterized by a male heterogametic sex determination system XY (Yano, et al. 2013). The gene that triggers the development of male gonads is *sdy* and is typically located on the Y chromosome. However, in whitefish this region was constantly detected also in females (Yano, et al. 2013). Consequently, the mechanism of sexual differentiation remains unknown. In order to shed some light on sexual determination in whitefish, we assembled and annotate the genome of a male to investigate the genomic architecture of the SDY region in both females and males. Analysis were performed using next generation sequencing techniques such as ddRAD sequencing, pool sequencing, and whole genome sequencing.

Chapter 7: The heritability of inbreeding coefficient, or heterozygosity, is rarely estimated and assumed to be zero. However, inbreeding coefficient can be heritable in small and structure populations (Neff and Pitcher 2008). In this chapter, we wanted to test whether the parental inbreeding coefficient influence the inbreeding coefficient of the progeny. We used 211 whitefish adults genotyped with ddRAD sequencing to estimate their inbreeding coefficient and the kinship, the expected mean inbreeding coefficient of the progeny.

General introduction references

Abrams EW, Mullins MC. 2009. Early zebrafish development: It's in the maternal genes. Current Opinion in Genetics & Development 19:396-403.

Agrawal AF, Whitlock MC. 2012. Mutation Load: The fitness of individuals in populations where deleterious alleles are abundant. Annual Review of Ecology, Evolution, and Systematics 43:115-135.

Ala-Honkola O, Uddström A, Pauli BD, Lindström K. 2009. Strong inbreeding depression in male mating behaviour in a poeciliid fish. Journal of Evolutionary Biology 22:1396-1406.

Andersson M. 1994. Sexual selection: Princeton University Press.

Auer SK, Anderson GJ, McKelvey S, Bassar RD, McLennan D, Armstrong JD, Nislow KH, Downie HK, McKelvey L, Morgan TAJ, et al. 2018. Nutrients from salmon parents alter selection pressures on their offspring. Ecology Letters 21:287-295.

Auld HL, Noakes DL, Banks MA. 2019. Advancing mate choice studies in salmonids. Reviews in Fish Biology and Fisheries 29:249-276.

Barneche DR, Robertson DR, White CR, Marshall DJ. 2018. Fish reproductive-energy output increases disproportionately with body size. Science 360:642-644.

Bernardo J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. American Zoologist 36:216-236.

Billing AM, Lee AM, Skjelseth S, Borg ÅA, Hale MC, Slate J, Pärn H, Ringsby TH, Saetern BE, Jensen H. 2012. Evidence of inbreeding depression but not inbreeding avoidance in a natural house sparrow population. Molecular Ecology 21:1487-1499.

Bonduriansky R, Day T. 2009. Nongenetic inheritance and its evolutionary implications. Annual Review of Ecology, Evolution, and Systematics 40:103-125.

Bradford MJ. 1995. Comparative review of Pacific salmon survival rates. Canadian Journal of Fisheries and Aquatic Sciences 52:1327-1338.

Brousseau DJ, Baglivo JA. 1988. Life tables for two field populations of soft-shell clam, *Mya arenaria*, (Mollusca: Pelecypoda) from Long Island Sound. Fishery Bulletin 86:567-580.

Büttiker B, Matthey G. 1986. Migration de la truite lacustre (*Salmo trutta lacustris* L.) dans le Léman et ses affluents. Swiss journal of hydrology 48:153-160.

Caballero A, Bravo I, Wang J. 2017. Inbreeding load and purging: implications for the short-term survival and the conservation management of small populations. Heredity 118:177-185.

Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237-268.

Charlesworth D, Willis JH. 2009. The genetics of inbreeding depression. Nature Reviews Genetics 10:783-796.

Cogliati KM, Unrein JR, Stewart HA, Schreck CB, Noakes DL. 2018. Egg size and emergence timing affect morphology and behavior in juvenile chinook salmon, *Oncorhynchus tshawytscha*. Ecology and Evolution 8:778-789.

Crean AJ, Marshall DJ. 2009. Coping with environmental uncertainty: Dynamic bet hedging as a maternal effect. Philosophical Transactions of the Royal Society B: Biological Sciences 364:1087-1096.

Darwin C. 1859. On the origins of species by means of natural selection. London: Murray J.

DeRose MA, Roff DA. 1999. A comparison of inbreeding depression in life-history and morphological traits in animals. Evolution 53:1288-1292.

Ebel ER, Phillips PC. 2016. Intrinsic differences between males and females determine sex-specific consequences of inbreeding. BMC Evolutionary Biology 16:16-36.

Einum S, Fleming IA. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): Norms of reaction to environmental quality. Proceedings of the Royal Society of London. Series B: Biological Sciences 266:2095-2100.

Einum S, Hendry AP, Fleming IA. 2002. Egg-size evolution in aquatic environments: does oxygen availability constrain size? Proceedings of the Royal Society B: Biological Sciences 269:2325-2330.

Falconer D. 1989. Introduction to quantitative genetics 3rd ed. Harlow: Longman Scientific & Technical.

Freeman S, Herron J. 2007. Evolutionary analysis. Upper Saddle River, NJ: Pearson Prentice Hall

Galla SJ, Moraga R, Brown L, Cleland S, Hoeppner MP, Maloney RF, Richardson A, Slater L, Santure AW, Steeves TE. 2020. A comparison of pedigree, genetic and genomic estimates of relatedness for informing pairing decisions in two critically endangered birds: Implications for conservation breeding programmes worldwide. Evolutionary Applications 13:991-1008.

Gallardo JA, García X, Lhorente JP, Neira R. 2004. Inbreeding and inbreeding depression of female reproductive traits in two populations of Coho salmon selected using BLUP predictors of breeding values. Aquaculture 234:111-122.

Garner BA, Hand BK, Amish SJ, Bernatchez L, Foster JT, Miller KM, Morin PA, Narum SR, O'Brien SJ, Roffler G. 2016. Genomics in conservation: case studies and bridging the gap between data and application. Trends in Ecology & Evolution 31:81-83.

Gavery MR, Nichols KM, Goetz GW, Middleton MA, Swanson P. 2018. Characterization of genetic and epigenetic variation in sperm and red blood cells from adult hatchery and natural-origin steelhead, *Oncorhynchus mykiss*. G3: Genes, Genomes, Genetics 8:3723-3736.

Ginzburg L. 1998. Population dynamics based on maternal effects. In. Maternal effects as adaptations. Oxford: Oxford University Press. p. 42-54.

Glasauer SM, Neuhauss SC. 2014. Whole-genome duplication in teleost fishes and its evolutionary consequences. Molecular Genetics and Genomics 289:1045-1060.

Goos JM, Swain CJ, Munch SB, Walsh MR. 2019. Maternal diet and age alter direct and indirect relationships between life-history traits across multiple generations. Functional Ecology 33:491-502.

Goudet J, Kay T, Weir BS. 2018. How to estimate kinship. Molecular Ecology 27:4121-4135.

Hamilton M. 2011. Population genetics. Oxford: Wiley-Blackwell.

Hayes CN, James AW, Stephenson AG. 2005. A comparison of male and female responses to inbreeding in *Cucurbita pepo* subsp. *Texana* (Cucurbitaceae). American Journal of Botany 92:107-115.

Hedrick PW, Garcia-Dorado A. 2016. Understanding inbreeding depression, purging, and genetic rescue. Trends in Ecology & Evolution 31:940-952.

Hendry AP, Day T, Cooper AB. 2001. Optimal size and number of propagules: Allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. American Naturalist 157:387-407.

Hill W, Weir B. 2011. Variation in actual relationship as a consequence of Mendelian sampling and linkage. Genetics Research 93:47-64.

Ho DH. 2014. Transgenerational epigenetics: The role of maternal effects in cardiovascular development. Integrative and Comparative Biology 54:43-51.

Huisman J, Kruuk LE, Ellis PA, Clutton-Brock T, Pemberton JM. 2016. Inbreeding depression across the lifespan in a wild mammal population. Proceedings of the National Academy of Sciences 113:3585-3590.

Hutchings JA, Myers RA. 1994. The evolution of alternative mating strategies in variable environments. Evolutionary Ecology 8:256-268.

Iverson JB. 1991. Patterns of survivorship in turtles (order Testudines). Canadian Journal of Zoology 69:385-391.

Kardos M, Åkesson M, Fountain T, Flagstad Ø, Liberg O, Olason P, Sand H, Wabakken P, Wikenros C, Ellegren H. 2018. Genomic consequences of intensive inbreeding in an isolated wolf population. Nature Ecology & Evolution 2:124-131.

Kardos M, Luikart G, Allendorf FW. 2015. Measuring individual inbreeding in the age of genomics: Marker-based measures are better than pedigrees. Heredity 115:63-72.

Keller LF. 1998. Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). Evolution 52:240-250.

Keller LF, Waller DM. 2002. Inbreeding effects in wild populations. Trends in Ecology & Evolution 17:230-241.

Kindsvater HK, Bonsall MB, Alonzo SH. 2011. Survival costs of reproduction predict age-dependent variation in maternal investment. Journal of Evolutionary Biology 24:2230-2240.

Kirkpatrick M, Lande R. 1989. The evolution of maternal characters. Evolution 43:485-503.

Kottelat M, Freyhof J. 2007. Handbook of European freshwater fishes: Publications Kottelat.

Lacey E. 1998. What is an adaptive environmentally induced parental effect. In. Maternal effects as adaptations. Oxford: Oxfor University Press. p. 54-66.

Leblanc CAL, Benhaïm D, Hansen BR, Kristjánsson BK, Skúlason S. 2011. The importance of egg size and social effects for behaviour of Arctic charr juveniles. Ethology 117:664-674.

Leigh DM, Hendry AP, Vázquez-Domínguez E, Friesen VL. 2019. Estimated six per cent loss of genetic variation in wild populations since the industrial revolution. Evolutionary Applications 12:1505-1512.

Liechti P. 1994. Der zustand der seen in der Schweiz. In: Bundesamt für Umwelt WuLB, editor. Bern.

Losdat S, Germain RR, Nietlisbach P, Arcese P, Reid JM. 2018. No evidence of inbreeding depression in sperm performance traits in wild Song sparrows. Ecology and Evolution 8:1842-1852.

Lynch M, Walsh B. 1998. Genetics and analysis of quantitative traits. Sunderland, MA: Sinauer Associated Inc.

Macqueen DJ, Johnston IA. 2014. A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification. Proceedings of the Royal Society B: Biological Sciences 281:20132881.

Manel S, Guerin P-E, Mouillot D, Blanchet S, Velez L, Albouy C, Pellissier L. 2020. Global determinants of freshwater and marine fish genetic diversity. Nature Communications 11:1-9.

Marsh JN, Vega-Trejo R, Jennions MD, Head ML. 2017. Why does inbreeding reduce male paternity? Effects on sexually selected traits. Evolution 71:2728-2737.

Marshall DJ, Bonduriansky R, Bussière LF. 2008. Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. Ecology 89:2506-2517.

Marshall DJ, Heppell SS, Munch SB, Warner RR. 2010. The relationship between maternal phenotype and offspring quality: Do older mothers really produce the best offspring? Ecology 91:2862-2873.

McLennan EA, Wright BR, Belov K, Hogg CJ, Grueber CE. 2019. Too much of a good thing? Finding the most informative genetic data set to answer conservation questions. Molecular Ecology Resources 19:659-671.

McQuillan R, Leutenegger A-L, Abdel-Rahman R, Franklin CS, Pericic M, Barac-Lauc L, Smolej-Narancic N, Janicijevic B, Polasek O, Tenesa A. 2008. Runs of homozygosity in European populations. The American Journal of Human Genetics 83:359-372.

Moore AJ, Brodie III ED, Wolf JB. 1997. Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. Evolution 51:1352-1362.

Mousseau TA, Fox CW. 1998. Maternal effects as adaptations. New York, USA: Oxford University Press.

Müller R, Stadelmann P. 2004. Fish habitat requirements as the basis for rehabilitation of eutrophic lakes by oxygenation. Fisheries Management and Ecology 11:251-260.

Neff BD, Pitcher TE. 2008. Mate choice for non-additive genetic benefits: A resolution to the lek paradox. Journal of Theoretical Biology 254:147-155.

Noel E, Fruitet E, Lelaurin D, Bonel N, Segard A, Sarda V, Jarne P, David P. 2019. Sexual selection and inbreeding: Two efficient ways to limit the accumulation of deleterious mutations. Evolution Letters 3:80-92.

Ou M, Hamilton TJ, Eom J, Lyall EM, Gallup J, Jiang A, Lee J, Close DA, Yun SS, Brauner CJ. 2015. Responses of pink salmon to CO2-induced aquatic acidification. Nature Climate Change 5:950-955.

Parker GA, Begon M. 1986. Optimal egg size and clutch size: Effects of environment and maternal phenotype. The American Naturalist 128:573-592.

Pauls SU, Nowak C, Bálint M, Pfenninger M. 2013. The impact of global climate change on genetic diversity within populations and species. Molecular Ecology 22:925-946.

Pianka ER, Parker WS. 1975. Age-specific reproductive tactics. The American Naturalist 109:453-464.

Pigliucci M. 2007. Do we need an extended evolutionary synthesis? Evolution: International Journal of Organic Evolution 61:2743-2749.

Pigliucci M. 2005. Evolution of phenotypic plasticity: Where are we going now? Trends in Ecology & Evolution 20:481-486.

Pigliucci M, Muller G. 2010. Evolution–the extended synthesis. Massachusetts: The MIT press.

Pinsky ML, Palumbi SR. 2014. Meta-analysis reveals lower genetic diversity in overfished populations. Molecular Ecology 23:29-39.

Proulx SR, Dey S, Guzella T, Teotónio H. 2019. How differing modes of non-genetic inheritance affect population viability in fluctuating environments. Ecology Letters 22:1767-1775.

Regnier 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 22:169-177.

Reznick DN. 1991. Maternal effects in fish life histories. Fourth International Congress of Systematic and Evolutionary Biology; Portland: Discorides Press. p. 780-793.

Richards EJ. 2006. Inherited epigenetic variation—revisiting soft inheritance. Nature Reviews Genetics 7:395-401.

Rijnsdorp A, Jaworski A. 1990. Size-selective mortality in plaice and cod eggs: a new method in the study of egg mortality. ICES Journal of Marine Science 47:256-263.

Roff D. 1992. Evolution of life histories: theory and analysis. New York, USA: Chapman & Hall.

Rossiter MC, Cox-Foster DL, Briggs MA. 1993. Initiation of maternal effects in *Lymantria dispar*: Genetic and ecological components of egg provisioning. Journal of Evolutionary Biology 6:577-589.

Rudolfsen G, Müller R, Urbach D, Wedekind C. 2008. Predicting the mating system from phenotypic correlations between life-history and sperm quality traits in the Alpine whitefish *Coregonus zugensis*. Behavioral Ecology and Sociobiology 62:561-567.

Sakai S, Harada Y. 2001. Why do large mothers produce large offspring? Theory and a test. The American Naturalist 157:348-359.

Sakaluk SK, Oldzej J, Poppe CJ, Harper JL, Rines IG, Hampton KJ, Duffield KR, Hunt J, Sadd B. 2019. Effects of inbreeding on life-history traits and sexual competency in decorated crickets. Animal Behaviour 155:241-248.

Schaberg PG, DeHayes DH, Hawley GJ, Nijensohn SE. 2008. Anthropogenic alterations of genetic diversity within tree populations: Implications for forest ecosystem resilience. Forest Ecology and Management 256:855-862.

Self KE, Schreck CB, Cogliati KM, Billman EJ, Noakes DLG. 2018. Egg size and growth in steelhead Oncorhynchus mykiss. Journal of Fish Biology 93:465-468.

Smith CC, Fretwell SD. 1974. The optimal balance between size and number of offspring. The American Naturalist 108:499-506.

Stearns SC, Hendry AP. 2004. Evolution illuminated: Salmon and their relatives. Oxford, New York: Oxford University Press, Inc.

Su G-S, Liljedahl L-E, Gall GA. 1996. Effects of inbreeding on growth and reproductive traits in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 142:139-148.

Szulkin M, Bierne N, David P. 2010. Heterozygosity-fitness correlations: a time for reappraisal. Evolution 64:1202-1217.

Teixeira JC, Huber CD. 2021a. The inflated significance of neutral genetic diversity in conservation genetics. Proceedings of the National Academy of Sciences 118:e2015096118.

Teixeira JC, Huber CD. 2021b. The inflated significance of neutral genetic diversity in conservation genetics. Proceedings of the National Academy of Sciences 118.

Uller T. 2008. Developmental plasticity and the evolution of parental effects. Trends in Ecology & Evolution 23:432-438.

Van den Berghe EP. 1984. Natural selection and reproductive success of female coho salmon (*Oncorhynchus kitsutch*): a study in female competition. [Burnaby, Canada]: Simon Fraser University.

Vonlanthen P, Bittner D, Hudson AG, Young KA, Müller R, Lundsgaard-Hansen B, Roy D, Di Piazza S, Largiadèr CR, Seehausen O. 2012. Eutrophication causes speciation reversal in whitefish adaptive radiations. Nature 482:357–362.

Wade MJ. 1998. The evolutionary genetics of maternal effects. In. Maternal effects as adaptations. New York: Oxford University Press. p. 5-21.

Wedekind C, Evanno G, Urbach D, Jacob A, Müller R. 2008. 'Good-genes' and 'compatible-genes' effects in an alpine whitefish and the information content of breeding tubercles over the course of the spawning season. Genetica 132:199-208.

Wells DA, Cant MA, Nichols HJ, Hoffman JI. 2018. A high-quality pedigree and genetic markers both reveal inbreeding depression for quality but not survival in a cooperative mammal. Molecular Ecology 27:2271-2288.

Wilkins AS. 2008. Waddington's unfinished critique of neo-Darwinian genetics: Then and now. Biological Theory 3:224-232.

Wolf JB, Wade MJ. 2016. Evolutionary genetics of maternal effects. Evolution 70:827-839.

Wright LI, Tregenza T, Hosken DJ. 2008. Inbreeding, inbreeding depression and extinction. Conservation Genetics 9:833.

Wright S. 1922. Coefficients of inbreeding and relationship. The American Naturalist 56:330-338.

Yanez JM, Bassini LN, Filp M, Lhorente JP, Ponzoni RW, Neira R. 2014. Inbreeding and effective population size in a coho salmon (*Oncorhynchus kisutch*) breeding nucleus in Chile. Aquaculture 420:15-19.

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 6:486-496.

Contribution to chapters

I contributed to the experimental design of chapter 1-2-3-4-5-6-7. I contributed to the collection of the data for all chapters (field work). I was responsible for the raising of the embryo in the climate chamber for all chapters and produced the treatments 1-2-3-4-5. I supervised/contributed to the generation of all datasets. I performed the molecular laboratory work for all chapters including ddRAD sequencing and whole genome sequencing libraries. I contributed to the whitefish genome assembly. I performed statistical and genomic analyses for all chapters. I contributed to the interpretation of the results for all chapters. I wrote the first draft of chapter 1-2-3-4-5-6 and revised chapter 7.

Chapter 1 - Inbreeding depression drives the evolution of elaborate female sexual ornaments in a lek-breeding fish

Christian de Guttry[1], Florin Stettler[1], Jannik Beninde[#1,2], Narelle Hill[1,3], Audrey Atherton[1,4], Roberto Sermier[1], Daniel L. Jeffries[1] & Claus Wedekind[1*]

[1] Department of Ecology & Evolution, University of Lausanne, Lausanne, Switzerland

[2] now at: Department of Biology, University of Konstanz, Konstanz, Germany

[3] now at: Centre for Biodiversity and Conservation Science, University of Queensland, Brisbane, Australia

[4] now at: Department of Environmental Systems Science, ETH, Zürich, Switzerland

([#] not yet agreed to the proposed co-authorship)

* correspondence: claus.wedekind@unil.ch

Chapter status: unpublished

Authors contribution

CdG, JB and CW organized and conducted the field and laboratory work with the help of further members of the group. CdG took the tissue samples, CW performed the experimental crosses, and FS and JB took the plaster casts. CdG and FS monitored embryo and larval development in the laboratory. CdG and RS performed DNA extraction and ddRADseq libraries preparation. CdG and DJ processed and analysed the genomic data. CdG determined the size of the breeding tubercles, and NH and AA determined larval size and growth. CdG and CW performed the statistical analysis and wrote the manuscript.

Abstract

Female sexual ornaments are still poorly understood, especially in lek-breeding species where strong selection against male choice is expected. Theory offers three possible explanations for such ornaments: (i) genetic constraints (e.g. pleiotropy), (ii) male preferences for high fecundity or (iii) for indirect genetic benefits. The latter possibility has received little theoretical or empirical support so far. However, it is often ignored that indirect genetic benefits can include inbreeding coefficients (*F*) if they show significant heritability. Since *F* are linked to inbreeding depression and therefore typically to fecundity and offspring survival, a combination of direct and indirect benefits could then lead to the evolution of male choice and elaborated female sexual ornaments. Whitefish (*Coregonus* spp.) are lek-breeding salmonids with no paternal care, but both sexes can display strong breeding tubercles during the breeding season. We sampled females from a natural population, measured their breeding tubercles with a 3D scanner, determined F_β from 16,633 SNPs, fertilized their eggs in a full-factorial breeding design (to separate maternal from paternal effects), and raised 5,616 embryos singly under stress or non-stress conditions until hatching. Female F_β significantly predicted the strength of the sexual ornamentation, egg number, and embryo development rate. We conclude that variation in F_β drives the evolution of female sexual ornaments in whitefish.

Key words: Sexual selection**,** sexual ornamentation, breeding tubercles, inbreeding depression, fecundity, maternal effects, egg quality.

Introduction

Female sexual ornaments can be observed in various taxa, but they are less likely to evolve than male sexual ornaments, and their evolution is not sufficiently understood yet (Clutton-Brock 2017; Fitzpatrick and Servedio 2018; Hare and Simmons 2019). Theory predicts that such ornaments can exist because of genetic constraints (e.g. pleiotropy of male and female signals and preferences) or because they have evolved in response to male preferences for highly fecund females, while potential indirect genetic benefits (that can be important for the evolution of male sexual ornaments) are believed to usually play no significant role here (Fitzpatrick and Servedio 2018; Hare and Simmons 2019). Selection against male choosiness is expected to be highest in species with a polygynous mating system and no parental care, and especially in species where males congregate in leks to display to, and possibly mate with, a large number of females. Female secondary sexual ornaments are therefore weak or absent in such species, with very few exceptions. Among these exceptions are whitefish (*Coregonus* spp., Salmonidae).

Whitefish belong to the large group of fishes that develop breeding tubercles, i.e. small keratinous epidermal structure that grow on the skin before a breeding season and fall off immediately afterwards (Wiley and Collette 1970). Breeding tubercles have been described in species of all three subfamilies of the salmonids (Kratt and Smith 1978) and are common in cyprinids and other teleost families (Wiley and Collette 1970). In most species, only males develop such breeding tubercles, or female tubercles are rare and tiny as compared to average male tubercles (Wiley and Collette 1970).

Tubercle size and number can vary much among the males of a populations (see below). This variation has sometimes been found to positively correlate with male body size, but the correlation is typically weak and depends on population and location (Kortet and Taskinen 2004; Perroud, et al. 2021). There are also sometimes positive, but overall non-consistent, correlations between breeding tubercles and male health indicators such as condition factor or infection status (Wedekind 1992; Taskinen and Kortet 2002; Kortet and Taskinen 2004; Kekäläinen, et al. 2014). Some behavioural studies concluded that breeding tubercles can be useful predictors of male swimming performance (Lai, et al. 2013) and male-male dominance (Kortet, et al. 2004; Jacob, et al. 2009; Poncin, et al. 2011; Rezucha, et al. 2012). The role that breeding tubercles play in female mate choice is not fully solved yet (Jacob, et al. 2009; Takahashi 2018). However, a series of breeding experiments revealed that the size of male breeding tubercles can be useful predictors of embryo survival or larval performance (Wedekind, et al. 2001; Wedekind, et al. 2008; Kekäläinen, et al. 2010; Kekäläinen, et al. 2015). Because these breeding experiments were full-factorial, i.e. controlling for potentially confounding maternal genetic and maternal environmental effects, the reported paternal effects revealed additive

genetic variance for genetic quality and hence a genetic correlation between aspects of genetic quality and breeding tubercles. Breeding tubercles on males can therefore be indicators of 'good genes' that would allow females to obtain indirect genetic benefits from mate choice (Neff and Pitcher 2005).

Contrary to most other fishes, female whitefish can develop breeding tubercles that may even be larger than those of many males (Wedekind and Müller 2004; Wedekind, et al. 2008; Kekäläinen, et al. 2010). This is all the more remarkable considering that whitefish are external fertilizer with no parental care. Females spawn their eggs in small batches (Karjalainen and Marjomäki 2018), multi-male spawning can frequently be observed (Karjalainen and Marjomäki 2018), and males invest heavily into milt, with total testis weight typically taking more than 5% of the total male body weight (Rudolfsen, et al. 2008). Spawning happens in leks where male displays and possibly male-male dominance are predicted to determine mating success (Fiske, et al. 1998). Therefore, selection against male choosiness seems strong in this species.

In species with lek-like breeding systems and no paternal care, the evolution of female mate preferences and male mate preferences is believed to be fundamentally different (Servedio 2007). Female mate preferences for male ornaments are likely to evolve because of indirect benefits, i.e. when male attractiveness is correlated with offspring fitness because ornaments signal genetic quality (Tomkins, et al. 2004; Jaquiéry, et al. 2009; Sardell, et al. 2014). Male mate preferences for female ornaments are predicted to rarely evolve, and only if there are strong direct benefits from mating with highly fecund females (Servedio and Lande 2006; Nakahashi 2008), i.e. if there are links between female sexual ornaments and fecundity. These links have to be strong to compensate for selection against male choice (Servedio and Lande 2006; Nakahashi 2008). Indirect (genetic) benefits from mating with well-ornamented females are believed to play a less important role here (Servedio and Lande 2006; Servedio 2007; Fitzpatrick and Servedio 2017). Here we suggest that a combination of direct and indirect benefits of male preferences could drive the evolution of female ornaments if variation in genetic quality affects fecundity and offspring viability via maternal environmental effects or genetic inheritance or both.

The heritability of genetic quality is often expected to be low but significant (Møller and Alatalo 1999). Such heritability can be maintained by co-evolutionary arms races, genetic constraints, mutations, or inbreeding depression (Ellegren and Sheldon 2008). The latter plays an important role in many wild vertebrates (Nietlisbach, et al. 2019). Inbreeding depression can be well predicted from individual inbreeding coefficients (F) (Nietlisbach, et al. 2019). Importantly, while F are not expected to be heritable in infinitely large populations, they can show significant heritability in small or structured populations (Neff and Pitcher 2008). F can therefore represent an important aspect of the heritable genetic quality that may be revealed in breeding tubercles. Indeed, male sexual ornaments

have repeatedly been found to reveal *F* in other species (Kempenaers 2007; Ilmonen, et al. 2009; Bolund, et al. 2010; Ferrer, et al. 2015), and Dugand et al. (2018) concluded from an experimental evolution study that female mate preferences significantly reduce inbreeding load in a population. However, it remains unclear whether female sexual ornaments can reveal *F* and would thereby allow males to reduce inbreeding depression of their progeny by preferring females with low *F*.

Nordeide et al. (2013) reviewed 43 papers (on birds, fish, and one reptile) that tested for links between female ornaments and offspring quality. They concluded that only about half of these studies revealed positive associations, while 9 studies even reported such associations to be negative (a more recent example includes Wilkins, et al. 2017). The review included 2 studies on whitefish that found positive links between female breeding tubercles embryo survival (Huuskonen, et al. 2011) or growth and larval swimming performance (Kekäläinen, et al. 2010). However, it remained unclear whether correlations between female ornaments and offspring quality were due to genetic effects, maternal environment effects, or a combination of genetic and environmental effects. As long as we do not even know how important genetic effects are in this context, it is even more unclear what type of genetic effects are most important. It is hence unclear whether, and to what degree, female sexual ornaments reveal *F* in whitefish or any other species.

We sampled female whitefish from a natural spawning place, determined their fecundity and the number and size of their breeding tubercles, fertilized their eggs with sperm of many males in a full-factorial breeding design to separate maternal effects from potentially confounding paternal effects, and raised the resulting embryos individually to determine growth and survival under stress or non-stress conditions. We then linked all these measurement to the females' individual *F* that were estimated from a large number of SNPs. We used these measurements to test whether and to what degree female *F* (i) are revealed in their sexual ornamentation, (ii) affect female fecundity, and (iii) influence offspring growth, survival, and stress tolerance. Answers to these questions will tell us whether there is significant variance in inbreeding depression in a large population of whitefish (that may be typical for populations in the pre-Alpine region), and whether this variance affects fecundity and thereby creates a link between indirect and direct benefits that could have promoted the evolution of male preferences and female ornaments even in lek-breeding species with no parental care.

Material and Methods

Adult whitefish (*Coregonus suidteri*) were caught with gillnets from lake Hallwil (Switzerland; 47.2772° N, 8.2173° E) at the beginning of their breeding season (21st December 2016). The gametes of 13 females and 18 males were collected, fish length and weight were measured, and a tissue sample from the anal fin was taken for genetic analysis. Plaster cast were then taken from one side of the fish as in Wedekind et al. (2008) to later determine the size of breeding tubercles.

The gametes were used for *in vitro* fertilizations in one full-factorial breeding block. This was done by first diluting the milt of each male with Storfish (IMV Technologies, France) to a concentration of 10%. The eggs of one female were about evenly distributed to 18 petri dishes and sperm from a different male was added to each one. Sperms were then activated in re-constituted standardized water (OECD 1992) that was also used for later incubation of embryos and larvae. After 2 hours of egg hardening, a total of 24 embryos per sib group (n_{total} = 5616) were transported in a climate chamber at 4°C and a 12h:12h light-dark cycle. A photo (Canon 70D) of the remaining eggs was taken in a custom-made photo box to later determine egg size and number. These eggs were then returned to the local supportive breeding program. Egg number was obtained using a custom-made macro in ImageJ v2.0.0 (https://imagej.net). The volume of 30 eggs per sib group (i.e. in total 540 eggs per female) was determined from egg diameters using the same software.

In the climate chamber, the 24 eggs per family were washed batch-wise in sterile sieves with flowing tap water (4 L/minutes) for 30 seconds. Eggs were then distributed singly to 24-well plates (Greiner bio-one, Germany) filled with 1.8 ml of autoclaved standardize water as in von Siebenthal et al. (2009) and left undisturbed for 15 days. By then, fertilization success could be determined based on the presence or absence of a spinal cord.

The treatments were added to the wells 21 days post fertilization. For each family, 12 eggs were exposed to the bacteria *Pseudomonas fluorescens* by adding a 200 µl solution containing 10^6 cell/µl prepared as in Clark et al. (2013). The remaining 12 eggs per family were sham exposed, i.e. the same volume of a solution containing only the nutrient broth used for bacteria growth was added. From then on, the eggs were checked daily for hatching and embryo mortality was registered considering only the eggs successfully fertilized. On the day of hatching the larvae were moved to a new 24-well plate filled with 2 mL of autoclaved standardize water.

To record the hatchling lengths and yolk sacs volume at hatching, 3 photos (Canon 70D) of each larva were taken in a custom-made photo box each on the day of hatching and 21 days after hatching. Therefore, the fish were temporarily transferred to a new plate filled with 300 µl standardize water to minimize distortions. Morphometric measurement on larvae were then conducted on the

photos using ImageJ v2.0.0 (https://imagej.net). When none of the three photos per sampling date allowed an accurate measurement of phenotype characteristics, the individual was discarded from the subsequent analysis (0.31% of total sample).

Breeding tubercles were measured on the plaster cast using the 3D macroscope scanner VR-3200 (Keyence International, Mechelen, Belgium) with a precision of 0.5 µm on each axis (Fig. 1). Each plaster cast was scanned with the stitching function of the VR-H2VE software (Keyence International). The scan was then transformed into a plane using the VR-H2AE software (Keyence International, Mechelen, Belgium) to determine the volume of each concavity produced by breeding tubercles.

Genomic DNA was extracted from the tissue sample using the DNAeasy Blood & Tissue kit (Quiagen, Venlo, Netherland). DNA integrity and concentration were measured respectively on agarose gel and with Qubit 2.0. The samples were then normalized to a concentration of 20 ng/µl. The library containing 31 individuals, each one identifiable with a different 5 bp length barcode, was prepared following the Brelsford et al. (2016) protocol adapted from Parchman et al. (2012). DNA digestion was performed using the enzymes EcoRI-HF and MspI (New England Biolab, Ipswich, Massachusetts, United States) and fragments size-selected with gel cutting in between 400-600 bp. The single-end genotyping was done on three lanes of Illumina HiSeq 2500 with fragments of 125 bp length at Lausanne Genomic Technologies Facility (University of Lausanne).

The quality control on raw-reads was done using FASTQC v0.11.7 (Andrews 2010). Given the inadequate per-base quality from 95 bp to 125 bp (<20 Phred score) the reads were trimmed to 90 bp length during *process_radtags* of Stacks version 1.48 (Catchen, et al. 2013). Reads were then mapped using BWA with the MEM algorithm (Li and Durbin 2010) to the newly assembled whitefish genome (De-Kayne, et al. 2020). For data processing the STACKS reference-aligned pipeline was used (Catchen, et al. 2013). Pstacks (Stacks v. 1.48) was done using the bounded SNP model with the default parameters to possibly distinguish between actual heterozygous sites and genotyping errors. The minimum depth to create a stack was set at 5 (*-m* 5). We built the catalogue of loci using Cstacks (Stacks v. 1.48) with 2 mismatches (*-n* 2) allowed between loci. Using Populations (Stacks v. 1.48), loci were filtered for a minimum depth of 10 (*-m* 10), presence in 80% of the individuals and a heterozygosity filter at 0.5 to avoid possible genotyping errors (Hohenlohe, et al. 2011) and to remove heterozygous loci resulting from a possible hidden paralogy. Considering the presence of paralogs in salmonid genomes and their possible influence on incorrect call of heterozygosity at a locus (type I and type II errors), a second filtering was done using *vcftools* v0.1.15 (Danecek, et al. 2011). Here, loci were filtered for a minimum mean depth of 15 X in order to decrease type I errors and for a

maximum depth of 50 X to discard possible paralogs still retained after the previous steps. The latter was applied after a visual inspection of the data where the distribution of coverage among the SNPs was plotted. Loci were then filtered for significant deviation from Hardy-Weinberg equilibrium with a threshold of $P < 0.05$. No filter for minor allele frequencies was applied since it is recommended to consider the entire allele frequency spectrum, including rare variants, for more accurate kinship or inbreeding coefficient estimates (Goudet, et al. 2018).

The inbreeding coefficient (*F*) was estimated using males and females with the function *beta.dosage* from the package *Hierfstat* (Goudet 2005). This function allows to estimate F_{β} based on small sample size (Goudet, et al. 2018). Compared to the other estimators, no need of prior information about the allele frequency in the population is needed. Females inbreeding coefficient ($F_{\beta.dam}$) and males inbreeding coefficient ($F_{\beta.sire}$) were extracted from the diagonal of the kinship matrix obtained using *beta.dosage* (dam and sire indicate respectively maternal and paternal identity). The observed multi-locus heterozygosity, i.e. the realized fraction of heterozygous SNPs across the genotyped fraction of the genome, was calculated per individuals using the function *het* in *vcftools* (Danecek, et al. 2011).

Linear models were used to estimate the effect of parental inbreeding coefficients on parental traits. Linear mixed-effects models (LMM) were used to tests for effects of $F_{\beta.dam}$ on eggs quality and offspring traits, with dam and sire identity as random factor and treatment and $F_{\beta.dam}$ as fixed factors. The significance of each factor was tested using a model lacking or including (in the case of interactions) the term of interest and comparing it to a reference model. Models fit were compared with Akaike's information criteria (AIC) and likelihood ratio tests (LRT). Analysis were done in R (R Development Core Team 2015) using the lme4 package (Bates, et al. 2015).

Results

As expected from the size-selectivity of the gill net, males and females of the present sample did not differ in body length ($t_{29} = 1.6$, $p = 0.13$). Females had on average smaller breeding tubercles ($t_{29} = 5.9$, $p > 0.001$; Fig. 1b; mean±sd= ♀ 0.15 ± 0.07 ♂ 0.31 ± 0.07) and less of them than males ($t_{29} = 2.4$, $p = 0.02$; Fig. 1c; mean±sd= ♀ 81 ± 29 ♂ 107 ± 30), but there was considerable overlap in the respective distributions, i.e. some of the females had more and larger tubercles than some of the males (Fig. 1b,c).

After filtering, a total of 16,633 SNPs could be kept with a mean presence 94.8% across 31 individuals and a mean coverage of 31 X. Given the high correlation between F_{β} and multi-locus

heterozygosity ($r^2 = 0.97$, $P < 0.001$; Fig. S1), only F_β was retained for further analysis. The estimated F_β ranged from -0.052 to 0.14 and did not differ between males and females ($t_{29} = 0.9$, $p = 0.39$).

$F_{\beta.dam}$ explained much of the variance in females secondary sexual ornament size with more inbred individuals having smaller breeding tubercles than the more outbred ones ($r^2 = 0.43$; $P = 0.009$; Fig. 2a). $F_{\beta.dam}$ was also linked to fecundity, with more inbred females producing fewer eggs than the more outbred ones ($r^2 = 0.46$; $P = 0.01$; Fig. 2b). The link between breeding tubercles volume and egg number was, however, not significant ($r^2 = 0.13$; $P = 0.21$; Fig. S2).

There was no evidence for a trade-off between egg number and egg volume ($r^2 = 0.01$; $P = 0.72$; Fig. S4), and no effect of $F_{\beta.dam}$ was detected on egg volume ($r^2 < 0.01$; $P = 0.32$; Fig. 2c). Therefore, more inbred females had not only fewer eggs, but also smaller total egg masses compare to more outbred individuals ($r^2 = 0.4$; $P = 0.02$; Fig. S3). No significant link was found between $F_{\beta.sire}$ and breeding tubercles volume in males ($r^2 = 0.003$; $P = 0.81$; Fig. S5).

In total 1.2% of identified embryos died before hatching. This mortality was dependent on dam identity but could not be linked to $F_{\beta.dam}$ (Table 1*A*). All other embryo and larvae characteristics were dependent on dam and sire identity and on the combination of dam x sire (Table 1). $F_{\beta.dam}$ affected embryo development rate: offspring of more inbred females hatched later (Table 1*B*; Fig. 2d) but at similar size (Table 1*C*; Fig. 2e) and with similar yolk sac volume (Fig. 2f; Table 1*D*) than offspring of less inbred ones.

The exposure to PF did not induce significant embryo mortality (Table 1*A*). However, exposure to PF delayed the hatching of larvae (Table 1*B*; Fig. S7A) that hatched at smaller size (Table 1*C*; Fig. S7B) and with larger yolk sacs (Table 1*D*; Fig. S7C) than the non-exposed controls. Females identity affected the effects of PF on egg volume 90 dpf, hatching time and length at hatching, but not on yolk sac volume at hatching (see D*t interactions in Table 1). However, the interaction effects of $F_{\beta.dam}$ and treatment on offspring performance were never significant (Table 1 *A-E*). Males identity did not affect the response to PF in any of the variables (see non-significant S*t interactions in Table 1).

Discussion

All females of our sample showed well developed and significant numbers of breeding tubercles. Some females had even more and larger tubercles than many males. This confirms previous observations in other whitefish species (see Introduction) and clearly distinguishes this genus from most other teleost fish where breeding tubercles on females are either absent or, especially in old females, tiny and in small numbers compared to the tubercles on males (Wiley and Collette 1970). Tiny tubercles on only old females suggest genetic constraints (e.g. due to pleiotropy) and decreasing selection on female

tubercles with increasing age. In our study population, however, females are caught during their first spawning seasons and long before they would have reached old age (Perroud, et al. 2021)(see also chapter 3 of this thesis). We therefore conclude that even if genetic constraints such as pleiotropy cannot be excluded as possible explanation for breeding tubercles in female whitefish, they seem unlikely to play a major role.

We found much variation in the number and size of tubercles among both, females and males. Within females, about 43% of this variance could be explained by variation in the $F_{\beta.dam}$. This was not the case in males, with the percentage of variance in breeding tubercles explained by $F_{\beta.sire}$ close to 0%. Such sex differences suggest fundamental differences in the evolution of male and female sexual ornaments, as expected from theory (Servedio 2007).

$F_{\beta.dam}$ was not only a useful predictor of sexual ornamentation but also of egg number, and because egg number and mean egg size were not correlated, $F_{\beta.dam}$ was even a useful predictors of total egg mass and hence total female fecundity. Variation in $F_{\beta.dam}$ explained at least 40% of the variance in fecundity. This estimate is likely to be conservative because females spawn their eggs in multiple batches (Karjalainen and Marjomäki 2018), and the observed variation in egg number may well be influenced by the number of batches a female spawned before being sampled. Variation in previous spawning activity is therefore likely to affect fecundity measures and obscure links to potential predictors of fecundity, for example, by creating outliers. Indeed, the female with the lowest number of eggs in our sample could have been such an outlier that could have had a strong effect on testing for a correlation between breeding tubercles and egg number. It therefore remains unclear whether breeding tubercles reveal female fecundity. Avoiding such possibly confounding effects would mean sampling females at exactly the moment when they are ready to spawn but have not started yet. Such a sampling protocol remains to be developed.

Potential outliers would be easier to identify if egg volume and egg number were correlated. However, this turned out not to be the case in the present sample. There was also no link between $F_{\beta.dam}$ and egg size. When sampling significantly larger numbers of whitefish towards the end of the breeding season, de Guttry et al. (chapter 5 of this thesis) found evidence for strong maternal environmental effects on embryo performance under stress conditions. These maternal environmental effects were, however, not linked to egg size, suggesting that egg quality was instead linked to the biochemical composition of the eggs. When combining their findings on late spawners with the present ones on early spawners to test for size and age-specific reproductive strategies, de Guttry et al. (chapter 3 of this thesis) concluded that variation in egg size reveals age-specific strategies. No conclusions were possible with regard to egg number and hence fecundity when sampling females towards the end

of the spawning season, because females had at time had significantly fewer eggs than the females sampled at the beginning of the season, possibly due to spawning activity before sampling that is likely to obscure links to fecundity. Here we note that there seems to be no trade-off between egg volume and egg number at the beginning of the spawning season, i.e. egg number seems not significantly constrained by the mean size that eggs reach after the 2 hours of hardening. Such a missing trade-off may not simply be explained by variation in the amount of water eggs take up during egg hardening, because de Guttry et al. (chapter 5 of this thesis) found a positive link between egg size and larval size at hatching.

While variation in female fecundity and its possible link to breeding tubercles remains to be further studied under conditions that control for possibly confounding factors, we found significant correlations between $F_{\beta.dam}$ and fecundity and between $F_{\beta.dam}$ and breeding ornamentation. We conclude that female breeding tubercles reveal inbreeding and hence inbreeding depression. Inbreeding depression is expected to affect fecundity through its effects on female health and vigor, and to affect offspring performance through maternal environmental and maternal genetic effects. Here we studied offspring performance under experimental conditions, controlling for paternal effects by the full-factorial breeding design. We found strong maternal and paternal effects on embryo development, confirming previous studies on other whitefish populations (e.g. von Siebenthal, et al. 2009; Clark, et al. 2014). Some of the significant maternal effects on embryo development rate could be predicted by $F_{\beta.dam}$. We conclude that $F_{\beta.dam}$ reveals inbreeding depression that either affects maternal genetic or maternal environmental effects on embryos, or both. The former is likely because of the significant heritability of inbreeding coefficients that de Guttry et al. (chapter 7 of the present thesis) found in 201 adults when combining the present sample with two other samples of males and females taken from the same population but at later stages of the spawning season. If maternal inbreeding load also affects maternal environmental effects on embryos, the non-significant correlation between $F_{\beta.dam}$ and egg size suggest that inbreeding load would then affect egg quality via the biochemical composition of the eggs. This still needs to be tested.

We also challenged the embryos by exposing them to PF, a pathogen that is known to affect gene expression (Wilkins, et al. 2015) and embryo development rate (von Siebenthal, et al. 2009; Clark, et al. 2014). PF also reduced embryo development rate here. This virulence effect was not significantly linked to host genetics, as revealed by the non-significant sire x treatment interactions on all embryo performance measures. Maternal sib groups reacted differently to the PF stressor (the significant dam x treatment interactions), but these maternal effects could not be linked to variance in $F_{\beta.dam}$ (the non-significant $F_{\beta.dam}$ x treatment interactions). A comparable treatment on embryos from

parents sampled later in the season (chapter 5 of this thesis) confirmed the non-significant sire x treatment interactions on all embryo performance measures and also demonstrated that egg size was no significant predictor of virulence. We conclude that PF-induced virulence is mainly linked to the biochemical composition of eggs while host genetics plays no significant role in this context. Immunocompetence genes seem to play no or a minor role when female breeding ornaments signal genetic quality.

In conclusion: A typical pattern in lek-breeding species is that males show sexual ornaments while females do not. Theory predicts strong selection again male choosiness in such cases and hence no sexual ornaments in females. The whitefish clearly contradict these expectancies, with females showing well developed breeding ornaments whose expression varies much among females. The distribution of female ornamentation even overlaps significantly with that of males. We found that the females in our sample varied also in $F_{\beta.dam}$, and that $F_{\beta.dam}$ was a significant predictor of female ornamentation, female fecundity, and offspring performance (but not offspring tolerance to infection). If males preferentially mate with strongly ornamented females, they are more likely to mate with less inbred females who are typically more fecund and whose embryos develop faster than those of more inbred females. Our findings suggest that male choice for strongly ornamented females has therefore led to the evolution of female ornaments in lek-breeding whitefish.

Ethics approval

The experimental breeding and the raising of embryos in the laboratory were approved by the Fishery Inspectorate of the Aargau canton. Approvals by the Veterinary Offices of the involved cantons were not required because the fish were caught in a commercial fishing program, measurements and tissue samples were taken from dead fish, and all embryos and larvae were euthanized before the end of the yolk sac period.

Data availability

Data will be deposited on the Dryad depository upon acceptance of the manuscript.

Funding

The project was supported by the Swiss National Science Foundation (31003A_182265) and the *Swisslos-Fonds* of the Aargau canton

Acknowledgments

We thank R. Stadelmann for catching the fish and D. Bittner for permits, N. Caloz, I. Castro, G. Fabre, L. Garaud, A. Jimenez, E. Longange, L. Marquez da Cunha, K. Mobley, D. Nusbaumer, E. Seddon, N. Sironi and C. Vincent for assistance, and J. Goudet for discussion.

References

Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Cambridge, UK: Babraham Bioinformatics.

Bates D, Machler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Bolund E, Martin K, Kempenaers B, Forstmeier W. 2010. Inbreeding depression of sexually selected traits and attractiveness in the Zebra finch. Animal Behaviour 79:947-955.

Brelsford A, Dufresnes C, Perrin N. 2016. High-density sex-specific linkage maps of a European tree frog (*Hyla arborea*) identify the sex chromosome without information on offspring sex. Heredity 116:177-181.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. 2013. Stacks: an analysis tool set for population genomics. Molecular Ecology 22:3124-3140.

Clark ES, Pompini M, Marques da Cunha L, Wedekind C. 2014. Maternal and paternal contributions to pathogen resistance dependent on development stage in a whitefish (Salmonidae). Functional Ecology 28:714-723.

Clark ES, Stelkens RB, Wedekind C. 2013. Parental influences on pathogen resistance in brown trout embryos and effects of outcrossing within a river network. PLoS ONE 8:e57832.

Clutton-Brock T. 2017. Reproductive competition and sexual selection. Philosophical Transactions of the Royal Society B-Biological Sciences 372:20160310.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, et al. 2011. The variant call format and VCFtools. Bioinformatics 27:2156-2158.

De-Kayne R, Zoller S, Feulner PGD. 2020. A de novo chromosome-level genome assembly of *Coregonus* sp. "*Balchen*": One representative of the Swiss Alpine whitefish radiation. Molecular Ecology Resources 20:1093-1109.

Dugand RJ, Kennington WJ, Tomkins JL. 2018. Evolutionary divergence in competitive mating success through female mating bias for good genes. Science advances 4:eaaq0369.

Ellegren H, Sheldon BC. 2008. Genetic basis of fitness differences in natural populations. Nature 452:169-175.

Ferrer ES, Garcia-Navas V, Bueno-Enciso J, Sanz JJ, Ortego J. 2015. Multiple sexual ornaments signal heterozygosity in male blue tits. Biological Journal of the Linnean Society 115:362-375.

Fiske P, Rintamäki PT, Karvonen E. 1998. Mating success in lekking males: A meta-analysis. Behavioral Ecology 9:328-338.

Fitzpatrick CL, Servedio MR. 2018. The evolution of male mate choice and female ornamentation: a review of mathematical models. Current Zoology 64:323-333.

Fitzpatrick CL, Servedio MR. 2017. Male mate choice, male quality, and the potential for sexual selection on female traits under polygyny. Evolution 71:174-183.

Goudet J. 2005. HIERFSTAT, a package for R to compute and test hierarchical F-statistics. Molecular Ecology Notes 5:184-186.

Goudet J, Kay T, Weir BS. 2018. How to estimate kinship. Molecular Ecology 27:4121-4135.

Hare RM, Simmons LW. 2019. Sexual selection and its evolutionary consequences in female animals. Biological Reviews 94:929-956.

Hohenlohe PA, Amish SJ, Catchen JM, Allendorf FW, Luikart G. 2011. Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. Molecular Ecology Resources 11:117-122.

Huuskonen H, Kekäläinen J, Panda B, Shikano T, Kortet R. 2011. Embryonic survival and larval predator-avoidance ability in mutually ornamented whitefish. Biological Journal of the Linnean Society 103:593-601.

Ilmonen P, Stundner G, Thoss M, Penn DJ. 2009. Females prefer the scent of outbred males: good-genes-as-heterozygosity? BMC Evolutionary Biology 9:104.

Jacob A, Evanno G, Renai E, Sermier R, Wedekind C. 2009. Male body size and breeding tubercles are both linked to intrasexual dominance and reproductive success in the minnow. Animal Behaviour 77:823-829.

Jaquiéry J, Broquet T, Aguilar C, Evanno G, Perrin N. 2009. Good genes drive female choice for mating partners in the lek-breeding European treefrog. Evolution 64:108-115.

Karjalainen J, Marjomäki TJ. 2018. Communal pair spawning behaviour of vendace (*Coregonus albula*) in the dark. Ecology of Freshwater Fish 27:542-548.

Kekäläinen J, Huuskonen H, Tuomaala M, Kortet R. 2010. Both male and female sexual ornaments reflect offspring performance in a fish. Evolution 64:3149-3157.

Kekäläinen J, Pirhonen J, Taskinen J. 2014. Do highly ornamented and less parasitized males have high quality sperm? An experimental test for parasite-induced reproductive trade-offs in European minnow (*Phoxinus phoxinus*). Ecology and Evolution 4:4237-4246.

Kekäläinen J, Soler C, Veentaus S, Huuskonen H. 2015. Male investments in high quality sperm improve fertilization success, but may have negative impact on offspring fitness in whitefish. PLoS ONE 10:e0137005.

Kempenaers B. 2007. Mate choice and genetic quality: A review of the heterozygosity theory. In: Brockmann HJ, Roper TJ, Naguib M, WynneEdwards KE, Barnard C, Mitani J, editors. Advances in the Study of Behavior, Vol 37. Amsterdam: Elsevier. p. 189-278.

Kortet R, Taskinen J. 2004. Parasitism, condition and number of front head breeding tubercles in roach (*Rutilus rutilus* L.). Ecology of Freshwater Fish 13:119-124.

Kortet R, Taskinen J, Vainikka A, Ylonen H. 2004. Breeding tubercles, papillomatosis and dominance behaviour of male roach (*Rutilus rutilus*) during the spawning period. Ethology 110:591-601.

Kratt LF, Smith RJF. 1978. Breeding tubercles occur on male and female Arctic grayling (*Thymallus arcticus*). Copeia 1:185-188.

Lai Y-T, Kekäläinen J, Kortet R. 2013. Male ornamentation in the European minnow (*Phoxinus phoxinus*) signals swimming performance. Ethology 119:1077-1085.

Li H, Durbin R. 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 26:589-595.

Møller AP, Alatalo R. 1999. Good-genes effects in sexual selection. Proceedings of the Royal Society of London Series B-Biological Sciences 266:85–91.

Nakahashi W. 2008. Quantitative genetic models of sexual selection by male choice. Theoretical Population Biology 74:167-181.

Neff BD, Pitcher TE. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Molecular Ecology 14:19-38.

Neff BD, Pitcher TE. 2008. Mate choice for non-additive genetic benefits: A resolution to the lek paradox. Journal of Theoretical Biology 254:147-155.

Nietlisbach P, Muff S, Reid JM, Whitlock MC, Keller LF. 2019. Nonequivalent lethal equivalents: Models and inbreeding metrics for unbiased estimation of inbreeding load. Evolutionary Applications 12:266-279.

Nordeide JT, Kekäläinen J, Janhunen M, Kortet R. 2013. Female ornaments revisited - are they correlated with offspring quality? Journal of Animal Ecology 82:26-38.

OECD. 1992. OECD Guideline for the testing of chemicals 203 (fish, acute toxicity test). In. Paris, France. p. 9.

Parchman TL, Gompert Z, Mudge J, Schilkey FD, Benkman CW, Buerkle CA. 2012. Genome-wide association genetics of an adaptive trait in lodgepole pine. Molecular Ecology 21:2991-3005.

Perroud G, Nusbaumer D, de Guttry C, Wedekind C. 2021. Male sexual signaling and expected effects of hatchery-induced sperm competition vary with water depth at which whitefish are caught. Current zoology zoab007.

Poncin P, Matondo BN, Termol C, Kestemont P, Philippart JC. 2011. Relationships between circulating androgens, aggressive behaviour and breeding tubercles in males of the common bream *Abramis brama* L. in an aquarium environment. Fish Physiology and Biochemistry 37:533-542.

R Development Core Team. 2015. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rezucha R, Smith C, Reichard M. 2012. Personality traits, reproductive behaviour and alternative mating tactics in male European bitterling, *Rhodeus amarus*. Behaviour 149:531-553.

Rudolfsen G, Müller R, Urbach D, Wedekind C. 2008. Predicting the mating system from phenotypic correlations between life-history and sperm quality traits in the Alpine whitefish *Coregonus zugensis*. Behavioral Ecology and Sociobiology 62:561-567.

Sardell RJ, Kempenaers B, Duval EH. 2014. Female mating preferences and offspring survival: testing hypotheses on the genetic basis of mate choice in a wild lekking bird. Molecular Ecology 23:933-946.

Servedio MR. 2007. Male versus female mate choice: sexual selection and the evolution of species recognition via reinforcement. Evolution 61:2772-2789.

Servedio MR, Lande R. 2006. Population genetic models of male and mutual mate choice. Evolution 60:674-685.

Takahashi D. 2018. Female mate choice based on male nuptial coloration in pale chub, *Zacco platypus*. Zoological Science 35:23-27.

Taskinen J, Kortet R. 2002. Dead and alive parasites: sexual ornaments signal resistance in the male fish, *Rutilus rutilus*. Evolutionary Ecology Research 4:919-929.

Tomkins JL, Radwan J, Kotiaho JS, Tregenza T. 2004. Genic capture and resolving the lek paradox. Trends in Ecology & Evolution 19:323-328.

von Siebenthal BA, Jacob A, Wedekind C. 2009. Tolerance of whitefish embryos to *Pseudomonas fluorescens* linked to genetic and maternal effects, and reduced by previous exposure. Fish Shellfish Immunol 26:531-535.

Wedekind C. 1992. Detailed information about parasites revealed by sexual ornamentation. Proceedings of the Royal Society of London Series B-Biological Sciences 247:169-174.

Wedekind C, Evanno G, Urbach D, Jacob A, Müller R. 2008. 'Good-genes' and 'compatible-genes' effects in an Alpine whitefish and the information content of breeding tubercles over the course of the spawning season. Genetica 132:199-208.

Wedekind C, Müller R. 2004. Parental characteristics versus egg survival: towards an improved genetic management in the supportive breeding of lake whitefish. Annales Zoologici Fennici 41:105-115.

Wedekind C, Müller R, Spicher H. 2001. Potential genetic benefits of mate selection in whitefish. Journal of Evolutionary Biology 14:980-986.

Wiley ML, Collette BB. 1970. Breeding tubercles and contact organs in fishes: their occurance, structure, and significance. Bulletin of the American Museum of Natural History 143:145-216.

Wilkins LGE, Clark ES, Farinelli L, Wedekind C, Fumagalli L. 2015. Embryonic gene expression of *Coregonus palaea* (whitefish) under pathogen stress as analyzed by high-throughput RNA-sequencing. Fish & Shellfish Immunology 47:130-140.

Wilkins LGE, Marques da Cunha L, Menin L, Ortiz D, Vocat-Mottier V, Hobil M, Nusbaumer D, Wedekind C. 2017. Maternal allocation of carotenoids increases tolerance to bacterial infection in brown trout. Oecologia 185:351-363.

Tables

Table 1. The effects of sire identity, dam identity, exposure to PF and $F_{\beta.dam}$ on: (*A*) Embryo mortality, (*B*) hatching time, (*C*) length at hatching and, (*D*) yolk sac volume at hatching. Likelihood ratio tests on mixed model regressions were used to compare a reference model (in bold) with models including or lacking the term of interest. Significant p-values are highlighted in bold.

Model	Effect tested	AIC	d.f.	X^2	*P*
(A) Embryo mortality					
t + $\boldsymbol{F_{\beta.dam}}$ + D + S		657	5		
t + $F_{\beta.dam}$ + S	D	700	4	45	**<0.001**
t + $F_{\beta.dam}$ + D	S	655	4	0	0.99
t + $F_{\beta.dam}$ + D*S	D*S	659	6	0.04	0.83
$F_{\beta.dam}$ + D + S	t	655	4	0.06	0.8
t + $F_{\beta.dam}$ + D + S + D*t	D*t	661	7	0.1	0.95
t + $F_{\beta.dam}$ + D + S + S*t	S*t	661	7	0	1
t + D + S	$F_{\beta.dam}$	665	4	0.01	0.9
t + $F_{\beta.dam}$ + D + S + $F_{\beta.dam}$ *t	$F_{\beta.dam}$*t	659	6	0	0.98
(B) Hatching time					
t + $\boldsymbol{F_{\beta.dam}}$ + D + S		24680	6		
t + $F_{\beta.dam}$ + S	D	25546	5	867	**<0.001**
t + $F_{\beta.dam}$ + D	S	25404	5	725	**<0.001**
t + $F_{\beta.dam}$ + D*S	D*S	24599	7	83	**<0.001**
$F_{\beta.dam}$ + D + S	t	24803	5	124	**<0.001**
t + $F_{\beta.dam}$ + D + S + D*t	D*t	24672	8	12	**0.002**
t + $F_{\beta.dam}$ + D + S + S*t	S*t	24683	8	1.1	0.57
t + D + S	$F_{\beta.dam}$	24683	5	4.7	**0.03**
t + $F_{\beta.dam}$ + D + S + $F_{\beta.dam}$ *t	$F_{\beta.dam}$*t	24682	7	0.1	0.73
(C) Length at hatching (mm)					
t + $\boldsymbol{F_{\beta.dam}}$ + D + S		5228	6		
t + $F_{\beta.dam}$ + S	D	6132	5	905	**<0.001**
t + $F_{\beta.dam}$ + D	S	5325	5	98	**<0.001**
t + $F_{\beta.dam}$ + D*S	D*S	5225	7	5.1	**0.02**
$F_{\beta.dam}$ + D + S	t	5285	5	58	**<0.001**
t + $F_{\beta.dam}$ + D + S + D*t	D*t	5222	8	10	**0.005**
t + $F_{\beta.dam}$ + D + S + S*t	S*t	5231	8	1.2	0.52
t + D + S	$F_{\beta.dam}$	5227	5	0.8	0.36
t + $F_{\beta.dam}$ + D + S + $F_{\beta.dam}$ *t	$F_{\beta.dam}$*t	5227	7	3	0.08

(D) Yolk sac volume at hatching (mm^3)

t + $F_{\beta.dam}$ + D + S		30	6		
t + $F_{\beta.dam}$ + S	D	828	5	799	**<0.001**
t + $F_{\beta.dam}$ + D	S	80	5	51	**<0.001**
t + $F_{\beta.dam}$ + D*S	D*S	27	7	5.5	**0.02**
$F_{\beta.dam}$ + D + S	t	33	5	4.9	**0.03**
t + $F_{\beta.dam}$ + D + S + D*t	D*t	33	8	1.1	0.56
t + $F_{\beta.dam}$ + D + S + S*t	S*t	34	8	0.04	0.97
t + D + S	$F_{\beta.dam}$	29	5	0.2	0.64
t + $F_{\beta.dam}$ + D + S + $F_{\beta.dam}$ *t	$F_{\beta.dam}$*t	32	7	0.4	0.51

Fixed effects: treatment (t) and $F_{\beta.dam}$; random effects: dam (D) and sire (S)

Figures

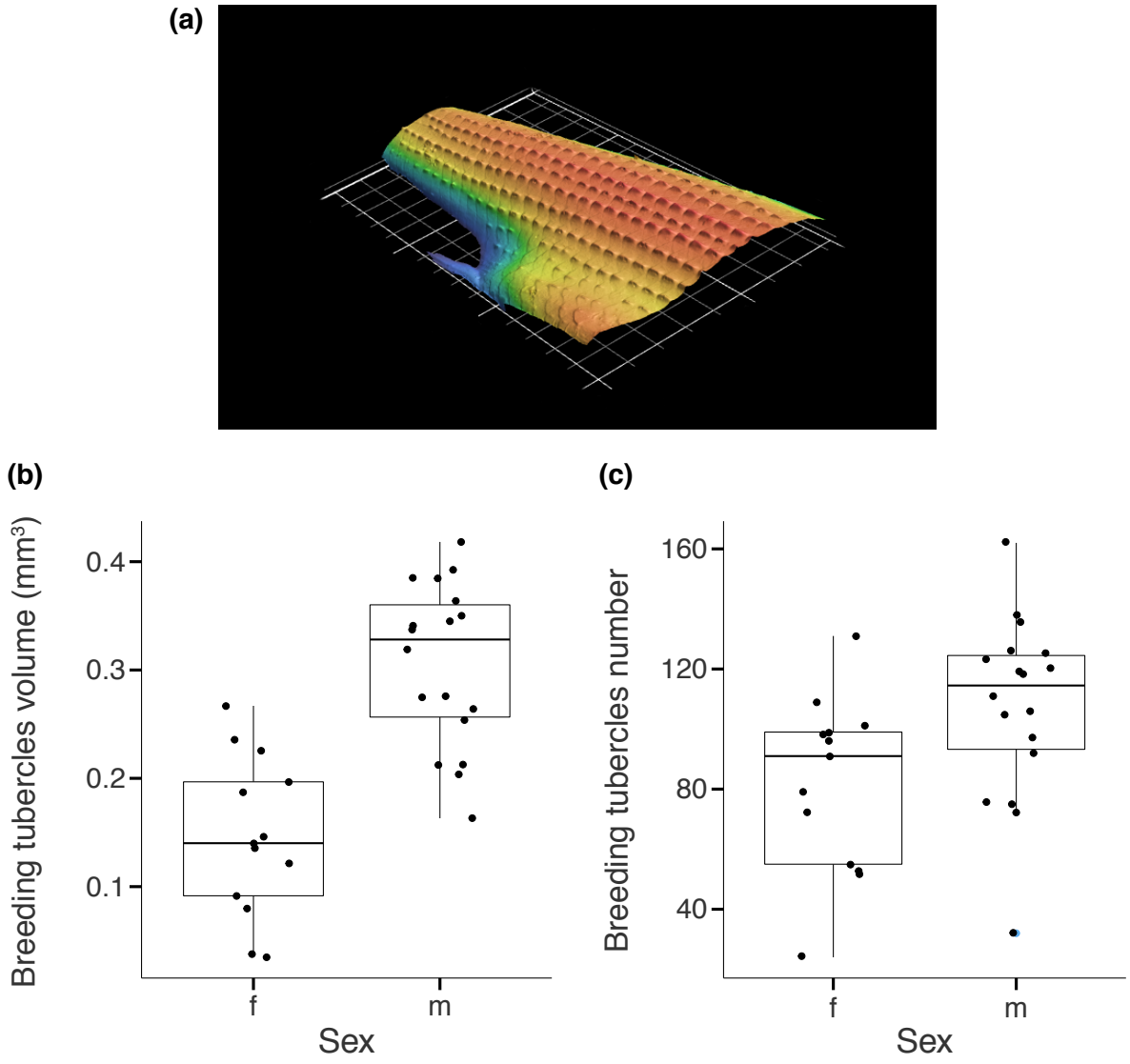


Figure 1. (a) Inverted 3D scan of a plaster cast revealing breeding tubercles on the posterior part of a whitefish right body side. The effect of sex on (a) breeding tubercles volume and (b) breeding tubercles number (Tukey outlier boxplots with quartiles, whiskers and individual values). See text for statistics.

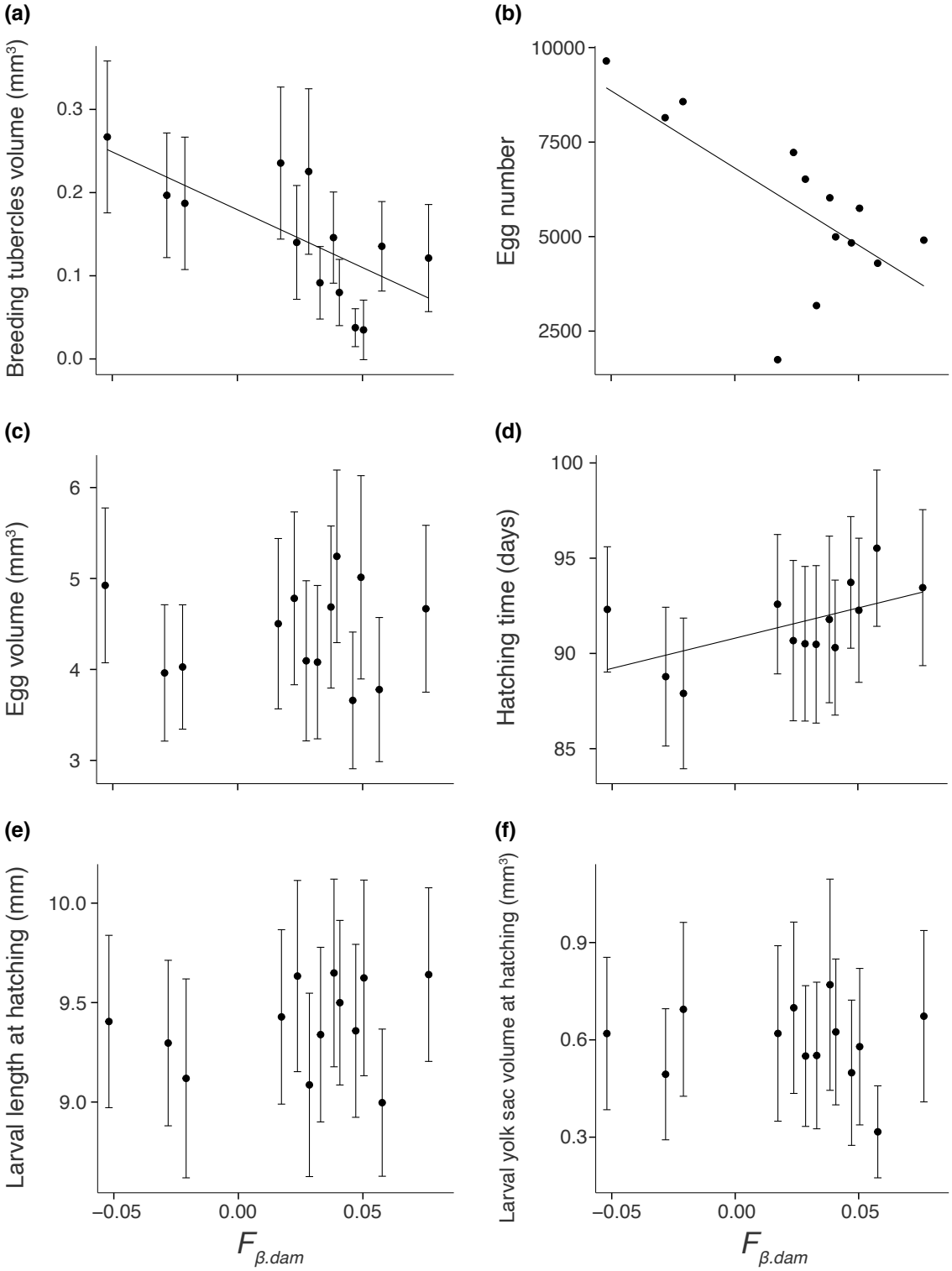


Figure 2. The relationship between $F_{\beta.dam}$ and: (a) mean breeding tubercles volume (± S.D.); (b) egg number; (c) mean egg volume after fertilization (± S.D.); (d) mean embryos hatching time (± S.D.); (e) mean larval length at hatching (± S.D.); (f) mean yolk sac volume at hatching (± S.D.). Regression lines are given if the correlation was significant. See text for statistics.

Supplementary information

Inbreeding depression drives the evolution of elaborate female sexual ornaments in a lek-breeding fish

Christian de Guttry[1], Florin Stettler[1], Jannik Beninde#[1,2], Narelle Hill[1,3], Audrey Atherton[1,4], Roberto Sermier[1], Daniel L. Jeffries[1] & Claus Wedekind[1*]

[1] Department of Ecology & Evolution, University of Lausanne, Lausanne, Switzerland

[2] now at: Department of Biology, University of Konstanz, Konstanz, Germany

[3] now at: Centre for Biodiversity and Conservation Science, University of Queensland, Brisbane, Australia

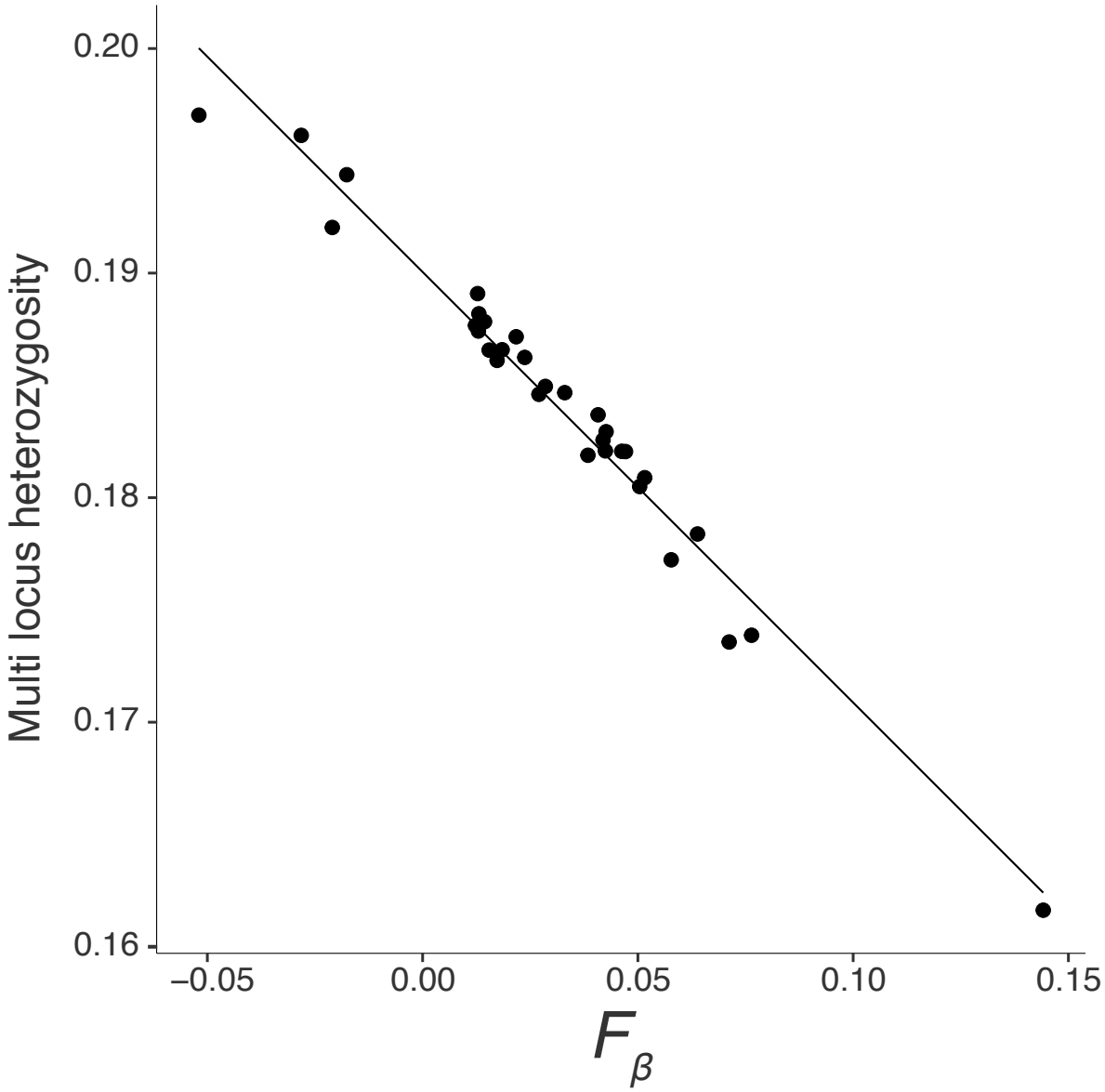
[4] now at: Department of Environmental Systems Science, ETH, Zürich, Switzerland

(# not yet agreed to the proposed co-authorship)

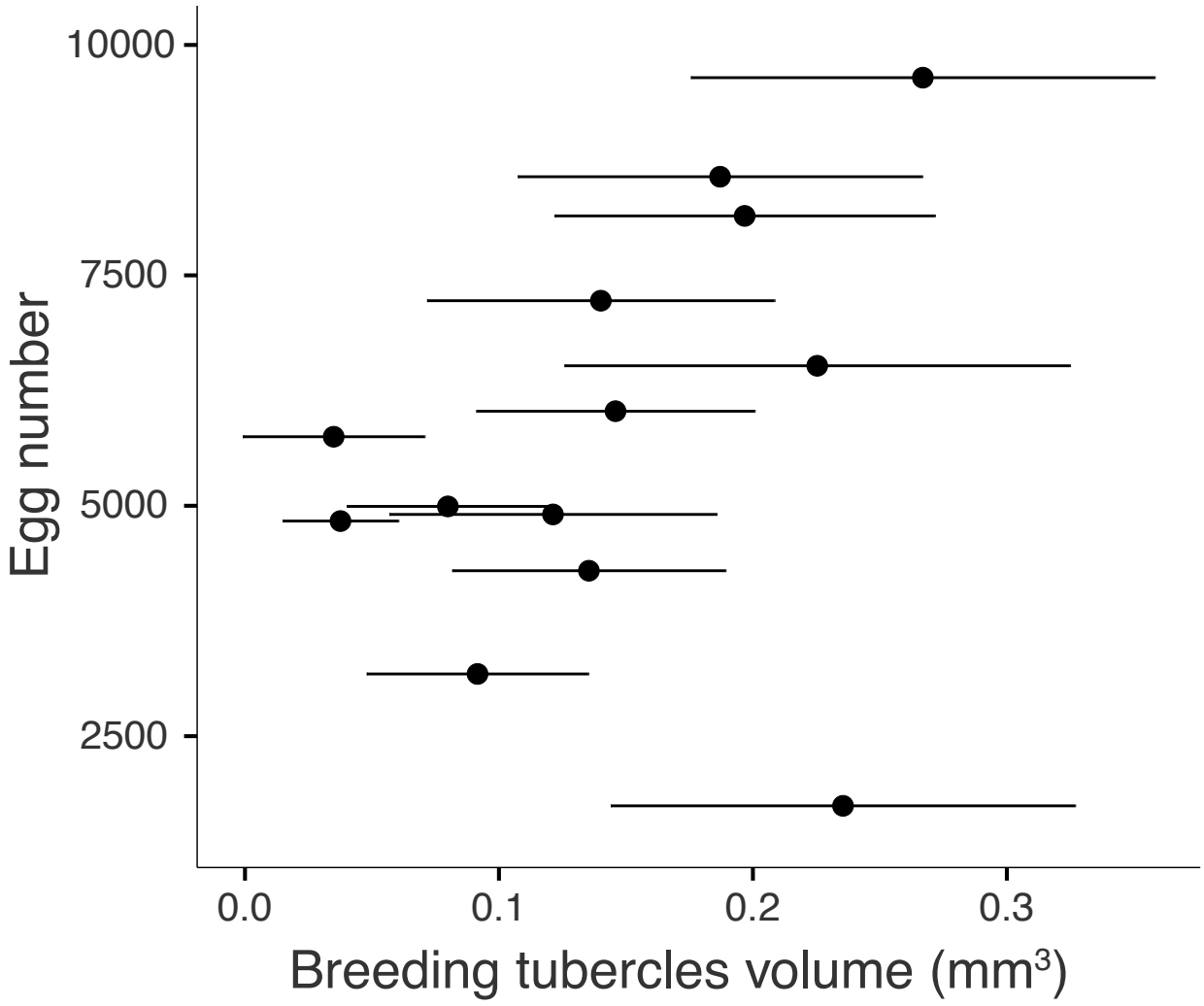
* correspondence: claus.wedekind@unil.ch

Chapter status: unpublished

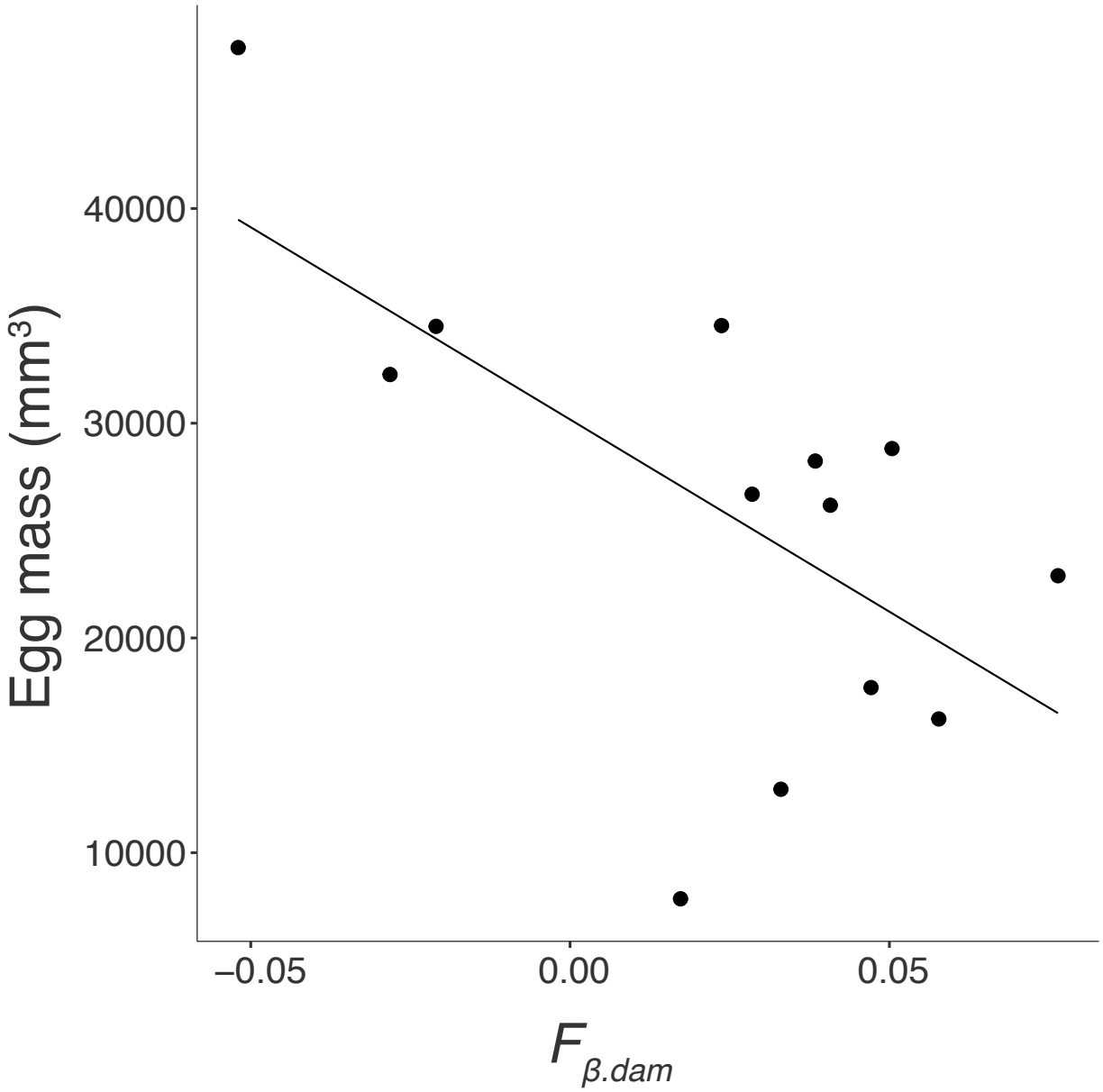
Content



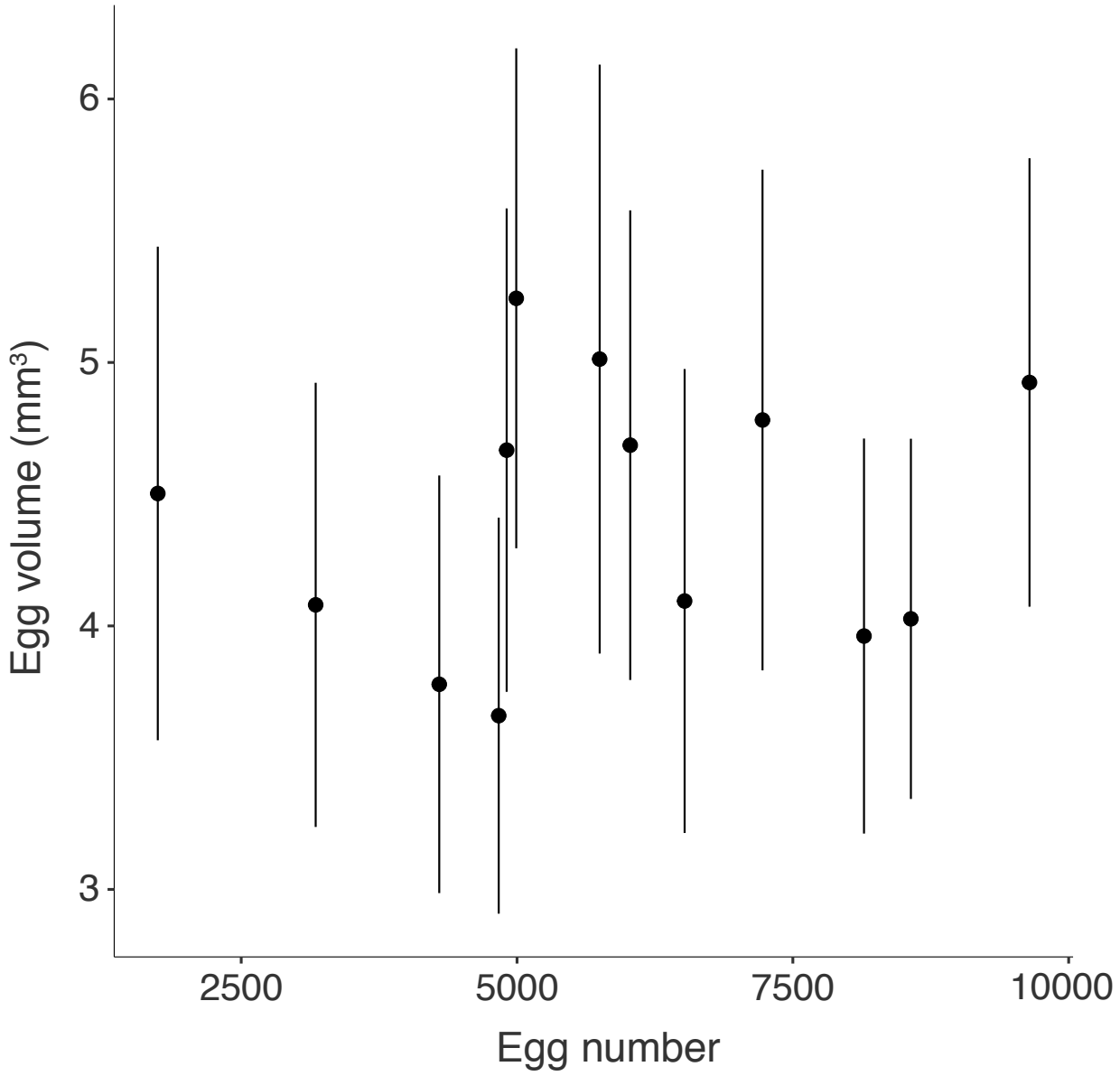
Supplementary figure S1. The correlation between F_β and multi-locus heterozygosity. The line gives the regression. See text for statistics.



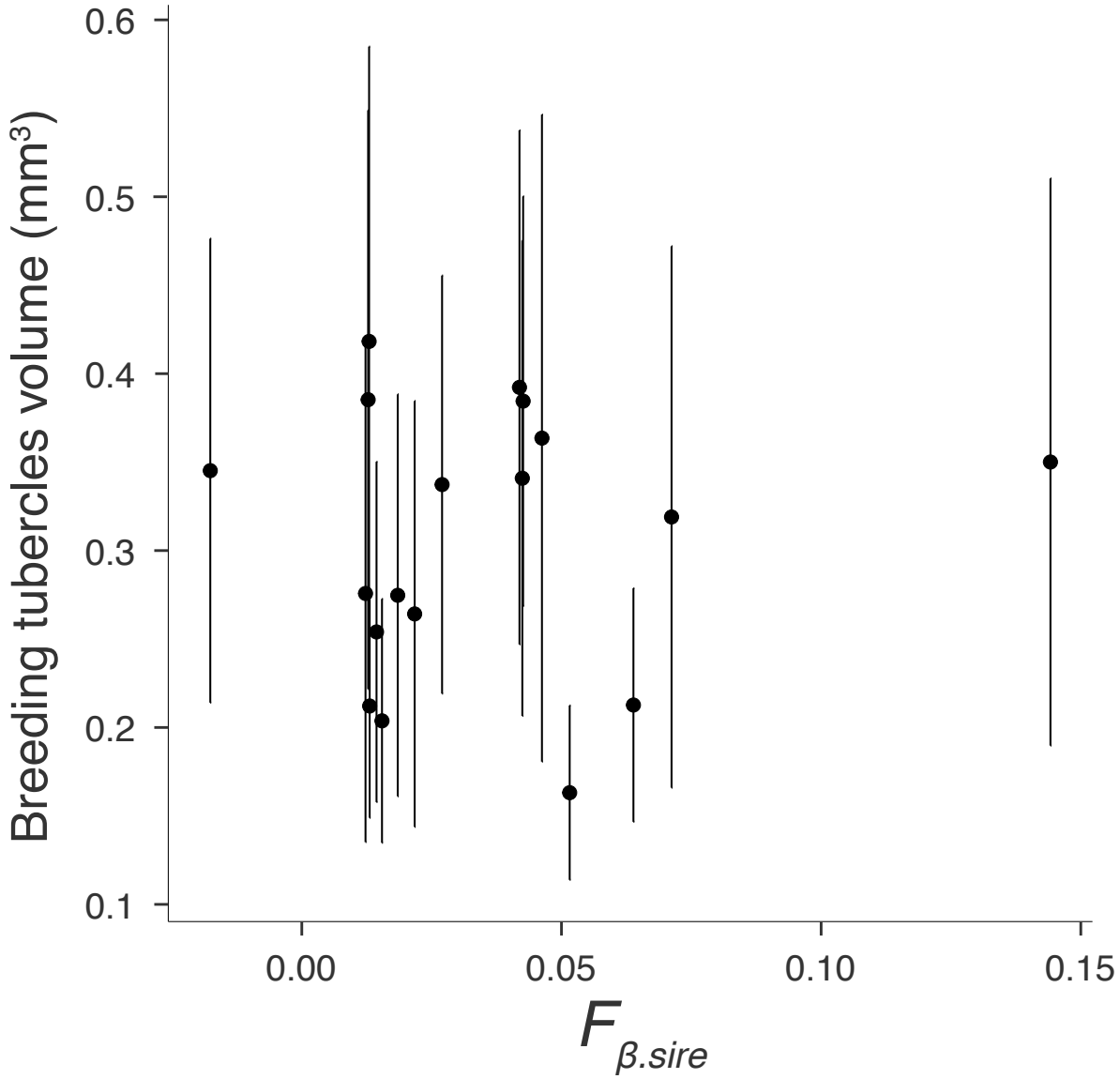
Supplementary figure S2. The non-significant link between egg number and mean breeding tubercles volume (± S.D.). See text for statistics.



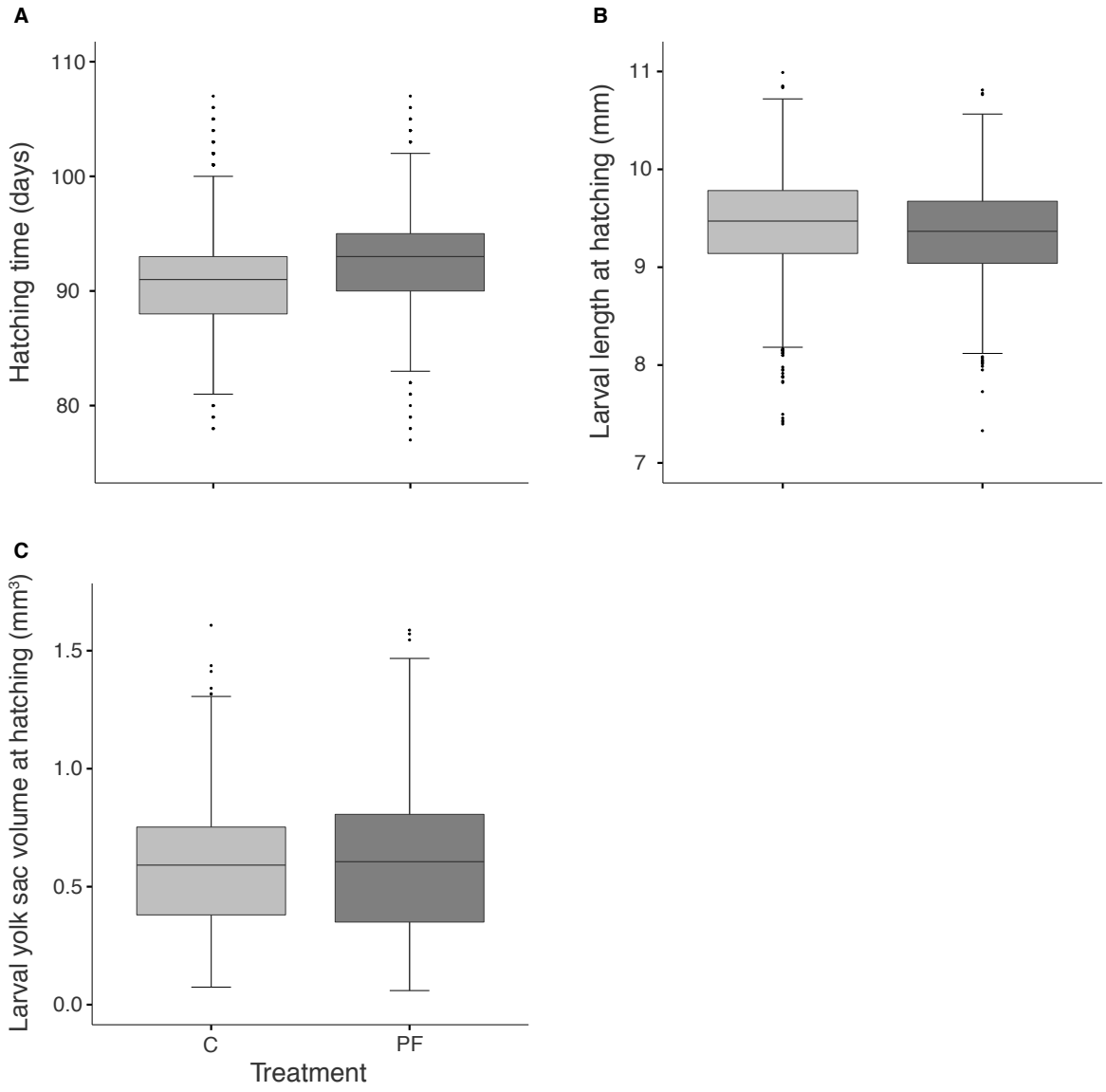
Supplementary figure S3. The link between $F_{\beta.dam}$ and total egg biomass. The regression line is displayed. See text for statistics.



Supplementary figure S4. The non-significant link between egg number and mean egg volume (± S.D.). See text for statistics.



Supplementary figure S5. The non-significant link between $F_{\beta.sire}$ and mean breeding tubercles volume (± S.D.). See text for statistics.



Supplementary Figure S6. The significant effect of the exposure to *Pseudomonas fluorescence* (PF) on (A) hatching time, (B) length at hatching and, (C) yolk sac volume at hatching. Tukey outlier boxplots with quartiles, whiskers, and outliers are plotted. For statistics see Table 1.

Chapter 2 - Maternal inbreeding increases within-clutch variation in egg size and reduces embryo development rate in whitefish

Christian de Guttry[1], Alberto Garcia Jimenez[1,2], Nathalie Caloz[1], Audrey Atherton[1,3] Eliane Longange[1] & Claus Wedekind[1*]

[1] Department of Ecology & Evolution, University of Lausanne, Lausanne, Switzerland

[2] now at Department of Computational Biology, University of Lausanne, Lausanne, Switzerland

[3] now at: Department of Environmental Systems Science, ETH, Zürich, Switzerland

* correspondence: claus.wedekind@unil.ch

Chapter status: unpublished

Author contributions

CdG and CW planned the study and conducted the field work with the help of further members of the group. CW performed the experimental crosses. CdG, AGJ, and NC monitored embryo and larval development in the climate chamber. AGJ and AA determined larval size. EL determined egg size. CdG and AGJ managed the data files. CdG took the tissue samples, extracted the DNA, prepared the ddRADseq libraries, and processed and analyzed the genomic data. CdG and CW made the statistical analyses and wrote the manuscript.

Abstract

Size variation within a brood is a commonly reported feature of which the causes still remain unclear. This defies models of offspring size evolution that predict the presence of an optimal offspring size influenced by environmental and maternal characteristics. It has been suggested that an unpredictable environment and maternal phenotype or condition influence within-brood size variation. Maternal inbreeding has rarely been studied in this context. In addition to its effect on maternal reproductive traits, it could also have an indirect influence on offspring via the quality/size of the eggs produced. Here we test for a relationship between maternal inbreeding coefficient ($F_{\beta.dam}$) on within-brood size variability and on offspring performance in whitefish. We used ddRADseq to sequence 30 adult female whitefish and estimated $F_{\beta.dam}$ based on 18,380 SNPs. These females and 60 males had been used for block-wise full-factorial breeding to produce 300 families and monitor 24 embryos per family (7,200 in total) in the context of another experiment on the effect of pathogen exposure (de Guttry et al., 2021 in prep). We found high between and within female variation in egg size. The within-brood variation in egg size increased with increasing $F_{\beta.dam}$, while no link was observed between mean egg size and $F_{\beta.dam}$. Embryos of more inbred females hatch later. We therefore conclude that inbreeding influences the capacity of a female to produce optimal egg size. We then suggest that embryo development is influenced by $F_{\beta.dam}$ mainly through reduced quality of food/energy stored in the egg during oocyte maturation. This result, suggest an intergenerational effect of maternal inbreeding in a species with no parental care. These results highlight the consequences of inbreeding on fitness and its potential influence on evolutionary processes.

Keywords: Maternal effects, ddRAD, inbreeding, intergenerational effects, females reproductive traits, egg quality, offspring performances.

Introduction

Elucidating the factors affecting offspring size variation and quality in early stages of life is key to the evolutionary processes and responses to selection of a population (Mousseau and Fox 1998; Wolf and Wade 2009). From a theoretical perspective, the evolution of offspring size was initially thought to be influenced by environmental factors (Smith and Fretwell 1974). There is evidence to suggest that mothers are able to collect information about their environment and optimize the size of their eggs in order to maximize their fitness (Fox, et al. 1997). Despite this, offspring size is reported to be astonishingly variable, be it at the level of single populations (Christians 2002; Marshall, et al. 2008) or even within a single brood (Chambers and Leggett 1996; Einum and Fleming 2004; Gossieaux, et al. 2019). At the population level, the between-females variation in offspring size has been widely investigated. This variation has been explained by including maternal phenotypes in optimality models (Parker and Begon 1986; Hendry, et al. 2001; Sakai and Harada 2001; Kindsvater, et al. 2011; Kindsvater and Otto 2014).

Few theoretical models and empirical work attempted to investigate within-brood size variability (Marshall, et al. 2008), despite the fact that offspring of the same brood are rarely homogeneous in size (Turnbull, et al. 2006; Marshall, et al. 2018). Within-brood size variation is thought to represent a diversifying bet-hedging strategy in response to unpredictable environments (Christians 2002; Einum and Fleming 2004; Marshall, et al. 2008; Haaland, et al. 2020). Under this strategy females relinquish their mean instant fitness (Olofsson, et al. 2009) in an attempt to reduce the variance in fitness between different reproductive seasons (Philippi and Seger 1989). In addition to an unpredictable environment, intrinsic factors such as physiological constrain and/or maternal status can lead to the production of different sized offspring (Bernardo 1996; Einum and Fleming 2004; Ford, et al. 2018; Gossieaux, et al. 2019). However, a relationship between maternal phenotypic characteristics and within-brood egg size variability has rarely been found (Crump 1981; Le Gouvello, et al. 2020).

Maternal characteristics are part of maternal effects, one of the major determinants of offspring's phenotypes in early-life stages (Bernardo 1996). The positive correlation between the phenotype of the mother and the size, survival and, performance of the offspring has frequently been reported (Sakai and Harada 2001; Green 2008; Burton, et al. 2016; Burton, et al. 2020). A maternal trait that has emerged as crucial is egg size. Egg size directly reflects the maternal investment in an individual and is a major determinant of its future survival and fitness (Quinn, et al. 1995). Despite this, there is one potential driver of egg size variability and quality that has received little attention, that is, the inbreeding status of the female.

It is well established that inbreeding can lead to a reduction in fecundity and other fitness-related traits, i.e. inbreeding depression (Charlesworth and Charlesworth 1987; Keller 1998; Gallardo, et al. 2004; Hayes, et al. 2005; Billing, et al. 2012; Ebel and Phillips 2016). Its consequences may appear early in life or be observed during the adult stage (Charlesworth and Charlesworth 1987). When an individual reaches sexual maturity its inbreeding affects not only its fitness related traits but also the performances of its offspring i.e. reduced parental care (Mattey, et al. 2013; Ford, et al. 2018; Mattey, et al. 2018). In presence of these dynamics, maternal inbreeding is considered as a maternal effect (Mousseau and Fox 1998).

In organisms lacking parental care, maternal inbreeding could directly impair egg production by decreasing their size and quality (Heath and Blouw 1998; Krist 2011). However, understanding how maternal inbreeding might indirectly affect offspring performances has not been mainstream in recent years. There is now the need to further explore this potential evolutionary dynamic also in species where the parents do not invest in offspring after fertilization. This may lead to more accurate estimates of the effects of inbreeding on fitness.

Therefore, we investigated whether the direct effect of maternal inbreeding coefficient can influence egg size and its variation within a brood. In addition, we tested the indirect effect of maternal inbreeding on offspring performances. We predicted embryos from eggs of more inbred females having reduced performances in the early stages of life. To achieve our goal we used whitefish population (*Coregonus suidteri*) from Lake Hallwil. This lake has had a relatively unstable environment over the last years, but its conditions are currently recovering so environment is not expected to increase within-brood variability in egg size. In addition, the local whitefish population is supported by a re-stocking program. The consistent restocking has likely increased inbreeding due to small source stocks. Lastly, salmonids produce thousands of eggs per reproductive event and are known to exhibit high within-population and within-brood variability (Einum and Fleming 1999; Leblanc, et al. 2016). As such salmonids are already an established model for examining the evolution of egg size (Stearns and Hendry 2004). This system is therefore ideal for testing the influence of inbreeding on maternal traits and determine whether beyond its direct effect, inbreeding may have indirect effects that eventually becomes intergenerational.

Material and Methods

Whitefish (*Coregonus suidteri*) were sampled, and their gametes used for experimental breeding and raising of embryos in the course of another study (chapter 5 of this thesis). Briefly, 60 males and 30 females were caught in gillnets (mesh size: 35 mm) towards the end of their breeding season (09/01/2017). Their gametes were collected to generate 6 full factorial breeding blocks each one consisting of 5 females and 10 males. Half of the embryos were exposed to the pathogen *Pseudomonas fluorescence* (PF) during incubation. Egg volume at day 0 (taken shortly after egg hardening), embryo mortality, hatching time, larval length at hatching and larval yolk sac volume at hatching were determined from photos in ImageJ v2.0.0 (https://imagej.net). Egg volume had been determined from 30 haphazardly selected eggs per sib group, i.e. a total from 300 eggs per female.

Tissue samples from the anal fin had been taken from all 90 adults and stored in 70% ethanol. Genomic DNA was extracted using DNAeasy Blood & Tissue kit (Quiagen, Venlo, Netherland). DNA integrity and concentration were then determined respectively on agarose gel and with the Qbit 2.0 fluorometer and samples were normalized to 20ng/µl. A first library with unique 5bp ECORI barcodes for each individuum was prepared following the Brelsford et al. (2016) protocol adapted from Parchman et al. (2012). A second library was prepared, using the same protocol but including only individuals with low genotyping rate. Shortly, both libraries were prepared as follow: 120 ng of DNA of each individual were digested with the enzymes EcoRI-HF and MspI (New England Biolab). After PCR amplification, fragment between 400-550bp were selected. The single-end genotyping was done with an Illumina Hiseq 2500 at the Lausanne Genomic Technologies Facility (University of Lausanne, Switzerland).

A quality control was done on the fastq files using FASTQC v0.11.7 (Andrews 2010). Given the insufficient per-base quality (<20 Phred) between 95bp and 125 bp reads were trimmed to 90bp using Trimmomatic (Bolger, et al. 2014). After the demultiplexing performed with *process_radtags* in Stacks version 1.48 (Catchen, et al. 2013), reads were mapped using BWA with the MEM algorithm (Li and Durbin 2010) to the whitefish genome (De-Kayne, et al. 2020). The retained reads were then processed with the Stacks reference-aligned pipeline (Stacks v. 1.48) as follow: Pstacks was done using the bounded SNP model with the default parameters to possibly distinguish between actual heterozygous sites and genotyping errors. The minimum depth to create a stack was set at 5 (*-m* 5). We built the catalogue of loci using Cstacks with 2 mismatches (*-n* 2) allowed between loci. Using Stacks' Populations, loci were filtered for a minimum depth of 10 (*-m* 10), presence in 80% of the individuals and a heterozygosity filter at 0.5 to avoid possible genotyping errors (Hohenlohe, et al. 2011) and to remove heterozygous loci resulting from a possible hidden paralogy. The VCF obtained

at the end of the Stacks pipeline has been filtered using *VCFTOOLS* v0.1.15 (Danecek, et al. 2011). Here, loci were filtered for a minimum mean depth of 15 X in order to decrease type I errors and for a maximum mean depth of 50 X to discard possible paralogs still retained after the previous steps. The latter was applied after a visual inspection of the data where the distribution of coverage among the SNPs was plotted. Only bi-allelic loci and loci that significantly deviate from Hardy-Weinberg equilibrium with a threshold of $P < 0.05$ were retained. No filter for minor allele frequencies was applied. Consider the entire allele frequency spectrum, including rare variants, is suggested for more accurate inbreeding coefficient estimates (Goudet, et al. 2018). The inbreeding coefficient (F_β)was estimated using the function *beta.dosage* from the package *Hierfstat* (Goudet 2005).

Linear models were used to estimate the correlation between $F_{\beta.dam}$, egg size, and the variance in egg size. In order to test for effects of $F_{\beta.dam}$ and the $F_{\beta.dam}$ * treatment interaction on offspring performance, the generalized linear mixed models (GLMM) and linear mixed models (LMM) that had been used in de Guttry *et al*. (chapter 5 of this thesis) were run again here, now newly including the $F_{\beta.dam}$ and the $F_{\beta.dam}$ * treatment interaction as fixed factors. Models fit were compared with Akaike's information criteria (AIC) and likelihood ratio tests (LRT). The significance of each factors was tested comparing a model lacking or including the term of interest with a reference model. Analysis were done in R (Core Team 2016) using the lme4 package (Bates, et al. 2015).

Results

After filtering, a total of 18,380 SNPs were kept with a mean presence of 92% across 84 individuals and a mean coverage of 26.7 X. Three females were excluded from further analysis due to low genotyping rate caused by low DNA quality or technical artefacts during library preparation or sequencing (O'Leary, et al. 2018). The estimated $F_{\beta.dam}$ ranged from 0.076 to -0.044.

Mean (±S.D.) egg size per female ranged from 4.2 mm^3 (± 0.73) to 8.6 mm^3 (± 1.13) (Fig. 1a). The mean egg size per female was not correlated with female standard length ($F_{1,28} = 0.17$, $p = 0.67$, $r^2 = 0.006$) nor with inbreeding coefficient ($F_{1,25} = 0.35$, $p = 0.56$, $r^2 = 0.013$; Fig. 1a). However, within-clutch variation in egg size increased with increasing $F_{\beta.dam}$ ($F_{1,28} = 3.9$, $p = 0.037$, $r^2 = 0.13$; Fig. 1b).

$F_{\beta.dam}$ was no significant predictor of embryo or larval mortality and showed no effects on embryo susceptibility to the pathogen treatment (Supplementary Table S1, see main effects of $F_{\beta.dam}$ and the $F_{\beta.dam}$ * treatment interaction terms). However, $F_{\beta.dam}$ vreduced embryo development, because offspring of more inbred females hatched later (Table S1b; Fig. 2a) while at similar size and with similar sized yolk sacs (Table S1c,d; Fig. 2c,d) than offspring of less inbred females.

Discussion

We investigated the direct effect of maternal inbreeding on within-brood size variability and the indirect effect of maternal inbreeding on offspring performances. We found that maternal inbreeding influences reproductive traits in whitefish. More inbred females have a larger within-brood variation in egg size compared to less inbred females. Egg size was not influenced by maternal inbreeding. We then find evidence for an indirect effect of maternal inbreeding on offspring performances. Embryos that develop in eggs from more inbred females hatched later compare to egg coming from less inbred females. Overall our results suggest that maternal inbreeding influence reproductive trait and its effect can be intergenerational also in species without parental care.

Inbreeding in whitefish is one of the factors affecting the variability of size within a brood. Its increase is followed by an increase in variance in egg size. Female inbreeding has previously been shown to affect reproductive traits (Su, et al. 1996; Gallardo, et al. 2004). However, its correlation with within-brood variation in egg size has never been demonstrated. Our results show that this variation is partially due to the genetic characteristics of females. Individual differences have already been invoked to explain variability in size within a brood such as the position of eggs in the ovary (Burton, et al. 2013) or maternal size (Moland, et al. 2010). In birds the observed within-brood size variation has been previously suggested to have a genetic component (Christians 2002). Despite this, the basis of this genetic component had not been clarified. In our experiment more inbred females might have a reduced ability to perceive the environmental stimuli. Indeed, inbreeding can affect perception of external stimuli and there is evidence that inbred individual could perceive a benign environment as harmful (Putten 1999; Bijlsma and Loeschcke 2005; Kristensen, et al. 2006). The mechanism used to cope with a changing environment is consequently triggered generating an unnecessary response (Pedersen, et al. 2005). We suggest that, in our experiment, this incorrect perception of the surrounding environment, could result in more inbred females having a higher variability for the size of the eggs produces. Under stable environmental conditions a female has been predicted to produce an optimal egg size to increase her fitness (Smith and Fretwell 1974). However, an unpredictable environment result in the production of different sized eggs. Adopting this "dynamic" bet-hedging strategy allow a female to minimize the difference in fitness for the future generations ensuring at least part of the individuals to have optimal performances (Fox, et al. 1997; Marshall, et al. 2008). The difficulty occurs when environmental effects, and those linked to the status of the female, must be disentangle. In our case, the females came from the same environment and were caught on the same day. Therefore, we were able to isolate the effect of inbreeding making the environmental unpredictability constant in our sample. On salmonids, it has been previously shown that when females

are moved into captivity, they tend to produce smaller eggs and/or increase the within-brood variation in size (Heath, et al. 2003; Einum and Fleming 2004; Jastrebski and Morbey 2009; Neely, et al. 2012). There, diversifying bet-hedging strategy has been invoked. However, the genetic status of females has not been considered in these studies. Its influence on within-brood variation in size, can then be confounded with the influence of an unpredictable environment. We recommend that future studies looking at the effect of habitat instability on egg size should account for the genetic effects. The latter should be considered especially when animals are in captivity, such as brood stock for conservation or commercial purposes, usually characterized by a higher level of inbreeding.

The strong maternal effect we found on hatching time was partly explained by $F_{\beta.dam}$. Interestingly, the $F_{\beta.dam}$ did not affect larval length at hatching and yolk sac volume at hatching. This confirms that embryos incubated in the eggs of more inbred mothers had an extended developmental time regardless of the genetic quality of the sire, which was anyway variable in the population (sire effect). We suggest that $F_{\beta.dam}$ might affect embryos development by a reduced quality of the nutriments, proteins, immune factors and hormones stocked in the eggs during oogenesis. The latter is also referred as an indirect genetic effect (Moore, et al. 1997). Indeed, egg content is linked to a female genome and to her capacity of acquire resources from the environment (McAdam, et al. 2014). Offspring that hatch later are usually subjected to greater selective pressures in salmonids (Einum and Fleming 2000). For instance, the longer exposure of incubating embryos to water disturbance and predation increases embryo mortality (Fausch 1984; Cutts, et al. 1999; Good, et al. 2001). Mortality, that has been found to increase even at the larval stage, due to late hatching, in another member of the coregoninae family (*Coregonus albula*) (Koho, et al. 1991). Therefore, embryos of more inbred females are disadvantage during incubation. To confirm our preliminary hypothesis, the content of the eggs coming from females that differ for their inbreeding coefficient should be determined.

We were able to demonstrate one of the few examples where the effects of maternal inbreeding are intergenerational without the presence of parental care. In our experiment, regardless of their genetic status, the offspring suffered the cost of maternal inbreeding. Previous evidence of this mechanism were found in species with the presence of parental care where an increase in inbreeding resulted in a decrease of cares (Mattey, et al. 2013). Since this also occur in species with external fertilization, and without parental care as whitefish, implies that more research is needed in this field. Not consider the indirect effect of inbreeding can lead to the underestimation of its consequences or to the misassignment of its effects to other factors. Only by further investigating the contribution of direct and indirect effect of inbreeding we will understand their role into the decrease in fitness typical of inbreeding depression.

No effect of $F_{\beta.dam}$ was found on egg size. This may seem an unexpected result but, to our knowledge, a connection between egg size and the level of inbreeding has rarely been reported (Wetzel, et al. 2012). The result that has most often emerged from these studies is that maternal inbreeding reduces fertility (Su, et al. 1996; Gallardo, et al. 2004) and hatchability of the offspring (Saccheri, et al. 1996; Su, et al. 1996; White, et al. 2015). In our experiment, $F_{\beta.dam}$ did not affect embryonic or larval mortality and did not reveal any influence on the embryo susceptibility to *Pseudomonas fluorescence.* The latter is an unexpected result since the influence of inbreeding depression on immune response is nearly ubiquitous in vertebrates (Reid, et al. 2003; Sellers, et al. 2012; Smallbone, et al. 2016) and insects (Rantala and Roff 2007; Drayton and Jennions 2011). In external fertilizer, a female provide the embryo with the only immune factors available during the early stages embryogenesis (Swain and Nayak 2009). We have exposed the embryos to the pathogen 21 days after fertilization and it is therefore possible that at this stage the immune response has already depended on the genes expressed by the embryo itself. Possibly, the indirect effect of maternal inbreeding is not so severe to induce mortality and, maybe, the lack of extreme inbreeding values in our sample may have influenced those result. A further experiment with higher and discrete classes of inbreeding could help to clarify these dynamics as well as the exposure to different pathogens already present in the natural population.

Conclusion

The finding of this study further confirms that inbreeding depression can have a strong influence on reproductive traits. Apart from affecting fecundity, as often demonstrated, also the variability of the size of eggs within a brood is influenced. Evolutionary theories and experiment that attempt to explain the variability within-brood variability in size must take into account the genetics of individuals. In addition, we were able to find evidence that maternal inbreeding, a part of having an effect on the same individual, also has an indirect detrimental effect on its offspring via the eggs produced. With these features the maternal inbreeding can also be described, in all respects, as a maternal effect (Wolf and Wade 2001). This leads us to two interesting conclusions that should enhance the interest of the scientific community in these dynamics. Firstly, since even in species without parental care there can be the presence of the indirect effect of maternal inbreeding on offspring performances, there is the need to account for it. Estimating the cost of inbreeding on individual fitness, or population dynamics, without accounting for indirect effect can lead to an underestimation of its true magnitude. Secondly, this category of effects has been overlooked in help explaining species and populations evolutionary trajectory. Understand whether, and how long, these effects persist in time and how they may affect

other phenotypic, behavioral, or social traits are almost unexplored fields, and their understanding must now become a priority.

Ethics

The experimental breeding and the raising of embryos in the laboratory were approved by the Fishery Inspectorate of the Aargau canton. Approvals by the Veterinary Offices of the involved cantons were not required because the fish were caught in a commercial fishing program, measurements and tissue samples were taken from dead fish, and all embryos and larvae were euthanized before the end of the yolk sac period.

Data availability

Data will be deposited on the dryad depository upon acceptance of the manuscript.

Funding

The project was supported by the lottery funds of the Aargau canton and the Swiss National Science Foundation (31003A_182265).

Acknowledgements

We thank R. Stadelmann for catching the fish, J. Beninde, I. Castro, G. Fabre, L. Garaud, L. Marques da Cunha, K. Mobley, D. Nusbaumer, E. Seddon, and C. Vincent for assistance in the field and in the laboratory, DL Jeffries for comments, D. Bittner for permits, and all the above for discussion.

References

Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Bernardo J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. American Zoologist 36:216-236.

Bijlsma R, Loeschcke V. 2005. Environmental stress, adaptation and evolution: An overview. Journal of Evolutionary Biology 18:744-749.

Billing AM, Lee AM, Skjelseth S, Borg ÅA, Hale MC, Slate J, Pärn H, Ringsby TH, Saetern BE, Jensen H. 2012. Evidence of inbreeding depression but not inbreeding avoidance in a natural house sparrow population. Molecular Ecology 21:1487-1499.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120.

Brelsford A, Dufresnes C, Perrin N. 2016. High-density sex-specific linkage maps of a European tree frog (*Hyla arborea*) identify the sex chromosome without information on offspring sex. Heredity 116:177-181.

Burton T, Hoogenboom MO, Beevers ND, Armstrong JD, Metcalfe NB. 2013. Among-sibling differences in the phenotypes of juvenile fish depend on their location within the egg mass and maternal dominance rank. Proceedings of the Royal Society B-Biological Sciences 280:20122441.

Burton T, Robertsen G, Stewart DC, McKelvey S, Armstrong JD, Metcalfe NB. 2016. Maternal age at maturation underpins contrasting behavior in offspring. Behavioral Ecology 27:1280-1287.

Burton T, Rollinson N, McKelvey S, Stewart DC, Armstrong JD, Metcalfe NB. 2020. Adaptive maternal investment in the wild? Links between maternal growth trajectory and offspring size, growth, and survival in contrasting environments. American Naturalist 195:678-690.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. 2013. Stacks: An analysis tool set for population genomics. Molecular Ecology 22:3124-3140.

Chambers RC, Leggett WC. 1996. Maternal influences on variation in egg sizes in temperate marine fishes. American Zoologist 36:180-196.

Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237-268.

Christians JK. 2002. Avian egg size: variation within species and inflexibility within individuals. Biological Reviews 77:1-26.

Core Team R. 2016. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Crump ML. 1981. Variation in propagule size as a function of environmental uncertainty for tree frogs. American Naturalist 117:724-737.

Cutts C, Metcalfe N, Taylor A. 1999. Competitive asymmetries in territorial juvenile Atlantic salmon, *Salmo salar*. Oikos 86:479-486.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST. 2011. The variant call format and VCFtools. Bioinformatics 27:2156-2158.

De-Kayne R, Zoller S, Feulner PG. 2020. A de novo chromosome-level genome assembly of *Coregonus* sp."*Balchen*": One representative of the Swiss Alpine whitefish radiation. Molecular Ecology Resources 20:1093-1109.

Drayton JM, Jennions MD. 2011. Inbreeding and measures of immune function in the cricket *Teleogryllus commodus*. Behavioral Ecology 22:486-492.

Ebel ER, Phillips PC. 2016. Intrinsic differences between males and females determine sex-specific consequences of inbreeding. BMC Evolutionary Biology 16:16-36.

Einum S, Fleming IA. 2004. Environmental unpredictability and offspring size: Conservative versus diversified bet-hedging. Evolutionary Ecology Research 6:443-455.

Einum S, Fleming IA. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): Norms of reaction to environmental quality. Proceedings of the Royal Society of London. Series B: Biological Sciences 266:2095-2100.

Einum S, Fleming IA. 2000. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). Evolution 54:628-639.

Fausch KD. 1984. Profitable stream positions for salmonids: Relating specific growth rate to net energy gain. Canadian Journal of Zoology 62:441-451.

Ford LE, Henderson KJ, Smiseth PT. 2018. Differential effects of offspring and maternal inbreeding on egg laying and offspring performance in the burying beetle *Nicrophorus vespilloides*. Journal of Evolutionary Biology 31:1047-1057.

Fox CW, Thakar MS, Mousseau TA. 1997. Egg size plasticity in a seed beetle: An adaptive maternal effect. The American Naturalist 149:149-163.

Gallardo JA, Garcia X, Lhorente JP, Neira R. 2004. Inbreeding and inbreeding depression of female reproductive traits in two populations of coho salmon selected using BLUP predictors of breeding values. Aquaculture 234:111-122.

Good S, Dodson J, Meekan M, Ryan DA. 2001. Annual variation in size-selective mortality of Atlantic salmon (*Salmo salar*) fry. Canadian Journal of Fisheries and Aquatic Sciences 58:1187-1195.

Gossieaux P, Leclerc M, Van de Walle J, Poisson Y, Toni P, Landes J, Bourret A, Garant D, Pelletier F, Belisle M. 2019. Offspring mass variation in tree swallows: A case of bet-hedging? Ecosphere 10:e02607.

Goudet J. 2005. Hierfstat, a package for R to compute and test hierarchical F-statistics. Molecular Ecology Notes 5:184-186.

Goudet J, Kay T, Weir BS. 2018. How to estimate kinship. Molecular Ecology 27:4121-4135.

Green BS. 2008. Maternal effects in fish populations. Advances in Marine Biology 54:1-105.

Haaland TR, Wright J, Ratikainen, II. 2020. Generalists versus specialists in fluctuating environments: a bet-hedging perspective. Oikos 129:879-890.

Hayes CN, James AW, Stephenson AG. 2005. A comparison of male and female responses to inbreeding in *Cucurbita pepo* subsp. *Texana* (Cucurbitaceae). American Journal of Botany 92:107-115.

Heath DD, Blouw D. 1998. Are maternal effects in fish adaptive or merely physiological side effects? In. Maternal effects as adaptations. New York: Oxford University Press. p. 178-201.

Heath DD, Heath JW, Bryden CA, Johnson RM, Fox CW. 2003. Rapid evolution of egg size in captive salmon. Science 299:1738-1740.

Hendry AP, Day T, Cooper AB. 2001. Optimal size and number of propagules: Allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. American Naturalist 157:387-407.

Hohenlohe PA, Amish SJ, Catchen JM, Allendorf FW, Luikart G. 2011. Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. Molecular Ecology Resources 11:117-122.

Jastrebski CJ, Morbey YE. 2009. Egg size variation in lake trout: Phenotype-habitat correlations show an effect of rearing environment. Transactions of the American Fisheries Society 138:1342-1351.

Keller LF. 1998. Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). Evolution 52:240-250.

Kindsvater HK, Bonsall MB, Alonzo SH. 2011. Survival costs of reproduction predict age-dependent variation in maternal investment. Journal of Evolutionary Biology 24:2230-2240.

Kindsvater HK, Otto SP. 2014. The Evolution of Offspring Size across Life-History Stages. American Naturalist 184:543-555.

Koho J, Karjalainen J, Viljanen M. 1991. Effects of temperature, food density and time of hatching on growth, survival and feeding of vendace (*Coregonus albula* (L.)) larvae. Aqua Fennica 21:63-63.

Krist M. 2011. Egg size and offspring quality: A meta-analysis in birds. Biological Reviews 86:692-716.

Kristensen TN, Sørensen P, Pedersen KS, Kruhøffer M, Loeschcke V. 2006. Inbreeding by environmental interactions affect gene expression in *Drosophila melanogaster*. Genetics 173:1329-1336.

Le Gouvello DZM, Nel R, Cloete AE. 2020. The influence of individual size on clutch size and hatchling fitness traits in sea turtles. Journal of Experimental Marine Biology and Ecology 527:151372.

Leblanc CAL, Kristjansson BK, Skulason S. 2016. The importance of egg size and egg energy density for early size patterns and performance of Arctic charr *Salvelinus alpinus*. Aquaculture Research 47:1100-1111.

Li H, Durbin R. 2010. Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics 26:589-595.

Marshall DJ, Bonduriansky R, Bussière LF. 2008. Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. Ecology 89:2506-2517.

Marshall DJ, Pettersen AK, Cameron H. 2018. A global synthesis of offspring size variation, its eco-evolutionary causes and consequences. Functional Ecology 32:1436-1446.

Mattey S, Strutt L, Smiseth P. 2013. Intergenerational effects of inbreeding in *Nicrophorus vespilloides*: Offspring suffer fitness costs when either they or their parents are inbred. Journal of Evolutionary Biology 26:843-853.

Mattey SN, Richardson J, Ratz T, Smiseth PT. 2018. Effects of offspring and parental inbreeding on parent-offspring communication. The American Naturalist 191:716-725.

McAdam AG, Garant D, Wilson AJ. 2014. The effects of others' genes: Maternal and other indirect genetic effects. In. Quantitative genetics in the wild. New York: Oxford University Press. p. 84-103.

Moland E, Olsen EM, Stenseth NC. 2010. Maternal influences on offspring size variation and viability in wild European lobster *Homarus gammarus*. Marine Ecology Progress Series 400:165-173.

Moore AJ, Brodie III ED, Wolf JB. 1997. Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. Evolution 51:1352-1362.

Mousseau TA, Fox CW. 1998. Maternal effects as adaptations. New York, USA: Oxford University Press.

Neely KG, Myers JM, Hard JJ. 2012. A comparison of early development between a domesticated stock of coho salmon and its parental stock. Transactions of the American Fisheries Society 141:1504-1509.

O'Leary SJ, Puritz JB, Willis SC, Hollenbeck CM, Portnoy DS. 2018. These aren't the loci you'e looking for: Principles of effective SNP filtering for molecular ecologists. Molecular Ecology 27:3193 - 3206.

Olofsson H, Ripa J, Jonzen N. 2009. Bet-hedging as an evolutionary game: the trade-off between egg size and number. Proceedings of the Royal Society B-Biological Sciences 276:2963-2969.

Parchman TL, Gompert Z, Mudge J, Schilkey FD, Benkman CW, Buerkle CA. 2012. Genome-wide association genetics of an adaptive trait in Lodgepole pine. Molecular Ecology 21:2991-3005.

Parker GA, Begon M. 1986. Optimal egg size and clutch size: Effects of environment and maternal phenotype. The American Naturalist 128:573-592.

Pedersen KS, Kristensen TN, Loeschcke V. 2005. Effects of inbreeding and rate of inbreeding in *Drosophila melanogaster*–Hsp70 expression and fitness. Journal of Evolutionary Biology 18:756-762.

Philippi T, Seger J. 1989. Hedging one's evolutionary bets, revisited. Trends in Ecology & Evolution 4:41-44.

Putten V. 1999. Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. Journal of Evolutionary Biology 12:1125-1137.

Quinn TP, Hendry AP, Wetzel LA. 1995. The influence of life history trade-offs and the size of incubation gravels on egg size variation in sockeye salmon (*Oncorhynchus nerka*). Oikos 74:425-438.

Rantala M, Roff D. 2007. Inbreeding and extreme outbreeding cause sex differences in immune defence and life history traits in Epirrita autumnata. Heredity 98:329-336.

Reid JM, Arcese P, Keller LF. 2003. Inbreeding depresses immune response in song sparrows (*Melospiza melodia*): Direct and inter–generational effects. Proceedings of the Royal Society of London. Series B: Biological Sciences 270:2151-2157.

Saccheri IJ, Brakefield PM, Nichols RA. 1996. Severe inbreeding depression and rapid fitness rebound in the butterfly *Bicyclus anynana* (Satyridae). Evolution 50:2000-2013.

Sakai S, Harada Y. 2001. Why do large mothers produce large offspring? Theory and a test. The American Naturalist 157:348-359.

Sellers R, Clifford C, Treuting P, Brayton C. 2012. Immunological variation between inbred laboratory mouse strains: Points to consider in phenotyping genetically immunomodified mice. Veterinary Pathology 49:32-43.

Smallbone W, Van Oosterhout C, Cable J. 2016. The effects of inbreeding on disease susceptibility: *Gyrodactylus turnbulli* infection of guppies, *Poecilia reticulata*. Experimental Parasitology 167:32-37.

Smith CC, Fretwell SD. 1974. The optimal balance between size and number of offspring. The American Naturalist 108:499-506.

Stearns SC, Hendry AP. 2004. Evolution illuminated: Salmon and their relatives. Oxford, New York: Oxford University Press, Inc.

Su GS, Liljedahl LE, Gall GAE. 1996. Effects of inbreeding on growth and reproductive traits in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 142:139-148.

Swain P, Nayak S. 2009. Role of maternally derived immunity in fish. Fish & Shellfish Immunology 27:89-99.

Turnbull LA, Santamaria L, Martorell T, Rallo J, Hector A. 2006. Seed size variability: From carob to carats. Biology Letters 2:397-400.

Wetzel DP, Stewart IR, Westneat DF. 2012. Heterozygosity predicts clutch and egg size but not plasticity in a house sparrow population with no evidence of inbreeding. Molecular Ecology 21:406-420.

White K, Eason DK, Jamieson IG, Robertson BC. 2015. Evidence of inbreeding depression in the critically endangered parrot, the kakapo. Animal Conservation 18:341-347.

Wolf JB, Wade MJ. 2001. On the assignment of fitness to parents and offspring: Whose fitness is it and when does it matter? Journal of Evolutionary Biology 14:347-356.

Wolf JB, Wade MJ. 2009. What are maternal effects (and what are they not)? Philosophical Transactions of the Royal Society B: Biological Sciences 364:1107-1115.

Figures

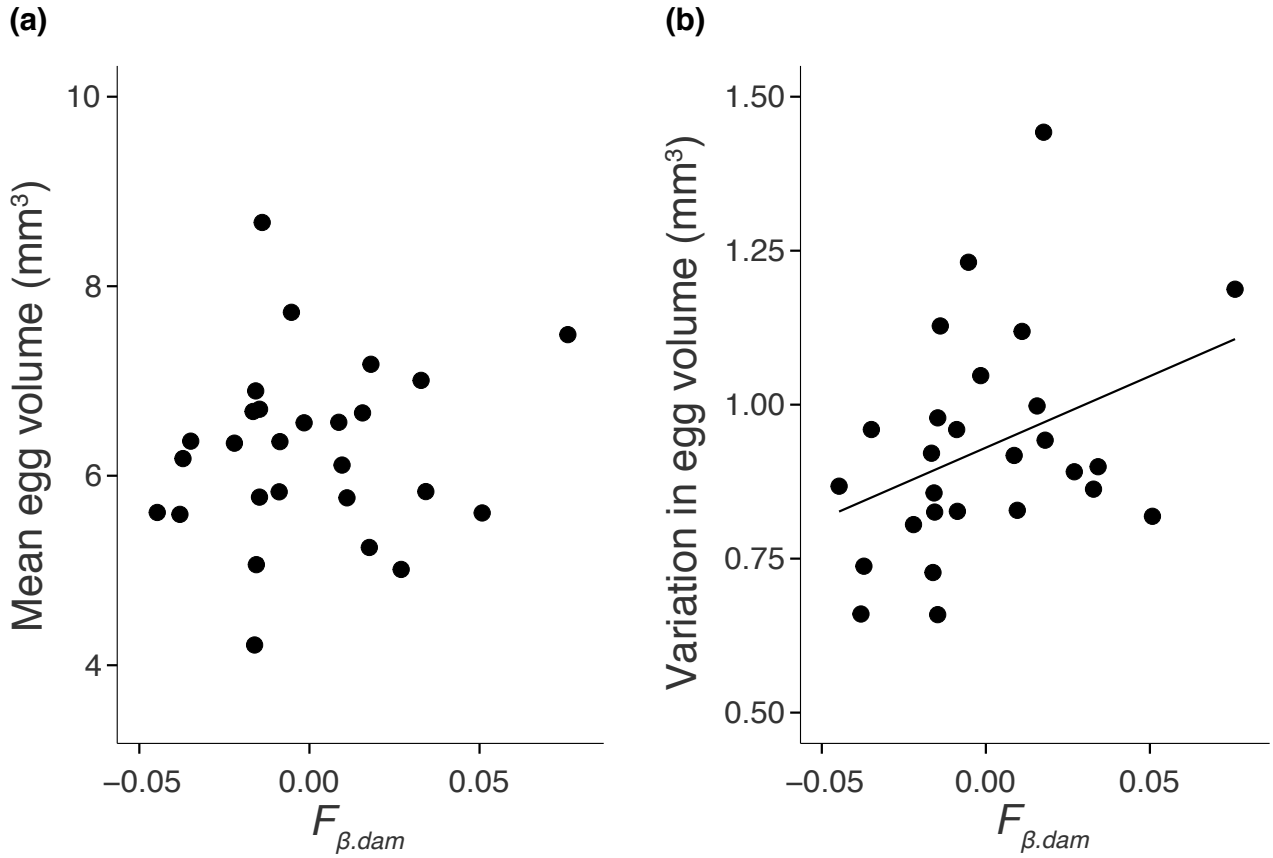


Figure 1. The link between maternal inbreeding coefficient ($F_{\beta.dam}$) and (a) mean egg volume per female (mm^3) and (b) within-clutch variation in egg volume per female (standard deviations). The regression line illustrates the significant correlation. See text for statistics.

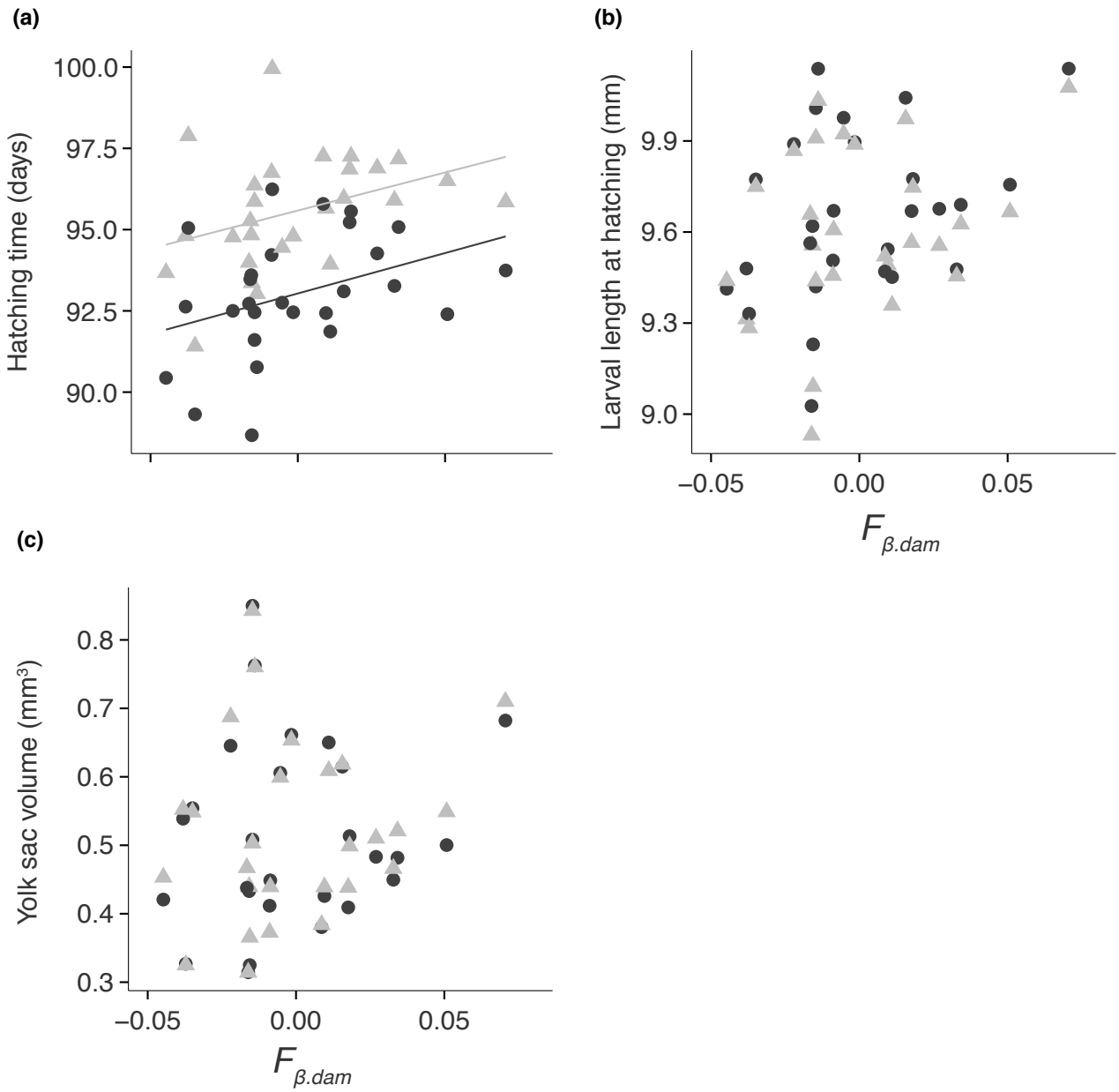


Figure 2. The effect of maternal inbreeding coefficient ($F_{\beta.dam}$) on (a) mean hatching time, (b) on mean larval length at hatching, and (c) mean yolk sac volume at hatching. Embryos exposed to PF are represented by light grey triangles and controls are represented with dark grey dots. The regression lines are given for significant correlations in the corresponding colors. For statistics see Supplementary Table S1b-d.

Supplementary information

Maternal inbreeding increases within-clutch variation in egg size and reduces embryo development rate in whitefish

Christian de Guttry[1], Alberto Garcia Jimenez[1,2], Nathalie Caloz[1], Audrey Atherton[1,3] Eliane Longange[1] & Claus Wedekind[1*]

[1] Department of Ecology & Evolution, University of Lausanne, Lausanne, Switzerland

[2] now at Department of Computational Biology, University of Lausanne, Lausanne, Switzerland

[3] now at: Department of Environmental Systems Science, ETH, Zürich, Switzerland

* correspondence: claus.wedekind@unil.ch

Chapter status: unpublished

Content

Table S1. Re-analysis of the data of Guttry *et al*. (Table 1 in chapter 5 of this thesis) in a new model that includes the dams' inbreeding coefficients $F_{\beta.dam}$ in order to test its effects on (*a*) embryo mortality, (*b*) hatching time, (c) length at hatching, (*d*) yolk sac volume at hatching and (*e*) larval mortality. Likelihood ratio tests on mixed model regressions were used to compare a reference model (in bold) with models including or lacking the term of interest. Significant new p-values are highlighted in bold.

Model	Effect tested	AIC	d.f.	X^2	P
(a) Embryo mortality					
t + $F_{\beta.dam}$ + S + D		1648	6		
t + $F_{\beta.dam}$ + S	D	1669	5	22	<0.001
t + $F_{\beta.dam}$ + D	S	1646	5	0	0.97
t + $F_{\beta.dam}$ + S + D + D*S	D*S	1650	7	0	1
$F_{\beta.dam}$ + S + D	t	1703	5	56	<0.001
t + $F_{\beta.dam}$ + **S** + D + D*t	D*t	1652	8	0.01	0.99
t + $F_{\beta.dam}$ + S + D + S*t	S*t	1651	8	1.6	0.44
t + S + D	$F_{\beta.dam}$	1647	5	0.4	0.51
t + $F_{\beta.dam}$ + S + D + t* $F_{\beta.dam}$	t* $F_{\beta.dam}$	1648	7	2.2	0.13
(b) Hatching time					
t + $F_{\beta.dam}$ + S + D		32880	7		
t + $F_{\beta.dam}$ + S	D	33680	6	802	<0.001
t + $F_{\beta.dam}$ + D	S	33639	6	761	<0.001
t + $F_{\beta.dam}$ + S + D + D*S	D*S	32882	8	0.1	0.70
$F_{\beta.dam}$ + S + D	t	33651	6	773	<0.001
t + $F_{\beta.dam}$ + **S** + D + D*t	D*t	32838	9	46	<0.001
t + $F_{\beta.dam}$ + S + D + S*t	S*t	32881	9	3.2	0.19
t + S + D	$F_{\beta.dam}$	32882	6	4	**0.04**
t + $F_{\beta.dam}$ + S + D + t* $F_{\beta.dam}$	t* $F_{\beta.dam}$	32882	8	0.1	0.70
(c) Length at hatching (mm)					
t + $F_{\beta.dam}$ + S + D		8680	7		
t + $F_{\beta.dam}$ + S	D	9890	6	1212	<0.001
t + $F_{\beta.dam}$ + D	S	8812	6	134	<0.001
t + $F_{\beta.dam}$ + S + D + D*S	D*S	8678	8	4.5	0.03
$F_{\beta.dam}$ + S + D	t	8695	6	17	<0.001
t + $F_{\beta.dam}$ + **S** + D + D*t	D*t	8684	9	0.2	0.86
t + $F_{\beta.dam}$ + S + D + S*t	S*t	8684	9	0.3	0.83
t + S + D	$F_{\beta.dam}$	8681	6	3.2	0.08

t + $F_{\beta.dam}$ + S + D + t* $F_{\beta.dam}$	t* $F_{\beta.dam}$	8682	8	0.2	0.64
(d) Yolk sac volume at hatching (mm^3)					
t + $F_{\beta.dam}$ + S + D		-2033	7		
t + $F_{\beta.dam}$ + S	D	-351	6	1684	<0.001
t + $F_{\beta.dam}$ + D	S	-2008	6	27	<0.001
t + $F_{\beta.dam}$ + S + D + D*S	D*S	-2033	8	1.6	0.20
$F_{\beta.dam}$ + S + D	t	-2033	6	2.8	0.10
t + $F_{\beta.dam}$ + **S** + D + D*t	D*t	-2030	9	0.5	0.79
t + $F_{\beta.dam}$ + S + D + S*t	S*t	-2232	9	2.7	0.25
t + S + D	$F_{\beta.dam}$	-2035	6	0.6	0.40
t + $F_{\beta.dam}$ + S + D + t* $F_{\beta.dam}$	t* $F_{\beta.dam}$	-2033	8	1.2	0.27
(e) Larval mortality					
t + $F_{\beta.dam}$ + S + D		1240	6		
t + $F_{\beta.dam}$ + S	D	1240	5	1.8	0.17
t + $F_{\beta.dam}$ + D	S	1239	5	1.2	0.26
t + $F_{\beta.dam}$ + S + D + D*S	D*S	1242	7	0.2	0.68
$F_{\beta.dam}$ + S + D	t	1302	5	63	<0.001
t + $F_{\beta.dam}$ + **S** + D + D*t	D*t	1243	8	0.8	0.67
t + $F_{\beta.dam}$ + S + D + S*t	S*t	1241	8	3	0.21
t + S + D	$F_{\beta.dam}$	1239	5	0.6	0.44
t + $F_{\beta.dam}$ + S + D + t* $F_{\beta.dam}$	t* $F_{\beta.dam}$	1242	7	0	0.95

Fixed: treatment (t), dam inbreeding coefficient ($F_{\beta.dam}$); random: sire (S), dam (D).

Chapter 3 - Maternal age rather than size predicts propagules size in whitefish

Christian de Guttry[1], Florin Stettler[1], Jonas Bylemans[1], Audrey Atherton[1,2], Steven Campana[3#] & Claus Wedekind[1*]

[1]Department of Ecology & Evolution, University of Lausanne, Lausanne, Switzerland

[2] now at: Department of Environmental Systems Science, ETH, Zürich, Switzerland

[3]Faculty of Life and Environmental Sciences, University of Iceland, Reykjavik, Iceland.

(# not yet agreed to the proposed co-authorship)

* correspondence: claus.wedekind@unil.ch

Chapter status: unpublished

Author contributions

CdG and CW organized and conducted the field and laboratory work with the help of further members of the group. CW performed the experimental crosses. CdG and FS monitored embryos and larval development in the climate chamber. FS extracted and processed the otoliths, CdG aged the parents and determined larval size together with AA. CdG generated the datasets and performed statistical analyses together with CW. CdG, JB and CW wrote the manuscript.

Abstract

In iteroparous salmonids, large females usually produce larger eggs and consequently larger offspring than small females. However, it is unclear whether this correlation reveals a size- or an age-dependent reproductive strategy because female size and age are typically correlated. Here we used otoliths to determine the age of female whitefish that had been sampled in only one type of gill nets at different times during the spawning season, and whose offspring had been monitored until late larval stages. Most of these females were 3+ or 4+. As expected from the size-selectivity of the gill net, the size distributions of young and old individuals overlapped significantly, i.e. effects of age and size could be well disentangled. We found that egg size was mainly affected by season and female age, while female size by itself played no significant role. Females sampled towards the end of the breeding season produced larger eggs than females sampled at the beginning of the season. Moreover, with increasing age, females produced on average larger eggs that led to larger yolk-sac larvae. We conclude that variation in egg size reveals age- but not size-dependent reproductive strategies of female whitefish.

Keywords: Maternal effects, maternal age, maternal size, egg size, offspring phenotypes, age-dependent reproductive investment.

Introduction

Understanding which mechanisms influence the evolution of propagules (i.e. eggs and larvae) size has long been a concern of evolutionary biologists. After decades of theories and empirical research it is now accepted that the environmental conditions and maternal effects are the most important factors driving propagules size evolution (Rollinson and Rowe 2016). It remains to be understood how the different maternal traits influence this process, because those traits are strongly correlated (Marshall, et al. 2010).

The most prominent optimality theory predicts that propagules size mainly results from the environmental stimuli perceived by the mothers (Smith and Fretwell 1974). The mother attempts to anticipate the environment that the propagules will face and modifies their size accordingly to increase their and hers fitness. While this would predict strong selective pressures on females producing offspring of sub-optimal propagules size, empirical studies mostly found within population variance in offspring size (Fleming and Gross 1990; Bernardo 1996; Berg, et al. 2001; Einum and Fleming 2002; Cogliati, et al. 2018). Hence, more recent optimality models included maternal effects, together with environmental conditions, as potential factors influencing offspring size evolution (Parker and Begon 1986; Hendry, et al. 2001; Sakai and Harada 2001; Kindsvater, et al. 2011; Kindsvater and Otto 2014).

Maternal effects (i.e. the influence of the phenotype, genotype and, the environment experience by the mother, on offspring) have been recognized to have a major role in determine propagules phenotypes and performances. Their influence is stronger in early-life stages and, depending on the species, they disappear over time (Vindenes, et al. 2016) or persist across generations (Goos, et al. 2019; Reichert, et al. 2020). Consequently, maternal effects can have a strong influence on population dynamics and evolutionary trajectories (Wolf and Wade 2009). Female size and/or age are two of the most studied maternal traits generating phenotypic variation in offspring (Bernardo 1996; Marshall, et al. 2010; Rollinson and Rowe 2016). For instance, it is common to observe larger and/or older females producing larger propagules than smaller and/or younger females (Roff 1992). A positive correlation between maternal size and offspring size has been found in different meta-analysis examining from animals (Roff 1992; Lim, et al. 2014) to insects (Fox and Czesak 2000). However, the increase in the size of the progeny, based on the increase in the size of the mother, cannot yet be generalized as global mechanism. Indeed, in numerous empirical observation a non-positive correlation has been observed (Roff 1992; Lim, et al. 2014; Rollinson and Rowe 2016). Offspring size in different species is, in fact, positively correlated with the age of the mother (Ito 1997; Berkeley, et al. 2004; Ameri, et al. 2019). Understanding how these two traits might influence independently, or through an interaction, the

evolution of propagules size in species with indetermined growth consequently remains an open challenge (Marshall, et al. 2010; Congdon, et al. 2013).

A variety of theoretical studies attempted to explain the observed trends and predict how maternal size influence offspring size (Pianka and Parker 1975; Parker and Begon 1986). It was initially suggested that a large mother would produce larger offspring that will prevail in the sibling competition generated by her large fecundity (Parker and Begon 1986). Larger offspring will therefore be more competitive than smaller ones in the early stages of life and increase maternal fitness (Marshall, et al. 2006). In addition to intraspecific competition, it has been suggested that larger mothers are able to better provide resources for their offspring than smaller mothers, due to differences in physiology (Sakai and Harada 2001). For instance, the better ability of a larger mother to compete for food compared to a smaller mother. This, results in larger mothers having a higher energetical budget to allocate to reproduction and consequently to the production of optimal sized propagules than a smaller mother (Barneche, Robertson, et al. 2018).

Regarding age it has been predicted to have an important effect on offspring size particularly in iteroparous species (Williams 1966; Pianka and Parker 1975; Kindsvater, et al. 2011). When a female reproduces more than once in its lifecycle a trade-off is expected between reproductive events (Williams 1966). As the likelihood of a future reproductive event decreases, a female increases her investment in reproduction (Pianka and Parker 1975). A set of more recent models, consider that a reduction in energy allocated to reproduction, in the early years of sexual maturity, increases the longevity of an individual (Kindsvater, et al. 2011; Kindsvater and Otto 2014). As a result, younger females are expected to decrease their reproductive allocation and produce smaller offspring. All of the aforementioned models still lack extensive empirical data that can validate them. Indeed, few empirical studies that have tried to disentangle the effect of maternal size from that of age on offspring size. Those that have succeeded have shown that maternal size is the main determinant of offspring size in organisms with determinate (Hepp and Kennamer 1993; Blanchard, et al. 2007) and indeterminate growth (O'Dea, et al. 2015). Despite these findings, the effect of maternal age on propagules size remains significant in many species and understanding if and which role age plays in this dynamic remains crucial.

In this experiment, our main aim was to disentangle the effects of size and age of the mother on the size and performances of the propagules by using the European whitefish (*Coregonus suidteri*). A strong size selective pressure on whitefish populations through gillnet fishing provides an opportunity to sample sexually mature females of approximately the same length but possibly belonging to different age classes. Moreover, whitefish are iteroparous characterize by indetermined growth. Therefore, we expect the possibility for the evolution of for an age-dependent allocation in

reproduction. Another important feature that can benefit this study is that whitefish produce thousands of eggs per reproductive season with a great variability in size per female and within population (Wedekind 2002; von Siebenthal, et al. 2009; Clark, et al. 2014). In oviparous organisms, egg size is one of the most widely studied traits to measure per-offspring energetical investment. Indeed, the size of an egg has a strong positive correlation with the energy invested by the mother on it (Marshall and Keough 2008) resulting in large eggs giving rise to larger individuals (Roff 1992; Einum and Fleming 1999, 2000b). Egg size is than an excellent trait to disentangle the effects of maternal size and age.

Material and Methods

Fish sampling, measurement of eggs and larvae

Mature female whitefish (*Coregonus suidteri*) were caught from lake Hallwil (Switzerland) using gillnets of 35 mm mesh size at the beginning (N = 13; 20/12/16) and towards the end of their breeding season (N = 30; 09/01/17) in the course of two other studies (see chapter 1 and chapter 5 of the thesis). Their size was determined and their gametes stripped to produce experimental sib groups. Details on the experimental production of maternal sib groups and the monitoring of the resulting embryos and larvae are given in de Guttry et al. (chapter 1) for the early, and in de Guttry et al. (chapter 5) for the late breeding season. Briefly, the eggs of each female were crosses with at least 10 different males in block-wise full-factorial *in vitro* breeding experiments, i.e. possible paternal effects are sufficiently controlled for when focusing on maternal effects here. Mean egg volume was determined from 30 eggs per sib group after egg hardening on day 0 (i.e. from at least 300 eggs per female). A subsample of 24 freshly fertilized eggs were then raised singly (one embryo per 2 mL well of 24-well plates) in the laboratory under standardized conditions until 21 days after hatching. In both experiments, half of the embryos were exposed to a stress treatment while the remaining 12 embryos per sib group always experienced the same control conditions, i.e. a sham treatment with nutrient broth used for bacterial growth as in Clark et al. (2014). These controls (n_{total} = 6,408) are used here to test for effects of female age and size on offspring development.

A third sample of 30 females had been caught from the same location and with the same type of nets on 11/01/17 in the course of a further study on the effects of various pH levels on embryo performance (see details in de Guttry et al., chapter 4 of this thesis). Egg sizes at day 0 were not taken in this 3rd study, only 29 of the females could be aged (see below), and embryos, including the controls raised at pH = 7.0, were incubated under conditions that differed from the first two studies (de Guttry et al. chapter 1 and 5). Embryo and larval performance measures from this 3rd study were therefore not included in the present analyses. However, the sizes of eggs that had been raised under non-stress

conditions had been determined 90 days post fertilization. These egg size measurements (at least 80 eggs per female) could be used here to test again for effects of female age and female size on egg size in an extended dataset (n_{total} = 72 females).

Age determination

Otoliths were extracted from all 72 females and age was determined using a slightly modified 'crack and burn' method (Campana, et al. 2016). Otoliths were broadside cut in half and shortly exposed to a blue flame to highlight the rings. A standardized photo was then taken using a Canon digital camera mounted on a binocular microscope. Age was determined by counting the number of annuli starting from the hatch check to the external margin of the otolith. A 3+ fish is then, for instance, a fish with 3 yearly rings, i.e. when sampled during spawning nearly 4 years old.

Statistical analyses

Student's t-tests were used to assess the effect of season and female age on the female standard length. The possible effects of female age and size on egg size, embryos, and larval performances were tested in linear mixed models (LMM) and generalized linear mixed models (GLMM). Embryo mortality was used as binomial response variables in GLMM. Hatching time, length at hatching, yolk sac volume at hatching, and length 21 days after hatching were analyzed as continue response variables in LMM. Dam identity and season were used as random factors. Models fit were compared with Akaike's information criteria (AIC) and likelihood ratio tests (LRT). The significance of each factors was tested comparing a model lacking or including the term of interest with a reference model. When using the extended dataset that included data from chapter 4 of this thesis, a multiple regression was used to test again the effects of female age and size on mean egg size of in total 72 females. Analysis were done in R (Core Team 2016) using the lme4 package (Bates, et al. 2015) and in JMP 15.2.1 (www.jmp.com).

Results

The females could be assigned to the 4 age classes 2+ (n = 1), 3+ (n = 30), 4+ (n = 10), and 5+ (n = 2). Given the low number of individuals in the 2+ and 5+ classes, the 2+ and the 3+ fish were summarized as "younger" and the 4+ and 5+ as "older". The two sampling dates did not differ in age (likelihood ratio test, χ^2 = 1.6, d.f. = 1, p = 0.21) nor female lengths (t = 0.9, d.f. = 41, p = 0.37). Older females were on average larger than younger ones (t = -2.1, d.f. = 41, p = 0.04), but the size distributions of the two age categories overlapped significantly (older: mean = 262.9 mm, range 243.3 to 286.5 mm; younger: mean = 256.3 mm, range 241.9 to 273.4 mm).

Mean egg volume was dependent on female age and season, with older females producing larger eggs than younger females, and both age classes producing larger eggs towards the end of the breeding season (Table 1a; Fig. 1a). The effects of female age and female size did not vary between the period of the season (see non-significant interactions in Table 1a). However, the size of the eggs seemed affected by a significant interaction between age and size (Table 1a): mean egg size tended to increase with body size in younger females while no such tendency was observed in older females (Supplementary Fig. S1a). This interaction between female age and size on egg size could, however, not be confirmed in the extended dataset that included 29 further females, while the effect of female age on egg size remained significant and the effect of female size on egg size remained non-significant (Table 2; Fig. S1b).

There was a significant season effect on larval length 21 days after hatching (Table 1f; Fig. 1d) but the analogous effects were not significant for the remaining embryo or larval performance measures (Table 1). The identity of the females ("dam") had an effect on all offspring performance measures (Table 1a-f). These dam effects could never be explained by female size alone (Table 1). However, while female age did not affect embryo mortality (Table 1b) nor the timing of hatching (Table 1c), it affected embryo growth: larvae of older females were larger at hatching (Table 1d; Fig. 1b), had larger yolk sacs (Table 1e; Figure 1c), and were larger 21 days after hatching (Table 1f; Fig. 1d) than larvae of younger females.

Discussion

Theories predict that the size and age of the mother can influence the size of the offspring. What is challenging, is to isolate their effects in species where the length of the female increases constantly with age. In our study, we were able to disentangle the effects of female age and length as size selective fishing pressures in whitefish have resulted in a more uniform size distribution in females varying in age. We then used this study system to evaluate the effects of maternal length and/or age on egg size and hatchlings phenotypes. Older females produced larger eggs, longer hatchlings and longer juveniles at 21 days post-hatching compared to younger females. This trend was present in both periods of the breeding season albeit the mean egg volume in the early reproductive season was significantly lower compared to the mean egg volume in the late reproductive season. We did not observe a significant effect of maternal size on any of the larval traits we investigated.

We found that older females continuously produced larger eggs indicating that reproductive allocation in female whitefish is age dependent. The energy invested by a female for reproduction is limited, generating the classic trade off of size/number of eggs (Smith and Fretwell 1974). Considering

that the production of larger eggs is energetically more expensive than the production of smaller eggs (Pick, et al. 2016), we therefore suggest that older females have a greater energetical budget to invest in reproduction than younger females. This trend might be explained by the fact that until sexual maturity younger females employ most of their energetical budget for somatic growth (Marshall, et al. 2018) resulting in the production of smaller eggs. Our results are consistent with the "Terminal investment hypothesis" (Williams 1966) and with optimality models that predict female age as one of the main drivers in determining offspring size (Pianka and Parker 1975; Kindsvater, et al. 2011; Kindsvater and Otto 2014). The common expected outcomes, of the above mentioned models, are females increasing the per-offspring allocation resulting in the production of larger individuals. Indeed, the production of larger eggs in our study corresponds to the production of larger larvae with a larger yolk sac volume at hatching compared to smaller eggs that result in smaller larvae. Larger offspring are usually more competitive in early life stages compared to the smaller one in common environmental conditions (Elgar 1990; Einum and Fleming 1999, 2000b, a; Benhaïm, et al. 2003; Leblanc, et al. 2011; Segers and Taborsky 2011; Leblanc, et al. 2016). These benefits eventually translate into an increase in the fitness of the progeny and consequently an increase in the fitness of the mother (Marshall, et al. 2010). Thus, this age dependent investment in reproduction may be a strategy to maximize fitness in later years of life. Clearly, we expect that the environment perceived by the mother influence the size of the eggs produced (Smith and Fretwell 1974; Barneche, Burgess, et al. 2018; Burton, et al. 2020). However, a benefit of our experimental setting was the possibility to standardize environmental conditions (i.e. all females are coming from the same lake and sampled during the same reproductive season). We can then conclude that, independently of the environment perceived by the mother, the size of the propagules is defined by maternal age in whitefish.

With our sampling method we were able to reduce the variation in female size, but by using this approach we reduced the number of age classes that could be sampled. Our females belonged to only 2 age groups made by 4 age classes. However, in the whitefish north American conspecific *Coregonus clupeaformis*, the increase in egg size with age has been demonstrated, in a survey study, investigating 10 different age classes (Johnston, et al. 2012). Whitefish are not the only salmonids where an effect of maternal age was found on egg size. In the brown trout (*Salmo trutta*) first time spawners were reported to produce smaller eggs compared to repeated spawner of similar size (Jonsson and Jonsson 1999). The same trend was observed in the lake trout (*Salvelinus namaycush*) where egg size was increasing with maternal age (Johnston 2018). Based on our experimental design, we cannot draw conclusions on the causal factor of why older females produce larger offspring and younger females produce smaller offspring. What we want to emphasized is the importance to consider maternal age predicting propagules size evolution, since in salmonids it seems to be an important

determinant. To be generalized, our work should be complemented by investigating a larger spectrum of age classes in order to possibly investigate a negative effect of senescence on old females (Barks and Laird 2020).

The size of the eggs, besides depending on the age of the mother, was also influenced by the period of the reproductive season in which the females were sampled. Eggs produced in the late breeding season were significantly larger than eggs produced in the early breeding season despite female size did not differ between the two sampling days. A typical whitefish breeding season in lake Hallwil last on average one month. Differences in the quality of breeders and of the clutch during the reproductive season can be expected and were observed in other fish species (Trippel 1998; Oliveira, et al. 1999; Tveiten, et al. 2001; Fleming and Reynolds 2004; Foster and Gilmour 2020). This trend varies depending on the species considered and by its ecology (Mandić and Regner 2014). For instance, it is common for fish that spawn in spring/summer to have a decrease in egg size along the breeding season and for fish that spawn in autumn/winter to have eggs that increase in size (Chambers 1997; Barneche, Burgess, et al. 2018). The production of larger eggs towards the end of the season may be a strategy to compensate for intraspecific competition (Pearson and Warner 2018) considering that larger larvae outcompete smaller larvae in the early stages of life (Marshall, et al. 2018). With this result we highlighted the need to control for the period of the season in which sampling is done. Assuming that there are no differences within a sampling season may lead to erroneous estimates and conclusions, when individual performances are compared.

In this study, maternal effects (‘dam’) were highly significant for all response variables analyzed. This indicates a strong contribution, of the maternal environment and additive genetic effects, on the phenotypes and performances of the propagules, as already observed in whitefish (von Siebenthal, et al. 2009; Brazzola, et al. 2014; Clark, et al. 2014). These effects, in our experiment, have never been explained by the size of the mother. The lack of correlation between maternal and offspring size has already been found in other salmonid species (Elgar 1990; Heath, et al. 1999; Berg, et al. 2001; Seamons and Quinn 2010). This could be the results of a species-specific allocation in reproduction, or it could be due by the difficulty of controlling the effects of other maternal traits that can be confused with size. For instance, it has been shown that the maternal condition and the nutritional status of the female also influence eggs and offspring phenotypes (Rollinson and Rowe 2016).

Surprisingly, we found a significant effect of the interaction between maternal size and maternal age on the size of eggs produced. This showed a tendency for younger females to produce larger eggs as their size increased, while in older females there seems to be no differences based on maternal size. This difference in the slope of correlation, between egg size and female size within

different age groups, has rarely been investigated. A recent study demonstrated that in the Artic charr (*Salvelinus alpinus*), egg number is overall positively correlated with female mass, however, this correlation was lost looking within age classes (Lasne, et al. 2018). Despite this, we want to be cautious in interpreting our result. When adding 29 females from a parallel study on the same species, we observed that this interaction was lost. By increasing the sample size both age groups show a tendency to produce larger eggs with increasing size. A study aimed at resolving the possible correlation between egg size and female size within different age classes is recommended to help clarify this dynamic.

Conclusion

The presence of an age-dependent reproductive allocation in whitefish highlight the importance of considering different maternal traits in evolutionary models other than maternal size. Several maternal characteristics can constrain the reproductive allocation of a female such as: genetics, epigenetics, behavior, physiology and morphology. All of them can differentially influence offspring phenotypes and, behavior. In planning future studies, there is a need to separating the effects of each maternal trait from the others so that we will understand how each of them affects maternal allocation in reproduction and consequently offspring size. From a conservation point of view, our study shows that protecting only the largest females in a population may not be sufficient. For instance, most conservation programs that attempt to protect endangered species tend to protect larger females assuming the link maternal size-offspring size/performances. To make these programs even more effective, targeted protection of other maternal traits than size should be implemented. Our results could also help inform the restocking authorities in which period of the season to take gametes in order to possibly increase offspring performances. For a better understanding of the contribution of maternal effect on population dynamics and evolution future study should also investigate if these effects in whitefish disappear over time or stay constant also in adulthood.

Ethics

The experimental breeding and the raising of embryos in the laboratory were approved by the Fisheries Administration of the Aargau canton. Approvals by the Veterinary Offices of the involved cantons were not required because the fish were caught in a commercial fishing program, measurements and tissue samples were taken from dead fish, and all larvae were euthanized before the end of the yolk sac period.

Data availability

Data will be deposited on the dryad depository upon acceptance of the manuscript.

Funding

The project was supported by the lottery funds of the Aargau canton and the Swiss National Science Foundation (31003A_182265).

Acknowledgements

We thank R. Stadelmann for catching the fish, J. Beninde, I. Castro, G. Fabre, L. Garaud, E. Longange, L. Marques da Cunha, K. Mobley, D. Nusbaumer, E. Seddon, and C. Vincent for assistance in the field and in the laboratory, D. Bittner for permits, and all the above for discussion.

References

Ameri M, Kemp DJ, Barry KL, Herberstein ME. 2019. Age-specific reproductive investment and offspring performance in an orb-web spider, *Argiope radon*. Evolutionary Biology 46:207-215.

Barks PM, Laird RA. 2020. Parental age effects and the evolution of senescence. American Naturalist 195:886-898.

Barneche DR, Burgess SC, Marshall DJ. 2018. Global environmental drivers of marine fish egg size. Global Ecology and Biogeography 27:890-898.

Barneche DR, Robertson DR, White CR, Marshall DJ. 2018. Fish reproductive-energy output increases disproportionately with body size. Science 360:642-644.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Benhaïm D, Skúlason S, Hansen B. 2003. Behavioural variation in juvenile Arctic charr in relation to body size. Journal of Fish Biology 62:1326-1338.

Berg O, Hendry A, Svendsen B, Bech C, Arnekleiv J, Lohrmann A. 2001. Maternal provisioning of offspring and the use of those resources during ontogeny: Variation within and between Atlantic salmon families. Functional Ecology 15:13-23.

Berkeley SA, Chapman C, Sogard SM. 2004. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology 85:1258-1264.

Bernardo J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. American Zoologist 36:216-236.

Blanchard P, Hanuise N, Dano S, Weimerskirch H. 2007. Offspring sex ratio in relation to parental structural size and body condition in the long-lived wandering albatross (*Diomedea exulans*). Behavioral Ecology and Sociobiology 61:767-773.

Brazzola G, Chèvre N, Wedekind C. 2014. Additive genetic variation for tolerance to estrogen pollution in natural populations of alpine whitefish (*Coregonus sp., Salmonidae*). Evolutionary Applications 7:1084-1093.

Burton T, Rollinson N, McKelvey S, Stewart DC, Armstrong JD, Metcalfe NB. 2020. Adaptive maternal investment in the wild? Links between maternal growth trajectory and offspring size, growth, and survival in contrasting environments. American Naturalist 195:678-690.

Campana SE, Valentin AE, MacLellan SE, Groot JB. 2016. Image-enhanced burnt otoliths, bomb radiocarbon and the growth dynamics of redfish (*Sebastes mentella* and *S. fasciatus*) off the eastern coast of Canada. Marine and Freshwater Research 67:925-936.

Chambers RC. 1997. Environmental influences on egg and propagule sizes in marine fishes. In. Early life history and recruitment in fish populations. London: Springer. p. 63-102.

Clark ES, Pompini M, Marques da Cunha L, Wedekind C. 2014. Maternal and paternal contributions to pathogen resistance dependent on development stage in a whitefish (Salmonidae). Functional Ecology 28:714-723.

Cogliati KM, Unrein JR, Stewart HA, Schreck CB, Noakes DL. 2018. Egg size and emergence timing affect morphology and behavior in juvenile chinook salmon, *Oncorhynchus tshawytscha*. Ecology and Evolution 8:778-789.

Congdon JD, Gibbons JW, Brooks RJ, Rollinson N, Tsaliagos RN. 2013. Indeterminate growth in long-lived freshwater turtles as a component of individual fitness. Evolutionary Ecology 27:445-459.

Core Team R. 2016. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Einum S, Fleming IA. 2002. Does within-population variation in fish egg size reflect maternal influences on optimal values? The American Naturalist 160:756-765.

Einum S, Fleming IA. 2000a. Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature 405:565-567.

Einum S, Fleming IA. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): Norms of reaction to environmental quality. Proceedings of the Royal Society of London. Series B: Biological Sciences 266:2095-2100.

Einum S, Fleming IA. 2000b. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). Evolution 54:628-639.

Elgar MA. 1990. Evolutionary compromise between a few large and many small eggs: Comparative evidence in teleost fish. Oikos 59:283-287.

Fleming IA, Gross MR. 1990. Latitudinal clines: a trade-off between egg number and size in Pacific salmon. Ecology 71:1-11.

Fleming IA, Reynolds JD. 2004. Salmonid breeding systems. In. Evolution illuminated: salmon and their relatives. New York: Oxford University Press. p. 264-294.

Foster T, Gilmour J. 2020. Egg size and fecundity of biannually spawning corals at Scott reef. Scientific Reports 10:1-9.

Fox CW, Czesak ME. 2000. Evolutionary ecology of progeny size in arthropods. Annual Review of Entomology 45:341-369.

Goos JM, Swain CJ, Munch SB, Walsh MR. 2019. Maternal diet and age alter direct and indirect relationships between life-history traits across multiple generations. Functional Ecology 33:491-502.

Heath DD, Fox CW, Heath JW. 1999. Maternal effects on offspring size: Variation through early development of chinook salmon. Evolution 53:1605-1611.

Hendry AP, Day T, Cooper AB. 2001. Optimal size and number of propagules: Allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. American Naturalist 157:387-407.

Hepp GR, Kennamer RA. 1993. Effects of age and experience on reproductive performance of wood ducks. Ecology 74:2027-2036.

Ito K. 1997. Egg-size and-number variations related to maternal size and age, and the relationship between egg size and larval characteristics in an annual marine gastropod, *Haloa japonica* (Opisthobranchia; Cephalaspidea). Marine Ecology Progress Series 152:187-195.

Johnston TA. 2018. Egg size and lipid content of lake trout (*Salvelinus namaycush*) in the wild and in captivity. Canadian Journal of Fisheries and Aquatic Sciences 75:2123-2135.

Johnston TA, Wong DMM, Moles MD, Wiegand MD, Casselman JM, Leggett WC. 2012. Reproductive allocation in exploited lake whitefish (*Coregonus clupeaformis*) and walleye (*Sander vitreus*) populations. Fisheries Research 125:225-234.

Jonsson N, Jonsson B. 1999. Trade-off between egg mass and egg number in brown trout. Journal of Fish Biology 55:767-783.

Kindsvater HK, Bonsall MB, Alonzo SH. 2011. Survival costs of reproduction predict age-dependent variation in maternal investment. Journal of Evolutionary Biology 24:2230-2240.

Kindsvater HK, Otto SP. 2014. The Evolution of Offspring Size across Life-History Stages. American Naturalist 184:543-555.

Lasne E, Leblanc CA, Gillet C. 2018. Egg size versus number of offspring trade-off: Female age rather than size matters in a domesticated Arctic charr population. Evolutionary Biology 45:105-112.

Leblanc CAL, Benhaïm D, Hansen BR, Kristjánsson BK, Skúlason S. 2011. The importance of egg size and social effects for behaviour of Arctic charr juveniles. Ethology 117:664-674.

Leblanc CAL, Kristjánsson BK, Skúlason S. 2016. The importance of egg size and egg energy density for early size patterns and performance of Arctic charr *Salvelinus alpinus*. Aquaculture Research 47:1100-1111.

Lim JN, Senior AM, Nakagawa S. 2014. Heterogeneity in individual quality and reproductive trade-offs within species. Evolution 68:2306-2318.

Mandić M, Regner S. 2014. Variation in fish egg size in several pelagic fish species. Studia Marina 27:31-46.

Marshall D, McAlister JS, Reitzel AM. 2018. Evolutionary ecology of parental investment and larval diversity. In. Evolutionary Ecology of Marine Invertebrate Larvae. New York: Oxford scholarship online. p. 34-49.

Marshall DJ, Cook CN, Emlet RB. 2006. Offspring size effects mediate competitive interactions in a colonial marine invertebrate. Ecology 87:214-225.

Marshall DJ, Heppell SS, Munch SB, Warner RR. 2010. The relationship between maternal phenotype and offspring quality: Do older mothers really produce the best offspring? Ecology 91:2862-2873.

Marshall DJ, Keough MJ. 2008. The relationship between offspring size and performance in the sea. The American Naturalist 171:214-224.

O'Dea RE, Vega-Trejo R, Head ML, Jennions MD. 2015. Maternal effects on offspring size and number in mosquitofish, *Gambusia holbrooki*. Ecology and Evolution 5:2945-2955.

Oliveira RF, Almada VC, Forsgren E, Gonçalves EJ. 1999. Temporal variation in male traits, nesting aggregations and mating success in the peacock blenny. Journal of Fish Biology 54:499-512.

Parker GA, Begon M. 1986. Optimal egg size and clutch size: Effects of environment and maternal phenotype. The American Naturalist 128:573-592.

Pearson P, Warner D. 2018. Early hatching enhances survival despite beneficial phenotypic effects of late-season developmental environments. Proceedings of the Royal Society B: Biological Sciences 285:20180256.

Pianka ER, Parker WS. 1975. Age-specific reproductive tactics. The American Naturalist 109:453-464.

Pick JL, Hutter P, Ebneter C, Ziegler A-K, Giordano M, Tschirren B. 2016. Artificial selection reveals the energetic expense of producing larger eggs. Frontiers in Zoology 13:1-10.

Reichert S, Berger V, Jackson J, Chapman SN, Htut W, Mar KU, Lummaa V. 2020. Maternal age at birth shapes offspring life-history trajectory across generations in long-lived Asian elephants. Journal of Animal Ecology 89:996-1007.

Roff D. 1992. Evolution of life histories: theory and analysis. New York, USA: Chapman & Hall.

Rollinson N, Rowe L. 2016. The positive correlation between maternal size and offspring size: fitting pieces of a life-history puzzle. Biological Reviews 91:1134-1148.

Sakai S, Harada Y. 2001. Why do large mothers produce large offspring? Theory and a test. The American Naturalist 157:348-359.

Seamons TR, Quinn TP. 2010. Sex-specific patterns of lifetime reproductive success in single and repeat breeding steelhead trout (*Oncorhynchus mykiss*). Behavioral Ecology and Sociobiology 64:505-513.

Segers FH, Taborsky B. 2011. Egg size and food abundance interactively affect juvenile growth and behaviour. Functional Ecology 25:166-176.

Smith CC, Fretwell SD. 1974. The optimal balance between size and number of offspring. The American Naturalist 108:499-506.

Trippel EA. 1998. Egg size and viability and seasonal offspring production of young Atlantic cod. Transactions of the American Fisheries Society 127:339-359.

Tveiten H, Solevåg S, Johnsen H. 2001. Holding temperature during the breeding season influences final maturation and egg quality in common wolffish. Journal of Fish Biology 58:374-385.

Vindenes Y, Langangen O, Winfield IJ, Vollestad LA. 2016. Fitness consequences of early life conditions and maternal size effects in a freshwater top predator. Journal of Animal Ecology 85:692-704.

von Siebenthal BA, Jacob A, Wedekind C. 2009. Tolerance of whitefish embryos to *Pseudomonas fluorescens* linked to genetic and maternal effects, and reduced by previous exposure. Fish & Shellfish Immunology 26:531-535.

Wedekind C. 2002. Induced hatching to avoid infectious egg disease in whitefish. Current Biology 12:69-71.

Williams GC. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. The American Naturalist 100:687-690.

Wolf JB, Wade MJ. 2009. What are maternal effects (and what are they not)? Philosophical Transactions of the Royal Society B: Biological Sciences 364:1107-1115.

Tables

Table 1. The effects of female (dam) identity, season, dam age and dam standard length on (*a*) egg volume, (*b*) embryo mortality, (*c*) hatching time, (*d*) larval length at hatching, (*e)* yolk sac volume and (*f)* larval length 21 days after hatching. Likelihood ratio tests on mixed model regressions were used to compare a reference model (in bold) with models including or lacking the term of interest. Significant p-values are highlighted in bold.

Model	Effect tested	AIC	d.f.	χ^2	*P*
(a) Egg volume (mm^3)					
D_{age} + D_{size} + D + season		43145	6		
D_{age} + D_{size} + season	D	50501	5	7358	**<0.001**
D_{age} + D_{size} + D	season	43170	5	26	**<0.001**
D_{size} + D + season	D_{age}	43150	5	6.3	**0.012**
D_{age} + D + season	D_{size}	43144	5	0.2	0.67
D_{age} + D_{size} + D_{age} * D_{size} + D + season	D_{age} * D_{size}	43142	7	5.4	**0.02**
D_{age} + D_{size} + D + D_{age} *season	D_{age} *season	43149	8	0.05	0.97
D_{age} + D_{size} + D + D_{size} *season	D_{size} *season	43149	8	0	1
(b) Embryo mortality					
D_{age} + D_{size} + D + season		670	5		
D_{age} + D_{size} + season	D	687	4	18	**<0.001**
D_{age} + D_{size} + D	season	668	4	0	1
D_{size} + D + season	D_{age}	668	4	0.01	0.9
D_{age} + D + season	D_{size}	668	4	0.07	0.8
D_{age} + D_{size} + D_{age} * D_{size} + D + season	D_{age} * D_{size}	672	6	0.01	0.93
(c) Hatching time					
D_{age} + D_{size} + D + season		31483	6		
D_{age} + D_{size} + season	D	32677	5	1995	**<0.001**
D_{age} + D_{size} + D	season	31484	5	2.2	0.13
D_{size} + D + season	D_{age}	31482	5	0.01	0.91
D_{age} + D + season	D_{size}	31482	5	0.2	0.66
D_{age} + D_{size} + D_{age} * D_{size} + D + season	D_{age} * D_{size}	31484	7	0.9	0.33
(d) Length at hatching (mm)					
D_{age} + D_{size} + D + season		7805	6		
D_{age} + D_{size} + season	D	8696	5	893	**<0.001**
D_{age} + D_{size} + D	season	7807	5	3.6	0.057
D_{size} + D + season	D_{age}	7812	5	9	**0.003**
D_{age} + D + season	D_{size}	7804	5	1.4	0.23
D_{age} + D_{size} + D_{age} * D_{size} + D + season	D_{age} * D_{size}	7806	7	1.4	0.24
(e) Yolk sac volume (mm^3)					
D_{age} + D_{size} + D + season		-1408	6		
D_{age} + D_{size} + season	D	-198	5	1211	**<0.001**
D_{age} + D_{size} + D	season	-1410	5	0	1
D_{size} + D + season	D_{age}	-1404	5	6.1	**0.013**

D_{age} + D + season	D_{size}	-1410	5	0.4	0.52
D_{age} + D_{size} + D_{age} * D_{size} + D + season	D_{age} * D_{size}	-1407	7	1.1	0.29
(f) Length 21 days after hatching (mm^3)					
D_{age} + D_{size} + D + season		7735	6		
D_{age} + D_{size} + season	D	9319	5	1585	**<0.001**
D_{age} + D_{size} + D	season	7737	5	4.4	**0.03**
D_{size} + D + season	D_{age}	7740	5	7.3	**0.007**
D_{age} + D + season	D_{size}	7735	5	2.1	0.13
D_{age} + D_{size} + D_{age} * D_{size} + D + season	D_{age} * D_{size}	7736	7	0.6	0.43

Fixed effect: dam age (D_{age}), dam size (D_{size}). Random effects: dam (D) and season.

Table 2. The effects of study (chapter 1, 4, and 5 of this thesis), dam age class (young or old), and dam standard length on mean egg volume after extending the dataset with 29 females from de Guttry et al. (chapter 4). Multiple regression on mean egg sizes per dam (N_{total} = 72). Significant p-values are emphasized in bold.

Effect tested	d.f.	F	*P*
Study	2	39.3	**<0.001**
D_{age}	1	8.5	**0.005**
D_{size}	1	0.2	0.67
D_{age} * D_{size}	1	2.7	0.10
D_{age} * study	2	0.6	0.56
D_{size} * study	2	0.3	0.76

Figures

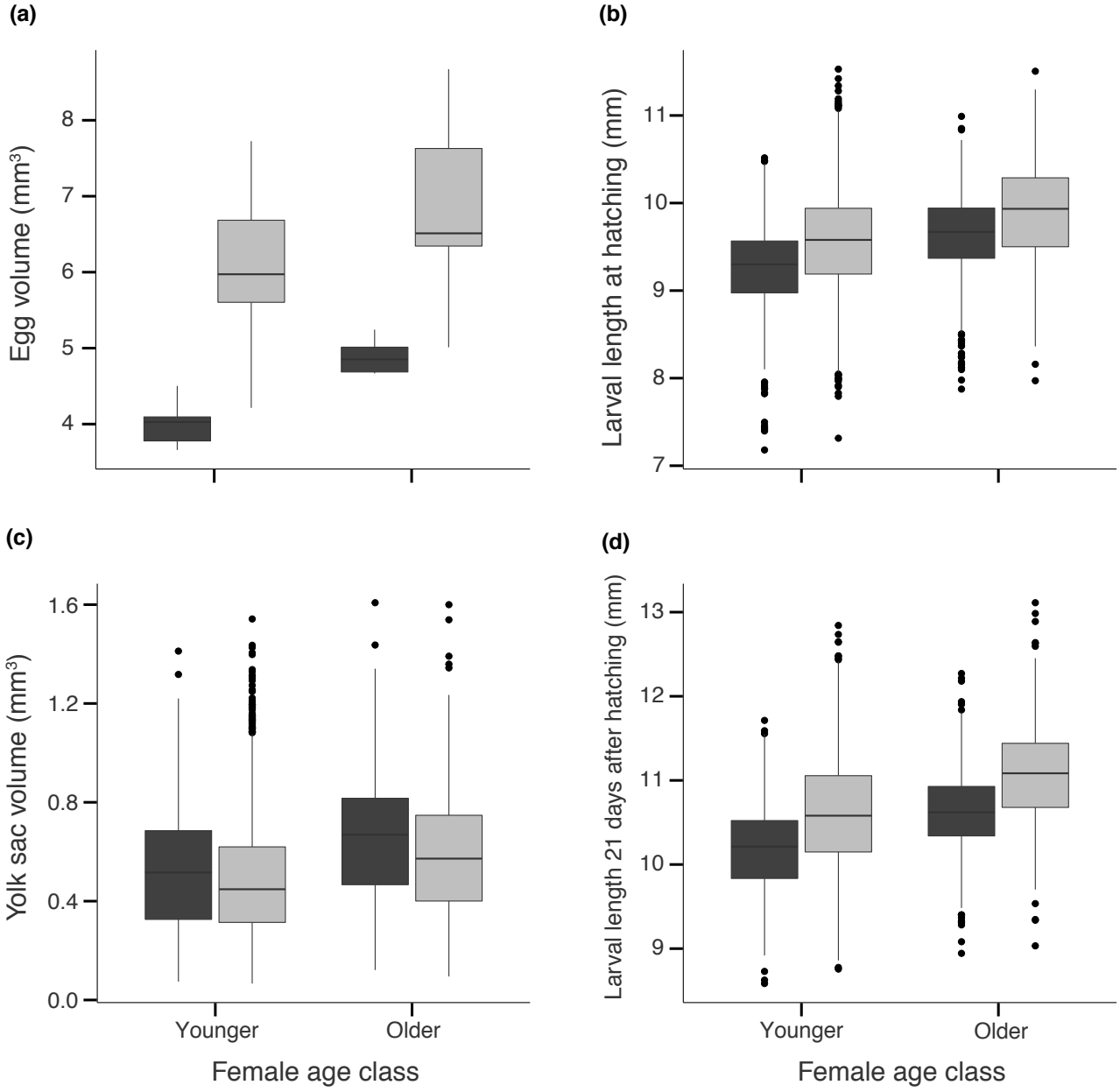


Figure 1. Effects of female age on (a) egg volume, (b) larval length at hatching, (c) yolk sac volume at hatching, and (d) larval length 21 days after hatching (Tukey outlier boxplots with quartiles, whiskers, and outliers). The effects are separately shown for females caught early (dark grey) and late (light grey) in the reproductive season to illustrate the significant season effects in panels (a) and (d). See Table 1 for statistics.

Supplementary information

Maternal age rather than size predicts propagules size in whitefish

Christian de Guttry[1], Florin Stettler[1], Jonas Bylemans[1], Audrey Atherton[1,2], Steven Campana[3#] & Claus Wedekind[1*]

[1]Department of Ecology & Evolution, University of Lausanne, Lausanne, Switzerland

[2] now at: Department of Environmental Systems Science, ETH, Zürich, Switzerland

[3]Faculty of Life and Environmental Sciences, University of Iceland, Reykjavik, Iceland.

(# not yet agreed to the proposed co-authorship)

* correspondence: claus.wedekind@unil.ch

Chapter status: unpublished

Content

Figures

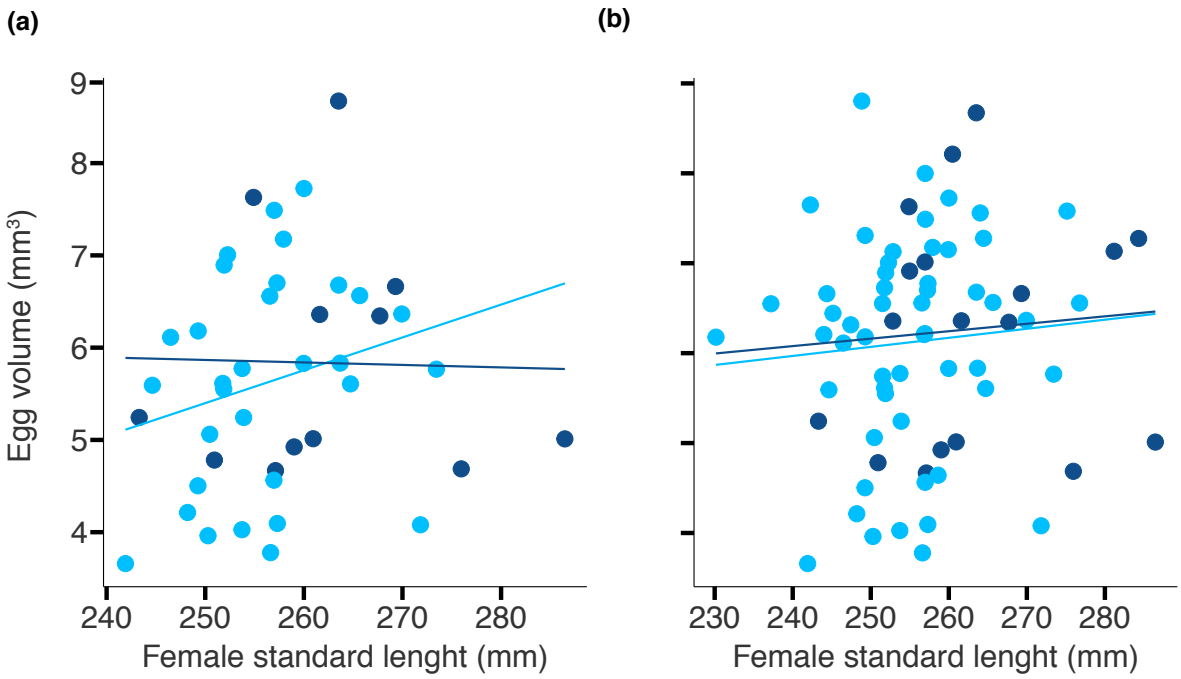


Figure S1. The effect of the interaction between female age and female length on egg volume (a) considering females from the present study (*N=43*), and (b) considering females from the extended sample (*N=72*). Younger females and the respective regression lines are represented in light blue while older females and the respective regression lines are represented in dark blue. See table 1a and table 2 for statistics.

Chapter 4 - The potential of a natural population of whitefish to rapidly adapt to freshwater acidification

Christian de Guttry[1], Eléonore Lavanchy[1], Florin Stettler[1], Nicolas Sironi[1,2] & Claus Wedekind[1*]

[1]Department of Ecology & Evolution, University of Lausanne, Lausanne, Switzerland
[2] Now at: Schweizerische Vogelwarte, Sempach, Switzerland.

* correspondence: claus.wedekind@unil.ch

Chapter status: unpublished

Author contributions

CdG and CW planned the study, conducted the field work, and distributed the fertilized eggs singly to 24-well plates with the help of FS and further helpers. CdG prepared the pH solutions. CdG, FS, and NS monitored pH, embryo, and larval development in the climate chamber. EL and NS determined egg and larval size. CdG and EL managed the data files. CdG and CW performed the statistical analysis and wrote the manuscript.

Abstract

Freshwater acidification is a widespread phenomenon caused mainly by anthropogenic activities. However, nearly all of the studies conducted on its effects have been done using marine organisms. Nowadays, it is still unclear whether and how freshwater vertebrates such as fish can adapt to a decrease in pH. We tested the effects of acidification on the early life stages of whitefish to investigate the potential for a rapid adaptation. We sampled 30 female and 60 male whitefish from a natural population. Their gametes were used to produce 6 full-factorial breeding blocks (5 females x 10 males) of which 24 freshly fertilized eggs per sib group (7,200 in total) were raised singly at one of three different pH: slightly acid (6.5), control (7.0) and basic (8.0). We found that embryos exposed to the acid treatment suffered an increased embryonic and larval mortality and a reduced embryo development rate as compared to embryos raised at control or basic conditions. The effect of acidification on embryo development varied between paternal sib group, i.e. there is significant additive genetic variance and hence the potential for rapid evolution towards increased tolerance to acidification in the study population. We also found significant maternal environmental effects on the reaction to treatment: larger eggs suffered more from acidity than smaller eggs. We conclude that water acidification reduces the viability of early life stages in whitefish, and that there is currently significant standing genetic variation and variance in maternal environmental effects to allow for rapid evolution in response to induced water acidification.

Key words: Freshwater acidification, climate change, additive genetic variance, maternal effects, egg size, conflicting selection pressures

Introduction

The acidification of water bodies is a global phenomenon that affects both saltwater and freshwater ecosystems (Beamish and Harvey 1972; Schindler 2001). In the oceans, acidification is largely due to the increase of atmospheric CO_2 that has been occurring since the beginning of the industrial revolution (Battarbee 1984; Villalobos, et al. 2020). In this process carbon dioxide dissolves into the water to form carbonic acid and releasing hydrogen ions which lowers the water pH. Mathematical models predict that this increase will continue until at least 2100 (Meinshausen, et al. 2011) and understanding how this affects aquatic organisms is today a priority. The same dynamics affects freshwaters bodies (Hasler, et al. 2018). In addition, the pH of rivers and lakes is also lowered by sporadic episodes of acid rain (Beamish 1976), high concentration of heavy metals and/or nutriments (Pedersen, et al. 2020) and runoff due to flash flooding (Barlaup and Atland 1996; Whitehead, et al. 2009). This combined with their limited buffering capacity makes these ecosystems extremely vulnerable. Today, little is known on the effects of freshwater acidification on the organisms that inhabit these ecosystems (Ou, et al. 2015; Hasler, et al. 2016).

Acidification effects are manifold on the whole trophic chain and it has been demonstrated that freshwater acidification also directly affects vertebrates as fish (Kitamura and Ikuta 2000, 2001; Serrano, et al. 2008; Ou, et al. 2015; Lin, et al. 2019). These effects, vary according to the period of life in which the exposure occurred. The early-life stages (Lacoue-Labarthe, et al. 2011; Tseng, et al. 2013; Ou, et al. 2015; Snyder, et al. 2018; Munday, et al. 2019) and the reproduction period (Schindler, et al. 1985; Ikuta and Kitamura 1995; Beaune, et al. 2018) are generally the most affected. Embryos or hatchlings exposed to an acidic environment have shown increased mortality (Munday, et al. 2010), reduced ability to use olfactory stimuli (Leduc, et al. 2013), increased expression of ion pumps on cell membranes (Dahlke, et al. 2020) and reduced growth (Franke and Clemmesen 2011; Baumann, et al. 2012; Ou, et al. 2015). To date, there is a lack of empirical studies on how acidification affects non-calcifying organisms, as fish, that perform their entire life cycle in freshwaters (Munday, et al. 2010; Baumann, et al. 2012). Understanding whether freshwater species have the ability to adapt to decreasing pH and what mechanisms allow this adaptation is necessary to evaluate appropriate conservation measures.

The presence of sufficient degree of genetic diversity influences the ability of a population to cope with environmental changes (Hendry, et al. 2011). Indeed, an evolutionary response depends primarily on the level of standing genetic variation present within the population (Barrett and Schluter 2008). With sufficient standing genetic variation, the frequency of alleles under directional selection could be high enough to allow the population to adapt to new conditions (Innan and Kim 2004). This

can be supported by the presence of maternal effects that offset selective pressures in early life, eventually becoming adaptive (Mousseau and Fox 1998). Maternal effects play a major role in determining survival and performances of individuals in their early stages of life (Mousseau and Fox 1998; Hendry, et al. 2011). Consequently, maternal effects play a key role in defining evolutionary trajectories in early-life stages of species with internal and external fertilization (Inchausti and Ginzburg 2009). The embryos of the latter might spend long periods of time incubating in eggs whose characteristics depends on the genetic quality of the mother (Parker and Begon 1986). For this reason, egg size has become an increasingly studied maternal trait used to investigate the performance of incubating embryos and post hatching larvae (Einum and Fleming 1999; Einum and Fleming 2002; Leblanc, et al. 2011; Leblanc, et al. 2016). These two life-stages are characterized by high selective pressures in external fertilizer with no parental care (Stearns and Hendry 2004). Typically, large eggs give rise to large larvae that perform better than small larvae after hatching (Marshall, et al. 2010). However, being of a large size can be detrimental under stressful conditions during incubation (Hendry, et al. 2001; Einum, et al. 2002; Rombough 2007; Regnier, et al. 2013). In the literature the stress factors tested to date are limited to hypoxic conditions (Einum, et al. 2002) and increased temperature (Regnier, et al. 2013). Despite this, several chemical or biological factors can have an influence on the evolution of egg size. To improve the prediction of evolutionary trajectories there is the need to investigate their possible effects. Understanding whether maternal effects, along with genetic diversity, play a role in the response to climate change is necessary to predict their role in the adaptation of a population to new environmental conditions (Bonduriansky, et al. 2012).

An ideal species for testing these effects are *Coregonus spp*.. Alpine whitefish are salmonids of great cultural and economic interest that complete their whole life cycle in fresh water. Previous studies revealed the susceptibility of anadromous salmonids to water acidification (Beamish and Harvey 1972; Ikuta, et al. 2001; Kitamura and Ikuta 2001; Ou, et al. 2015). They are external fertilizer showing no parental care, and their numerous gametes allow for large full-factorial breeding crosses that are ideal to evaluate the presence of additive genetic variation and to test possible evolutionary response to stressors (Brazzola, et al. 2014; Nusbaumer, et al. 2020). In addition, whitefish produce thousands of eggs per spawning season and the quality of these eggs varies within and among females, making it a good model species to investigate how maternal effects influence offspring performances and population dynamics (Einum and Fleming 1999; Einum and Fleming 2002; Leblanc, et al. 2016).

The main purpose of our experiment was to understand whether and how an ecologically relevant acidification affects the performance of embryos and larvae of a keystone species of Swiss Alpine lakes. Importantly, we wanted to test if standing genetic variation in the population along with maternal effects allow potential rapid adaptation to water acidification. To do so, we sampled sexually

mature individual from a natural population, collect their gametes and crossed them to generate several full factorial breeding blocks. The resulting embryos were exposed to 3 pH levels based on the natural optimum for freshwater fish that range from a pH of 6.0 to a pH of 8.0 (Wetzel 2001). We used pH 6.5 corresponding to the average pH concentration of Swiss lake after the last eutrophication crisis (Steingruber and Colombo 2006) and normally registered on the deeper layer of lakes (Wetzel 2001). A pH.7.0 that correspond to the standardize water commonly used to raise embryos in experimental conditions and a pH 8.0 to mimic the average pH of an healthy European lake (Kristensen and Hansen 1994).

Material and Methods

Whitefish spawners (*Coregonus suidteri*) were caught in gillnets (mesh size: 35 mm) from Lake Hallwil (Switzerland; 47.2772° N, 8.2173° E) on January 11, 2017. A haphazardly chosen sample of 30 females and 60 males were euthanized and stripped for eggs and milt before weight and length was determined. Egg and milt was split to Petri dishes so that five females were crossed *in vitro* with 10 males each in a full-factorial breeding block. This was repeated to obtain in total 6 breeding blocks and 300 sib groups. Sperm were activated with reconstituted standardized water (Kitano 1992) that was also used for later manipulations. Freshly fertilized eggs were left undisturbed for at least two hours to allow egg hardening before 24 eggs per sib group (n_{tot} = 7,200) were transferred to 50 mL Falcon tubes (Corning, Gurgaon, India) and transported on ice to a climate chamber. Eggs were family-wise washed in a sterilized sieve under running tap water (4 L/minute) for 30 seconds and then singly distributed to 24-well plates (Greiner Bio-One, Kremsmünster, Austria) filled with 1.8 ml water at one of three pH levels and incubated at constant 4°C under a 12h:12h light-dark cycle until 14 days after hatching.

Autoclaved and oxygenated standardize water (Kitano 1992) was used as a control solution (C; pH 7). This water has been routinely used for embryo incubation in our laboratory (von Siebenthal, et al. 2009). The acidic solution (A) was prepared by acidifying standardized water to pH 6.5 with a phosphate buffer (monosodium phosphate 0.1M titrated with potassium dibasic phosphate 0.1M). The basic solution (B) was prepared by alkalizing standardized water to pH 7.9 with a bicarbonate buffer (sodium bicarbonate 0.1M titrated with hydrochloric acid 1M). Because fluctuations in temperature can affect pH (Langelier 1946), all treatments had been prepared several days before eggs were added, at 4°C with a cold-operating pH electrode InLab Micro (Mettler-Toledo, Bussigny, Switzerland).

Developing embryos were then left untouched for the first 40 days. From then on, they were monitored in 3-days intervals for mortality and hatching, and daily when hatching started. From 42

days post fertilization (dpf) on, pH levels were measured in regular intervals in all wells of 6 plates per treatment group. A photo of the eggs was taken 90 dpf, i.e. shortly before hatching started, to test for changes in mean egg volume due to the exposure to different pH.

At the day of hatching, the freshly hatched larvae were singly transferred to new 24-well plates filled with water at the corresponding pH, i.e. treatment was maintained over the larval period. Three consecutives photos of the larvae were taken at the day of hatching. To improve the quality of the photos, the larvae were temporarily transferred to a new well filled with only 300 µl standardized water to minimize optical distortions. Morphometric measurement on eggs and larvae were taken in ImageJ v2.0.0 (https://imagej.net). When none of the three photos allowed an accurate measurement of phenotype characteristics, the individual was discarded from the subsequent analysis (1.8% of all larvae). Larvae were monitored for 14 day after hatching to record mortality during the early yolk-sac period.

Effects of treatment and egg size on offspring performance were tested in generalized linear mixed models (GLMM) and linear mixed models (LMM) fitted using maximum likelihood. Embryo mortality and larval mortality were used as binomial response variables in GLMM. Egg size at 90 dpf, day of hatching ("hatching time"), length at hatching, and yolk sac volume were analyzed as continuous response variables in LMM. Dam and sire identity were used as random factors. Models fit were compared with Akaike's information criteria (AIC) and likelihood ratio tests (LRT). The significance of each factors was tested comparing a model lacking or including the term of interest with a reference model. Analysis were done in R (Core Team 2016) using the lme4 package (Bates, et al. 2015).

Results

The pH in the wells (mean ±SD) remained close to the nominal pH during the incubation period for the A (6.42 ±0.037), C (7.05 ±0.12), and B treatment (8.02 ± 0.16) (Fig. S1). The variation in pH caused strong effect on all offspring performance measures (Table 1*;* Fig. 1). Low pH caused increased embryo and larval mortality (Table 1a,f; Fig. 1a,f), a higher loss of egg volume (Table 1b; Fig. 1b), and late hatching (Table 1c; Fig 1c). At hatching, larvae from more acidic environments were slightly longer than the hatchlings from the other treatments (Table 1d; Fig. 1d) but had smaller yolk sacs (Table 1e; Fig. 1e).

Dam and sire identity had an effect on all offspring performance measures except larval mortality (Table 1). The dam*sire interaction influenced all the embryo-related response variable but not the larval ones (Table 1). The dam identity influenced the response to pH (D*t interaction in Table

1) on egg volume at 90 dpf (Table 1b), hatching time (Table 1c), and yolk sac volume at hatching (Table 1e) but not the remaining variables (Table 1). The sire identity affected the response to pH (S*t interactions in Table 1) on hatching time of similar sized hatchlings but with different yolk sac volume at hatching. Importantly, these sire- and treatment-specific effects on embryo growth reveal additive genetic variance for susceptibility to acidity in the study population. There were, however, no sire- and treatment-specific effects on embryo or larval mortality.

Egg size was a significant predictor of larval size and mortality (see egg size effect in Table 1d-f), while the strength of the effects of egg size on embryo performance mostly varied with pH. Larger eggs suffered higher embryo mortality than small eggs, but only when exposed to acidic environment (Table 1a; Fig. 2a). Larger eggs also hatched later in the acidic environment (Table 1c; Fig. 2b), with smaller yolk sac (Table 1e; Fig. 2c) than smaller eggs. Table 1f suggests that larvae from larger eggs suffered higher mortality, but this effect seems driven by only few females in the most acidic treatment, while larval mortality was generally low (Fig. 1f; Supplementary Fig. S2).

Discussion

It is still unclear if and how freshwater fish can adapt to acidification. Here, we exposed whitefish to 3 different, ecologically relevant, pH levels to test the effect of acidification on embryos and larvae. We wanted to investigate for the potential adaptation to acidification and understand the role of maternal environmental effects in this process. We found additive genetic variance and a significant influence of egg size (maternal environmental effect) on the resistance to acidification. A slightly acidic pH (pH 6.5) increased embryos mortality rate and induced a reduction in egg volume at 90 dpf. Embryos exposed to acidic pH also suffered a delay in hatching time resulting in slightly larger larvae at hatching, with considerably smaller yolk sac and with higher larval mortality rate compared to the two other levels of pH. The magnitude of the effect of acidification were stronger on larger eggs for embryo mortality, hatching time and yolk sac volume at hatching. With our experiment we also demonstrated how the pH of a healthy freshwater ecosystem (8.0) maximize embryo and offspring performances.

The full factorial breeding design we used allowed the analysis of parental effects on susceptibility to acidification. Therefore, we were able to detect that the identity of the sires had a significant effect on almost all response variables. Since the father in external fertilizers contributes only with his DNA, we can then suggest that males differ in their genetic quality as already demonstrated in swiss alpine whitefish (Wedekind, et al. 2001; Wedekind, et al. 2007; Wedekind, et al. 2008; Clark, et al. 2014). The significance of the sire effect did not change over developmental

stages, showing the importance of paternal genetic contribution also during the early stages of development. A fundamental result is that paternal sib groups vary in their response to acidification i.e. we found the presence of additive genetic variance in response to different pH levels on hatching time and yolk sac volume, a critical variable in this framework (see below). A similar result was found for the resistance of the whitefish *Coregonus palaea* and *Coregonus albellus* offspring to the synthetic estrogen EE2 (Brazzola, et al. 2014). We then confirm the presence of paternal genotypes more capable to withstand the selective pressures generated by acidification. This can induce an evolutionary response, one of the main prerequisites necessary for a population to quickly adapt to environmental changes (Hermisson and Pennings 2005; Hendry, et al. 2011). Our result confirm the presence of enough genetic diversity in the population for a possible fast adaptation to water acidification.

We also found a significant effect of the identity of the mother (dam) on almost all response variables analyzed. We expected this result since a female in addition to its DNA, contributes with the incubation environment of the embryos (maternal environmental effect). This makes the magnitude of the maternal effects larger than the one of paternal effects and therefore easier to detect (Lynch and Walsh 1998). Interestingly, also female identity influenced the tolerance to acidification (D*t) for egg volume at 90 dpf, hatching time and yolk sac volume at hatching. Typically the causal factors generating this result are difficult to determine. It has been suggested that a different investment of the mother in the eggs can generate it (Wilkins, et al. 2015). In our experiment egg size generated a different response to acidification. Since in species with external fertilization the size of eggs correspond to the per-offspring investment (Marshall, et al. 2010) with our result we confirm that a differential maternal investment in the eggs influence the response to the treatment. It is possible to conclude that whitefish females differ in their genetic quality and in the quality of the eggs produced. Considering that egg size is a plastic trait modulated by the females in response to environmental stimuli (Smith and Fretwell 1974), and assuming that the mother can perceive changes in pH, adaptive maternal effects (Mousseau and Fox 1998) might contribute to the fast adaptation to more acidic environment, during the incubation period. We also found a significant dam x sire (D*S) effect on embryonic related response variables but not on larval performances. This result indicates that some haplotypes of males prove to be better when paired with some female haplotypes (Neff and Pitcher 2005) and indicate also the presence of non-additive genetic variation in the population.

Larval mortality is the only response variable on which an effect of parental identity was not found. This response variable was influenced by treatment and egg volume. However, this result should be interpreted with caution given that only 40 larvae died after hatching. Consequently, it is possible that we had a lack of statistical power to properly analyze the observed results.

During incubation, the slightly acidic treatment (pH 6.5) induced significant embryo mortality compared to the other two pH levels. This result confirms what was found by Lin et al. (2019) using *Dario renio* embryos which were exposed to pH 4 for short periods of time. In our case we exposed the embryos from the day of fertilization until hatching as it has been done in Villalobos et al. (2020). The latter found that exposing *Clupea pallasi* embryos to a slightly lower pH throughout the incubation period increased mortality. The main difference with previous studies is the difference in sample size i.e. we used 7200 eggs from 30 females. This allowed us to have sufficient statistical power to confidently interpret our results. Despite this, we exposed embryos to the treatments in a laboratory-based study. Other stressors that can be found in natural conditions (i.e. temperature) may interact with variations in pH intensifying or weakening the results we obtained (Villalobos, et al. 2020).

During incubation, eggs exposed to pH 6.5 had a reduction in volume compared to eggs of the other 2 pH levels. The loss of volume might be due to the difference in osmotic pressure between the inside and outside of the egg membrane. When the external environment is more acidic it is characterized by a higher ionic concentration. The osmotic gradient might force the water outwards as the pH of a developing embryo is 7.5 (Mölich and Heisler 2005). As a result, embryos may be affected by dehydration and this might explain their reduced performance in terms of growth and increase embryo mortality (Baumann, et al. 2012; Ou, et al. 2015). Indeed it has been demonstrated that embryos need a large amount of water to complete maturation (Dolomatov, et al. 2012). Since the surface in contact with the external environments determines the quantity of exchanges (Rombough 2007; Nusbaumer, et al. 2020), this can also be interpreted as a defense mechanism act to reduce the surface in contact with the external medium. In our study, we could not compare whether the loss of volume differed according to egg volume given the lack of post fertilization photos.

The treatment also affected offspring performances after hatching. Hatching time was strongly delayed by an increasing acidic environment. The larvae exposed to the standardize water (pH 7.0) had a slight delay in hatching time compared with the larvae exposed to a pH 8.0. The most significant delay in hatching was caused by the more acidic pH where the larvae hatched on average almost 20 days later than the others. The resulting larvae were slightly longer but with a considerably smaller yolk sac. In our case we suggest that the delay in hatching is due to a tradeoff, for the yolk sac reserved depleted, between growth and the acclimatation to acidic pH. A similar result has already been found when embryos of *Salmo trutta* were exposed to a common salmonid pathogen (Clark, et al. 2013). Our results are also coherent with what has been found when embryos of pink salmon (*Oncorhynchus gorbuscha*) were exposed to acidic environment (Ou, et al. 2015). The percentage of yolk sac converted into tissue was 25% lower in their exposed embryos suggesting that yolk sac energies were used for the response to the stress. A very similar response has been recorded for *Paralichthys dentatus* where

larvae exposed to low pH stress were slightly larger but with markedly reduced resources in the yolk sac at hatching (Chambers, et al. 2014). To confirm these patterns, Dahlke et al. (2017) demonstrated that acclimatation to acidic conditions require resources allocation at the expense of embryonic growth. In our experiment the proportion of yolk sac consumed is noticeably greater than the difference in length at hatching. Thus, our study confirms that embryo development is severely slowed when the larvae are exposed to a slightly acidic environment.

Large eggs were more sensitive to acidic conditions during the incubation period. This effect also appears to be reflected in yolk sac volume; a variable strongly correlated with egg size. With regard to the embryo mortality, it can be clearly observed that larger eggs undergo a greater selective pressure during incubation. This effect tended to increase with increasing acidity of the medium. Here, we observed that the mortality rate is close to 30% for females producing larger eggs. Concerning hatching time, we can observe that the time required for development is proportional to the size of the eggs. However, when the eggs are exposed to an acidic environment, the slope of the regression line increases compared to the other two treatments. Therefore, large eggs are further penalized by the treatment. As a consequence, these had a smaller yolk sac with a less pronounced correlation with egg size as was observed in the other 2 levels of the treatment.

Our results clearly suggest that selective pressures exerted by an acidic pH are strongest on larger eggs. In most salmonids it has been shown that egg size determines the size of the larvae in the early stages of life. Therefore, a female that produces larger eggs will also produce larger larvae, that will be more competitive than smaller larvae. These females are therefore supposed to have higher fitness than those that produce smaller eggs. However, we showed that large eggs are disadvantaged during the incubation period, hence confirming the “bigger is not always better” theory (Krogh 1941). This result demonstrates that the best strategy for a female, in the presence of stressful conditions, is to produce smaller eggs, as initially suggested by evolutionary models (Hendry, et al. 2001). Indeed, the results of an increasing number of studies suggest that the response to waterborne stressors depends on the absolute surface area of the egg (Einum, et al. 2002; Rombough 2007; Nusbaumer, et al. 2020). In this context, the larger the egg is, the more it will suffer the consequences of the stressor be it a pollutant (Nusbaumer, et al. 2020), rising temperature (Regnier, et al. 2013), hypoxic conditions (Einum, et al. 2002) or the presence of an acidic pH, as in our experiment. Through this finding, we can therefore assume the presence of contrasting selection pressures (Schluter, et al. 1991) acting on embryonic and larval stage of whitefish.

With this study we demonstrated that in our whitefish population there is the possibility of rapid adaptation to acidification. This is possible due to the presence of a sufficient degree of standing genetic variation and to maternal environmental effects. Our results suggest that theories trying to

predict the evolution of offspring size, in external fertilizer, should include the incubation stage and not only the post hatching period. Various factor can exert a selective pressure on incubating eggs and not considering them can lead to incorrect estimates of the fitness of a female and wrong predictions on how offspring size is evolving in a given environment. Our results, also helps to understand how the variability in the size of eggs produced within a population is a characteristic to be preserved. This feature can enable a population a fast response to environmental changes. We highlighted how important is the incubation period for freshwater fish and how only a slight acidification can cause strong selective pressure on the population. To maintain the diversity of freshwater species, it is necessary to increase control of multiple stressors that are often not emphasized over others as pH. Only acting in this way the genetic diversity of wild population can be preserved.

Ethics

Capture and manipulation of the fish was approved by Fisheries Administration of the Aargau canton.

Data availability

Data will be deposited on the Dryad depository upon acceptance of the manuscript.

Funding

The project was supported by the Swiss National Science Foundation (31003A_182265) and the *Swisslos-Fonds* of the Aargau canton

Acknowledgements

We thank R. Stadelmann for catching the fish, D. Bittner for permits and discussion, J. Beninde, N. Caloz, I. Castro, G. Fabre, L. Garaud, A. Jimenez, E. Longange, L. Marquez da Cunha, K. Mobley, D. Nusbaumer, E. Seddon, and C. Vincent for assistance, and G. Cercignani for suggestions on pH solutions.

References

Barlaup BT, Atland A. 1996. Episodic mortality of brown trout (*Salmo trutta L*) caused by sea-salt-induced acidification in western Norway: effects on different life stages within three populations. Canadian Journal of Fisheries and Aquatic Sciences 53:1835-1843.

Barrett RD, Schluter D. 2008. Adaptation from standing genetic variation. Trends in Ecology & Evolution 23:38-44.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Battarbee RW. 1984. Diatom analysis and the acidification of lakes. Philosophical Transactions of the Royal Society B-Biological Sciences 305:451-477.

Baumann H, Talmage SC, Gobler CJ. 2012. Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. Nature Climate Change 2:38-41.

Beamish RJ. 1976. Acidification of lakes in Canada by acid precipitation and resulting effects on fishes. Water Air and Soil Pollution 6:501-514.

Beamish RJ, Harvey HH. 1972. Acidification of La Cloche mountain lakes, Ontario, and resulting fish mortalities. Journal of the Fisheries Research Board of Canada 29:1131-1143.

Beaune D, Sellier Y, Luquet G, Grandjean F. 2018. Freshwater acidification: an example of an endangered crayfish species sensitive to pH. Hydrobiologia 813:41-50.

Bonduriansky R, Crean AJ, Day T. 2012. The implications of nongenetic inheritance for evolution in changing environments. Evolutionary Applications 5:192-201.

Brazzola G, Chèvre N, Wedekind C. 2014. Additive genetic variation for tolerance to estrogen pollution in natural populations of alpine whitefish (*Coregonus sp., Salmonidae*). Evolutionary Applications 7:1084-1093.

Chambers RC, Candelmo AC, Habeck EA, Poach ME, Wieczorek D, Cooper KR, Greenfield CE, Phelan BA. 2014. Effects of elevated CO2 in the early life stages of summer flounder, *Paralichthys dentatus*, and potential consequences of ocean acidification. Biogeosciences 11:1613-1626.

Clark ES, Pompini M, Marques da Cunha L, Wedekind C. 2014. Maternal and paternal contributions to pathogen resistance dependent on development stage in a whitefish (Salmonidae). Functional Ecology 28:714-723.

Clark ES, Stelkens RB, Wedekind C. 2013. Parental influences on pathogen resistance in brown trout embryos and effects of outcrossing within a river network. PLoS One 8:e57832.

Core Team R. 2016. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Dahlke F, Lucassen M, Bickmeyer U, Wohlrab S, Puvanendran V, Mortensen A, Chierici M, Portner HO, Storch D. 2020. Fish embryo vulnerability to combined acidification and warming coincides with a low capacity for homeostatic regulation. Journal of Experimental Biology 223:jeb212589.

Dahlke FT, Leo E, Mark FC, Portner HO, Bickmeyer U, Frickenhaus S, Storch D. 2017. Effects of ocean acidification increase embryonic sensitivity to thermal extremes in Atlantic cod, *Gadus morhua*. Global Change Biology 23:1499-1510.

Dolomatov S, Zukow W, Novikov NY, Muszkieta R, Bulatowicz I, Dzierzanowski M, Kazmierczak U, Strojek K. 2012. The regulation of osmotic and ionic balance in fish reproduction and in the early stages of ontogeny. Russian Journal of Marine Biology 38:365-374.

Einum S, Fleming IA. 2002. Does within-population variation in fish egg size reflect maternal influences on optimal values? American Naturalist 160:756-765.

Einum S, Fleming IA. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): Norms of reaction to environmental quality. Proceedings of the Royal Society of London. Series B: Biological Sciences 266:2095-2100.

Einum S, Hendry AP, Fleming IA. 2002. Egg-size evolution in aquatic environments: does oxygen availability constrain size? Proceedings of the Royal Society B: Biological Sciences 269:2325-2330.

Franke A, Clemmesen C. 2011. Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus L.*). Biogeosciences 8:3697-3707.

Hasler CT, Butman D, Jeffrey JD, Suski CD. 2016. Freshwater biota and rising pCO2? Ecology Letters 19:98-108.

Hasler CT, Jeffrey JD, Schneider EVC, Hannan KD, Tix JA, Suski CD. 2018. Biological consequences of weak acidification caused by elevated carbon dioxide in freshwater ecosystems. Hydrobiologia 806:1-12.

Hendry AP, Day T, Cooper AB. 2001. Optimal size and number of propagules: Allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. American Naturalist 157:387-407.

Hendry AP, Kinnison MT, Heino M, Day T, Smith TB, Fitt G, Bergstrom CT, Oakeshott J, Jørgensen PS, Zalucki MP. 2011. Evolutionary principles and their practical application. Evolutionary Applications 4:159-183.

Hermisson J, Pennings PS. 2005. Soft sweeps: Molecular population genetics of adaptation from standing genetic variation. Genetics 169:2335-2352.

Ikuta K, Kitamura S. 1995. Effects of low pH exposure of adult salmonids on gametogenesis and embryo development. Water Air and Soil Pollution 85:327-332.

Ikuta K, Munakata A, Aida K, Amano M, Kitamura S. 2001. Effects of low pH on upstream migratory behavior in land-locked sockeye salmon *Oncorhynchus nerka*. Water Air and Soil Pollution 130:99-106.

Inchausti P, Ginzburg LR. 2009. Maternal effects mechanism of population cycling: a formidable competitor to the traditional predator–prey view. Philosophical Transactions of the Royal Society B: Biological Sciences 364:1117-1124.

Innan H, Kim Y. 2004. Pattern of polymorphism after strong artificial selection in a domestication event. Proceedings of the National Academy of Sciences 101:10667-10672.

Kitamura S, Ikuta K. 2000. Acidification severely suppresses spawning of hime salmon (land-locked sockeye salmon, *Oncorhynchus nerka*). Aquatic Toxicology 51:107-113.

Kitamura S, Ikuta K. 2001. Effects of acidification on salmonid spawning behavior. Water Air and Soil Pollution 130:875-880.

Kitano M. 1992. Updating of OECD guidelines for the testing of chemicals. Water Science and Technology 25:1-465.

Kristensen P, Hansen HO. 1994. European rivers and lakes. Assessment of their environmental state. In: EEA Environmental Monograph. p. 122.

Krogh A. 1941. The comparative physiology of respiratory mechanisms. Philadelphia, USA: University of Pennsylvania press.

Lacoue-Labarthe T, Reveillac E, Oberhansli F, Teyssie JL, Jeffree R, Gattuso JP. 2011. Effects of ocean acidification on trace element accumulation in the early-life stages of squid *Loligo vulgaris*. Aquatic Toxicology 105:166-176.

Langelier W. 1946. Effect of temperature on the pH of natural waters. American Water Works Association 38:179-185.

Leblanc CAL, Benhaïm D, Hansen BR, Kristjánsson BK, Skúlason S. 2011. The importance of egg size and social effects for behaviour of Arctic charr juveniles. Ethology 117:664-674.

Leblanc CAL, Kristjansson BK, Skulason S. 2016. The importance of egg size and egg energy density for early size patterns and performance of Arctic charr *Salvelinus alpinus*. Aquaculture Research 47:1100-1111.

Leduc A, Munday PL, Brown GE, Ferrari MCO. 2013. Effects of acidification on olfactory-mediated behaviour in freshwater and marine ecosystems: a synthesis. Philosophical Transactions of the Royal Society B-Biological Sciences 368:20120447.

Lin LY, Hung GY, Yeh YH, Chen SW, Horng JL. 2019. Acidified water impairs the lateral line system of zebrafish embryos. Aquatic Toxicology 217:105351.

Lynch M, Walsh B. 1998. Genetics and analysis of quantitative traits. Sunderland, MA: Sinauer Associated Inc.

Marshall DJ, Heppell SS, Munch SB, Warner RR. 2010. The relationship between maternal phenotype and offspring quality: Do older mothers really produce the best offspring? Ecology 91:2862-2873.

Meinshausen M, Smith SJ, Calvin K, Daniel JS, Kainuma ML, Lamarque J-F, Matsumoto K, Montzka SA, Raper SC, Riahi K. 2011. The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. Climatic Change 109:213-241.

Mölich A, Heisler N. 2005. Determination of pH by microfluorometry: intracellular and interstitial pH regulation in developing early-stage fish embryos (*Danio rerio*). Journal of Experimental Biology 208:4137-4149.

Mousseau TA, Fox CW. 1998. Maternal effects as adaptations. New York, USA: Oxford University Press.

Munday PL, Dixson DL, McCormick MI, Meekan M, Ferrari MCO, Chivers DP. 2010. Replenishment of fish populations is threatened by ocean acidification. Proceedings of the National Academy of Sciences of the United States of America 107:12930-12934.

Munday PL, Jarrold MD, Nagelkerken I. 2019. Ecological effects of elevated CO2 on marine fishes: from individual to community effects. In: Martin Grosell PLM, Anthony P. Farrell, Colin J. Brauner, editor. Carbon Dioxyde. Cambridge, Massachusetts: Academic Press. p. 323-368.

Neff BD, Pitcher TE. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Molecular Ecology 14:19-38.

Nusbaumer D, Marques da Cunha L, Wedekind C. 2020. Testing for population differences in evolutionary responses to pesticide pollution in brown trout (*Salmo trutta*). Evolutionary Applications 00:1-14.

Ou M, Hamilton TJ, Eom J, Lyall EM, Gallup J, Jiang A, Lee J, Close DA, Yun SS, Brauner CJ. 2015. Responses of pink salmon to CO2-induced aquatic acidification. Nature Climate Change 5:950-955.

Parker GA, Begon M. 1986. Optimal egg size and clutch size: Effects of environment and maternal phenotype. The American Naturalist 128:573-592.

Pedersen HC, Nybo S, Sandercock BK. 2020. Exposure of white-throated dippers to heavy metals in acidified and non-acidified streams in Norway. Journal of Ornithology 161:915-921.

Regnier 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 22:169-177.

Rombough PJ. 2007. Oxygen as a constraining factor in egg size evolution in salmonids. Canadian Journal of Fisheries and Aquatic Sciences 64:692-699.

Schindler DW. 2001. The cumulative effects of climate warming and other human stresses on Canadian freshwaters in the new millennium. Canadian Journal of Fisheries and Aquatic Sciences 58:18-29.

Schindler DW, Mills KH, Malley DF, Findlay DL, Shearer JA, Davies IJ, Turner MA, Linsey GA, Cruikshank DR. 1985. Long-term ecosystem stress - The effects of years of experimental acidification on a small lake. Science 228:1395-1401.

Schluter D, Price TD, Rowe L. 1991. Conflicting selection pressures and life history trade-offs. Proceedings of the Royal Society of London. Series B: Biological Sciences 246:11-17.

Serrano I, Buffam I, Palm D, Brannas E, Laudon H. 2008. Thresholds for survival of brown trout during the spring flood acid pulse in streams high in dissolved organic carbon. Transactions of the American Fisheries Society 137:1363-1377.

Smith CC, Fretwell SD. 1974. The optimal balance between size and number of offspring. The American Naturalist 108:499-506.

Snyder JT, Murray CS, Baumann H. 2018. Potential for maternal effects on offspring CO2 sensitivities in the Atlantic silverside (*Menidia menidia*). Journal of Experimental Marine Biology and Ecology 499:1-8.

Stearns SC, Hendry AP. 2004. Evolution illuminated: Salmon and their relatives. Oxford, New York: Oxford University Press, Inc.

Steingruber S, Colombo L. 2006. Impacts of air pollution on alpine lakes and rivers. Chemistry and biology in alpine lakes and rivers in southern Switzerland related to acidification from long-range transboundary air pollution: Monitoring results from 1980–2004. In. Environmental studies no. UW–0619. Switzerland: Federal Office for the Environment.

Tseng YC, Hu MY, Stumpp M, Lin LY, Melzner F, Hwang PP. 2013. CO2-driven seawater acidification differentially affects development and molecular plasticity along life history of fish (*Oryzias latipes*). Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 165:119-130.

Villalobos C, Love BA, Olson MB. 2020. Ocean acidification and ocean warming effects on Pacific herring (*Clupea pallasi*) early life stages. Frontiers in Marine Science 7:597899.

von Siebenthal BA, Jacob A, Wedekind C. 2009. Tolerance of whitefish embryos to *Pseudomonas fluorescens* linked to genetic and maternal effects, and reduced by previous exposure. Fish & Shellfish Immunology 26:531-535.

Wedekind C, Evanno G, Urbach D, Jacob A, Müller R. 2008. 'Good-genes' and 'compatible-genes' effects in an alpine whitefish and the information content of breeding tubercles over the course of the spawning season. Genetica 132:199-208.

Wedekind C, Müller R, Spicher H. 2001. Potential genetic benefits of mate selection in whitefish. Journal of Evolutionary Biology 14:980-986.

Wedekind C, Rudolfsen G, Jacob A, Urbach D, Müller R. 2007. The genetic consequences of hatchery-induced sperm competition in a salmonid. Biological Conservation 137:180-188.

Wetzel RG. 2001. Limnology: lake and river ecosystems. Amsterdam,Netherlands: Elsevier.

Whitehead PG, Wilby RL, Battarbee RW, Kernan M, Wade AJ. 2009. A review of the potential impacts of climate change on surface water quality. Hydrological Sciences Journal-Journal Des Sciences Hydrologiques 54:101-123.

Wilkins LG, Rogivue A, Schütz F, Fumagalli L, Wedekind C. 2015. Increased diversity of egg-associated bacteria on brown trout (*Salmo trutta*) at elevated temperatures. Scientific Reports 5:17084.

Tables

Table 1. The effects of dam and sire identity, exposure to treatment and egg volume at 90 dpf on: (a) embryo mortality, (b) egg volume 90dpf, (c) hatching days, (d) larval length at hatching, (e) yolk sac volume at hatching, and (f) larval mortality. Likelihood ratio tests on mixed model regressions were used to compare a reference model (in bold) with models including or lacking the term of interest. Significant p-values are highlighted in bold.

Model	Effect tested	AIC	d.f.	X^2	*P*
(a) Embryo mortality					
t + egg volume + S + D		2113	6		
t + egg volume + S	D	2136	5	24	**<0.001**
t + egg volume + D	S	2123	5	11	**<0.001**
t + egg volume + S + D + D*S	D*S	2111	7	4.1	**0.04**
egg volume + S + D	t	2878	4	769	**<0.001**
t + egg volume + S + D + D*t	D*t	2116	11	7	0.2
t + egg volume + S + D + S*t	S*t	2118	11	5	0.4
t + S + D	egg volume	2112	5	1	0.31
t + egg volume + S + D + egg volume*t	egg volume*t	2106	8	11	**0.003**
(b) Egg volume 90 dpf (mm^3)					
t + S + D		12605	6		
t + S	D	18600	5	6097	**<0.001**
t + D	S	12721	5	217	**<0.001**
t + S + D + D*S	D*S	12454	7	53	**<0.001**
S + D	t	12570	4	69	**<0.001**
t + S + D + D*t	D*t	12497	11	18	**0.003**
t + S + D + S*t	S*t	12511	11	4.4	0.5
(c) Hatching time					
t + egg volume + S + D		35119	7		
t + egg volume + S	D	35534	6	416	**<0.001**
t + egg volume + D	S	35335	6	217	**<0.001**
t + egg volume + S + D + D*S	D*S	35101	8	19	**<0.001**
egg volume + S + D	t	43728	5	8613	**<0.001**
t + egg volume + S + D + D*t	D*t	34604	12	524	**<0.001**
t + egg volume + S + D + S*t	S*t	34927	12	202	**<0.001**
t + S + D	egg volume	35117	6	0.04	0.83
t + egg volume + S + D + egg volume*t	egg volume*t	34987	9	135	**<0.001**
(d) Larval length at hatching (mm)					
t + egg volume + S + D		7171	7		
t + egg volume + S	D	7934	6	764	**<0.001**
t + egg volume + D	S	7228	6	58	**<0.001**
t + egg volume + S + D + D*S	D*S	7173	8	0.2	0.62
egg volume + S + D	t	7193	5	25	**<0.001**
t + egg volume + S + D + D*t	D*t	7174	12	7.4	0.18
t + egg volume + S + D + S*t	S*t	7179	12	2.6	0.75

t + S + D	egg volume	7418	6	248	**<0.001**
t + egg volume + S + D + egg volume*t	egg volume*t	7174	9	1.3	0.51
(e) Larval yolk sac volume at hatching (mm^3)					
t + egg volume + S + D		-1709	7		
t + egg volume + S	D	-662	6	1049	**<0.001**
t + egg volume + D	S	-1697	6	14	**<0.001**
t + egg volume + S + D + D*S	D*S	-1708	8	1.2	0.27
egg volume + S + D	t	-288	5	1425	**<0.001**
t + egg volume + S + D + D*t	D*t	-1741	12	41	**<0.001**
t + egg volume + S + D + S*t	S*t	-1715	12	15	**0.008**
t + S + D	egg volume	-1688	6	23	**<0.001**
t + egg volume + S + D + egg volume*t	egg volume*t	-1717	9	11	**0.003**
(f) Larval mortality					
t + egg volume + S + D		452	6		
t + egg volume + S	D	450	5	0	1
t + egg volume + D	S	450	5	0	1
t + egg volume + S + D + D*S	D*S	454	7	0	1
egg volume + S + D	t	485	4	37	**<0.001**
t+ egg volume + S + D + D*t	D*t	462	11	0	1
t + egg volume + S + D + S*t	S*t	459	11	2.2	0.8
t + S + D	egg volume	456	5	6.6	**0.01**
t + egg volume + S + D + egg volume*t	egg volume*t	455	8	0.4	0.8

Fixed effect: treatment (t) and egg volume. Random effects: sire (S) and dam (D).

Figures

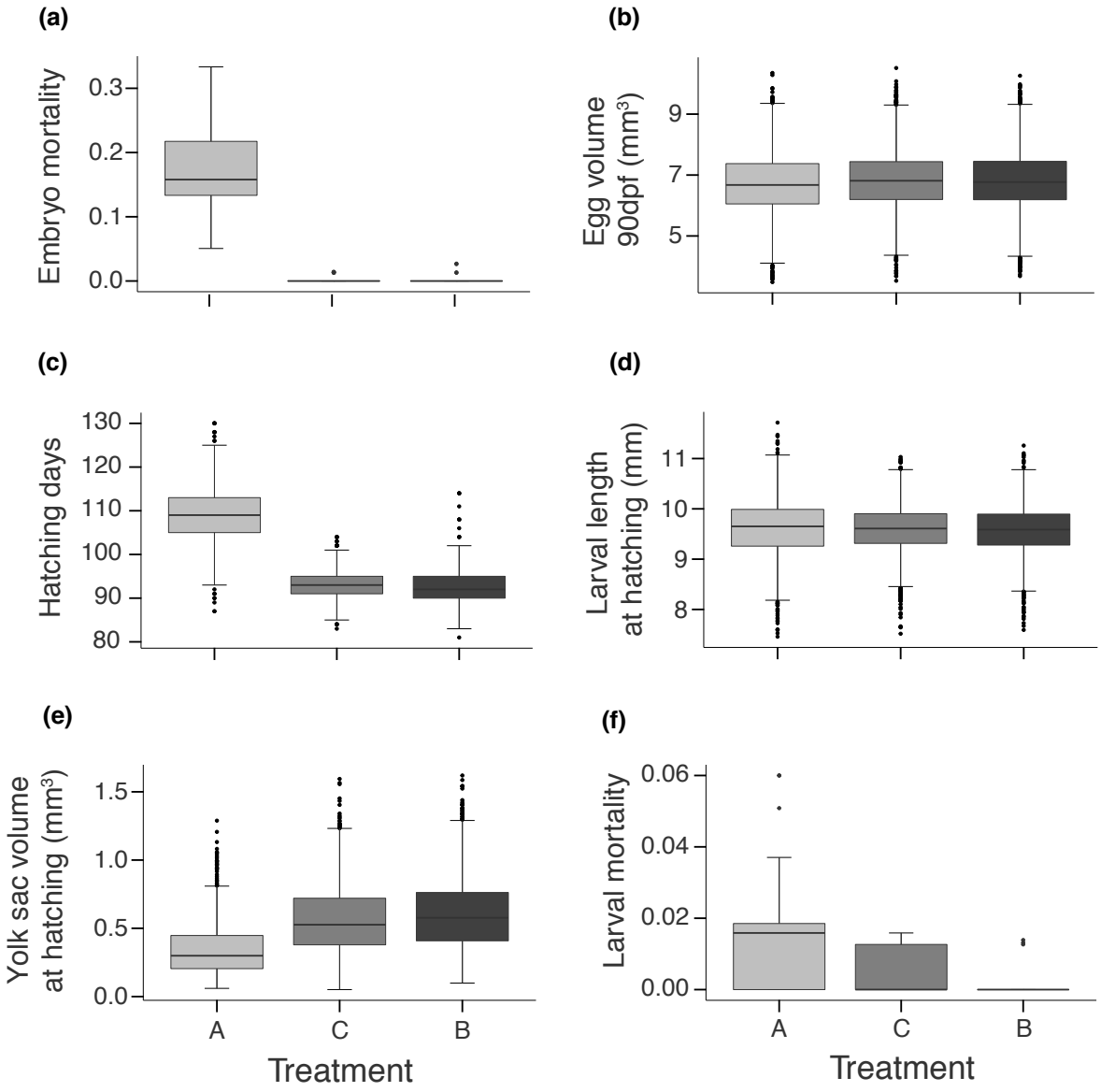


Figure 1. The effect of the exposure to different pH level on (a) embryo mortality, (b) egg volume 90 dpf, (c) hatching days, (d) larval length at hatching, (e) yolk sac volume at hatching, and (f) larval mortality. The levels of the treatment are represented as follow: A = acid, light grey (pH = 6.5 to 6.4); C = control, intermediate grey (pH = 7.0); B = Basic, dark grey (pH = 8.0; Tukey outlier boxplots with quartiles, whiskers, and outliers). See Table 1 for statistics.

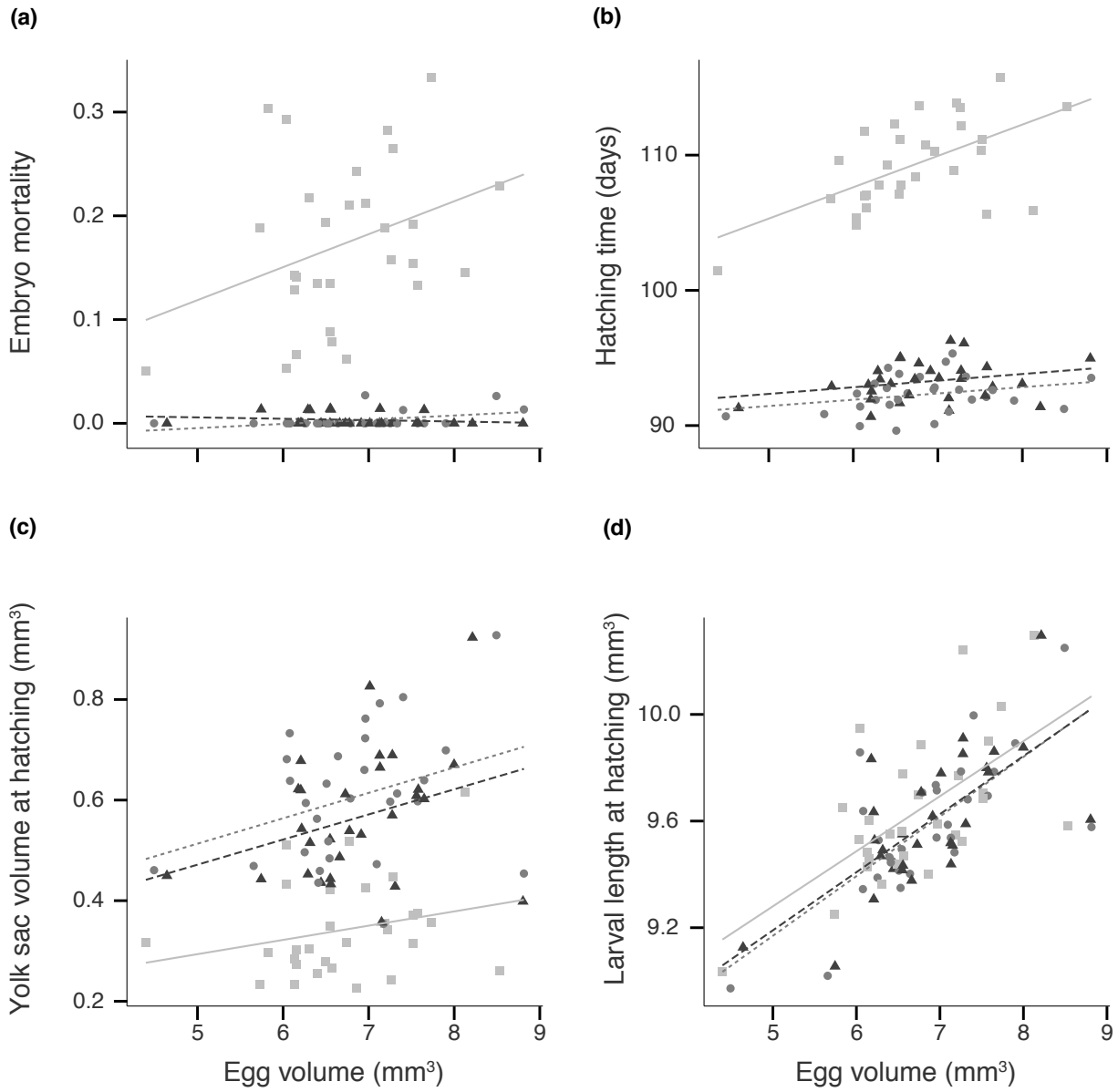


Figure 2. The significant interaction between egg volume and treatment on (a) mean embryo mortality, (b) mean hatching days, (c) mean yolk ac volume at hatching, and (d) the non-significant interaction between egg volume and treatment on mean larval length at hatching. The levels of the treatment are represented as follow: A = Acid, light grey squares and continuous regression line; C = control, intermediate grey, circles and dashed line; B = Basic, dark grey, triangles and dotted line. See Table 1 for statistics.

Supplementary information

The potential of a natural population of whitefish to rapidly adapt to freshwater acidification

Christian de Guttry[1], Eléonore Lavanchy[1], Florin Stettler[1], Nicolas Sironi[1,2] & Claus Wedekind[1*]

[1]Department of Ecology & Evolution, University of Lausanne, Lausanne, Switzerland

[2] Now at: Schweizerische Vogelwarte, Sempach, Switzerland.

* correspondence: claus.wedekind@unil.ch

Chapter status: unpublished

Content

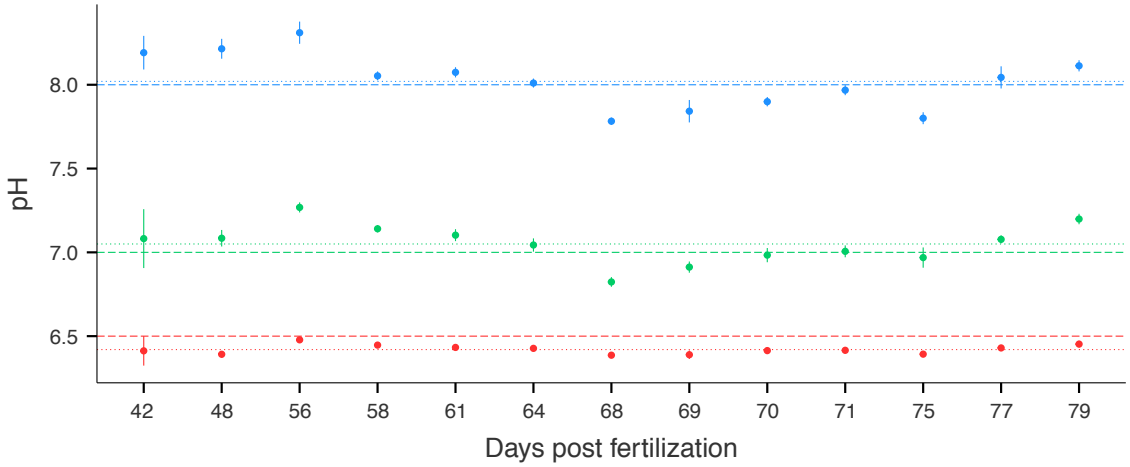


Figure S1. The mean (±SD) pH levels per treatment group and days after fertilization as measured during late embryo incubation. To determine the pH stability of each treatment in time, 95% prediction interval (pi) were calculated on the registered values: acidic treatment 95% pi= 6.35 to 6.49 (red symbols); control treatment 95% pi= 6.98 to 7.13 (green); basic treatment 95% pi = 7.94 to 8.09 (blue; it remains open whether the variation over time within treatment groups is linked to pH meter calibration or is caused in interaction with the embryos). Dashed lines give the concentrations at which each treatment group started on day 0, dotted lines the average concentrations recorded during the measurement period shortly before hatching.

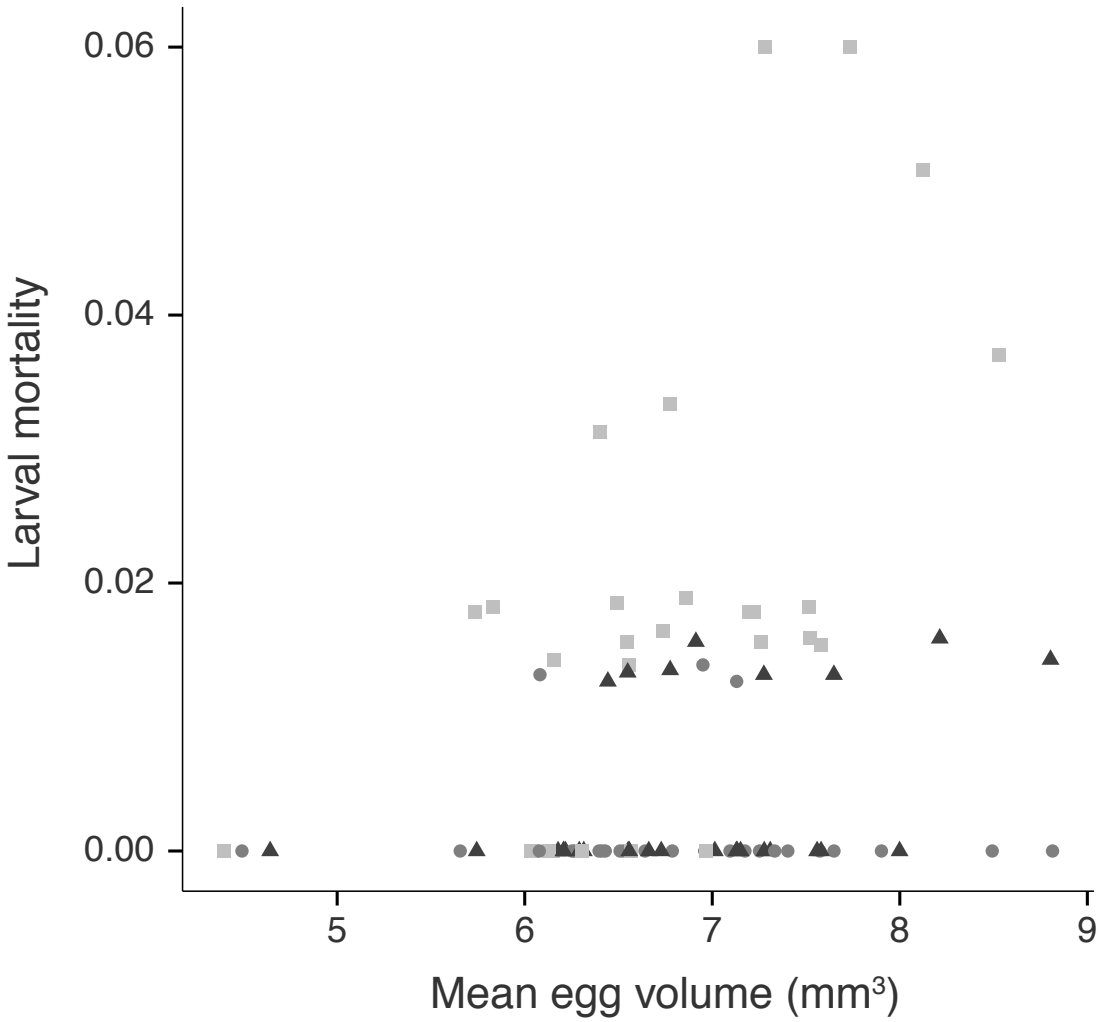


Figure S2. The non-significant interaction between mean egg volume and treatment on mean larval mortality. The treatment are represented as follow: A = acid, light grey squares; C = control, intermediate grey circles; B = basic, dark grey triangles. See text for statistics.

Chapter 5 - Egg size does not predict pathogen tolerance in whitefish embryos

Christian de Guttry[1], Alberto Garcia Jimenez[1,2], Nathalie Caloz[1], Audrey Atherton[1,3], Eliane Longange[1] & Claus Wedekind[1*]

[1] Department of Ecology & Evolution, University of Lausanne, Lausanne, Switzerland

[2] now at Department of Computational Biology, University of Lausanne, Lausanne, Switzerland

[3] now at: Department of Environmental Systems Science, ETH, Zürich, Switzerland

* correspondence: claus.wedekind@unil.ch

Chapter status: unpublished

Authors contributions

CdG and CW organized and conducted the field and laboratory work with the help of further members of the group. CW performed the experimental crosses. CdG and AGJ monitored embryo and larval development in the climate chamber with the help of NC. AA and AGJ determined larval and yolk sac size. EL determined egg size. CdG and AJ managed the data files. CdG and CW performed the statistical analysis and wrote the manuscript.

Abstract

The bigger is not always better hypothesis predict that in aquatic environment selection act against large eggs in stressful conditions. To date, this has been predominantly demonstrated in experiments using oxygen deficiency as stressor. Nonetheless, different sources of stress present in the water column could influence egg size evolution. We tested whether the reaction to pathogen exposure varies according to egg size during incubation. We sampled Alpine whitefish (*Coregonus suidteri*) at the end of their breeding season, used their gametes in 6 full-factorial breeding blocks and incubated 7,200 eggs singly under control or stress conditions (i.e. exposure to *Pseudomonas fluorescence*, a common salmonid pathogen). The influences of the stressor on offspring performances were monitored up to 21 days after hatching. The exposure to *Pseudomonas fluorescence* did not significantly affect embryo mortality but it reduced egg volume and embryo development rate and led to increased larval mortality. We found strong maternal but no paternal effects on virulence, suggesting strong maternal environmental effects on virulence. However, these differences in virulence could not be predicted by egg size. We conclude that the response to pathogen exposure, in the context of host-microbe interactions, is determined by the biochemical composition of the eggs while not linked to egg size.

Introduction

Egg size is a key reproductive trait that connects maternal and offspring fitness (Bernardo 1996). Early studies investigating the influence of egg size found a ubiquitous strong positive correlation between egg size and offspring performances (Roff 1992; Mousseau and Fox 1998; Einum and Fleming 1999). That is, larger eggs produce larger offspring which in turn will have a competitive advantage in early life stages over their smaller congeners (Fox and Mousseau 1996; Marshall and Keough 2008; Kosman and Pernet 2011; Leblanc, et al. 2016). Despite this, the evolutionary trajectory of this female reproductive trait is not advancing toward the production of larger eggs thus indicating the presence of balancing selective forces (Einum, et al. 2002).

Optimality theories on the evolution of offspring size predict the presence of an optimal per-offspring maternal investment. This investment is determined by the environmental stimuli perceived by the mother (Smith and Fretwell 1974) and influenced by maternal traits (i.e. size, age, condition) (Parker and Begon 1986; Sakai and Harada 2001; Kindsvater, et al. 2011). Those models are based on the observation that 'bigger is better' in the post-hatching period. However, predict evolutionary trajectories in species with complex life cycles requires an understanding of the selective pressures acting on each phase (Allen and Marshall 2014; Sun, et al. 2015). Indeed, it has been suggested that contrasting selection pressures can act on specific traits (i.e. size) at different life stages generating conflicting fitness benefits (Schluter, et al. 1991). If correct, this can help explain why theorical predictions often do not match empirical evidence as they fail to explain within population and within clutch egg size variability (Bernardo 1996; Marshall, et al. 2008).

The so called 'Bigger is not always better' hypothesis was often called into play to explain offspring size evolution in aquatic environment. It was initially assumed that oxygen acquisition was a limiting factor for large eggs during the incubation (Krogh 1941). This intuition was based on the surface-to-volume ratio of a sphere, and thus an increase in egg size would result in a decrease in the surface-to-volume ratio which in turn could reduce oxygen uptake and the embryo's metabolic rate. The possible presence of contrasting selection pressures between incubation period and post-hatching period on different-sized eggs was then included in optimality models by Hendry et al. (2001). Here, a negative correlation between egg size and survival during incubation and a positive correlation between offspring size and survival after hatching is expected. The first successful experimental test was carried out by Einum et al. (2002) and contrary to the expectations, larger eggs performed better under hypoxic conditions compared to smaller eggs. Indeed, developing embryos do not fully occupy the volume inside the egg shell and several parts of the embryo (e.g. the yolk sac) are metabolically inactive (Rollinson and Rowe 2018). This suggested, that to an increase of the egg surface corresponds

an increase of the contact with the external environment regardless of the internal morphology of the embryo (Einum, et al. 2002; Rombough 2007). Within this new scenario females are expected to produce larger eggs in hypoxic environment (Hendry and Day 2003). Despite these interesting findings, few studies investigated the potential selective pressures exerted during incubation, by different exogenous stressors present in the water column, on egg size.

Salmonids are an excellent model to test theories on egg size evolution (Stearns and Hendry 2004). They are external fertilizer that lay a high number of eggs during the breeding season with a substantial variability in size within clutches and populations (Quinn, et al. 1995; Marshall, et al. 2008; Lasne, et al. 2018). Those eggs can spend more than 2 moths incubating before hatching (Nusslé, et al. 2009; Kekäläinen, et al. 2010; Clark, et al. 2013; Pompini, et al. 2013; Clark, et al. 2014; Wilkins, et al. 2015). During this period, embryo mortality can be extremely high and selective pressure can be directed towards specific traits of the eggs such as size (Sifa and Mathias 1987; Johnson, et al. 2014). In addition, a strong positive correlation between egg size and offspring size after hatching has been observed in many species of in this family (Einum and Fleming 1999, 2000; Klemetsen, et al. 2003; Leblanc, et al. 2016; Cogliati, et al. 2018) but not yet in whitefish.

In this study we wanted to test the 'bigger is not always better' hypothesis by exposing whitefish (*Coregonus suidteri*) embryos during incubation to the common pathogen *Pseudomonas fluorescence (PF).* Different negative effects were found on salmonids when incubating embryos were exposed to bacteria (Clark, et al. 2013; Pompini, et al. 2013; Clark, et al. 2014), organic and inorganic sediments (Mari, et al. 2016; Gatch, et al. 2020) and chemical pollutants (von Siebenthal, et al. 2009; Marques da Cunha, Maitre, et al. 2019; Marques da Cunha, Uppal, et al. 2019; Nusbaumer, et al. 2020; Selmoni, et al. 2020). However, none of these controlled for a specific effect on different-sized eggs. Our prediction was that larger eggs, with a larger surface of contact with the external environment, may suffer more from the effects of pathogen exposure. To test this we collected eggs from 30 females, we estimated the average size of the eggs they produced and fertilized about 7200 of them with the gametes of 60 males. Half of the eggs were then exposed during incubation to the pathogen *PF*, and embryos were followed up to a later larval stage. Our results are presented in the context of 'bigger is not always better' hypothesis.

Material and Methods

Adult whitefish (*Coregonus suidteri*) were collected from Lake Hallwil (Switzerland; 47.2772° N, 8.2173° E) using gillnets (mesh size: 35 mm) towards the end of their reproductive season (09/01/2017). Gametes were stripped from haphazardly selected adults, immediately after euthanasia and before weight and length was measured. *In vitro* fertilizations were performed to create 6 x (5♀ x

10♂) breeding block, thus obtaining 300 different parental full-sib groups. After egg hardening (at least two hours), 24 eggs for each full-sib group (n_{tot} = 7,200) were transported to a climate room for incubation under standardized conditions. All remaining eggs were photographed (Canon 70D) in a custom-made photo box to later determine size measurements and passed on to the local supplementary breeding program. For each of the full-sib groups, egg volume at day 0 ("egg volume") of 30 haphazardly selected eggs were calculated from diameter measurements, using ImageJ v2.0.0 (https://imagej.net).

In the climate chamber, eggs were family-wise washed in a sterilized sieve under running tap water (4 L/minute) for 30 seconds. They were then singly distributed to 24-well plates (Greiner Bio-One, Kremsmünster, Austria) filled with 1.8 ml of autoclaved, reconstituted standardize water (Kitano 1992) as in von Siebenthal et al (2009) and incubated at 4°C. Eggs were treated 21 days post fertilization by adding 200 µL of either standardized water with nutrient broth containing 10^6 cell/µL of *Pseudomonas fluorescens (PF*, an opportunistic bacterial pathogen) or not containing *PF* (control treatment) as in Clark et al. (2013). This treatment was full-factorially and fully balanced with regard to the sib group. To assess egg volumes at a late development stage, eggs were photographed shortly before hatching at 90 dpf. At hatching, the larvae were temporarily transferred to a new 24-well plate filled with 300 µl standardize water and a series of three photos of were taken. Larvae were then singly transferred to new wells of 24-well plates containing only 2.0 mL of standardized water. After hatching, larval mortality was daily monitored over a period of 21 days. Larval length (from snout to the fork) and yolk sac volumes (ellipsoid based on two axes) were taken from the photos using ImageJ v2.0.0 (https://imagej.net). In 2.6 % of all larvae, photo quality did not allow for sufficiently accurate measurements.

The effects of pathogen exposure and egg size on offspring performance were tested in linear mixed-effect models (LMM) and generalized linear mixed-effect models (GLMM). Larval mortality was included as a binomial response variable in GLMM. Egg volume at 90 dpf, hatching time (days since fertilization), larval length at hatching, larval yolk sac volume at hatching, and larval length 21 days after hatching were analyzed as continue response variables in LMM. The dam and sire identity and their interaction were included as random factors. Likelihood ratio testing (LRT) was used to evaluate the significance of each factors by comparing a model lacking or including the term of interest with a reference model and the model fit was assessed with Akaike's information criteria (AIC). Analysis were done in R v. 4.0 (Core Team 2016) using the lme4 package (Bates, et al. 2015).

Results

The exposure to *PF* did not affect embryo mortality (Table 1a) but significantly reduced egg volume at 90 dpf (Table 1b; Fig. 1a). Larger eggs lost more volume (Fig. 1b) and more so in response to *PF* than smaller eggs (egg volume*t interaction in Table 1b). However, no egg volume * t interaction was significant for any remaining measures of offspring growth and survival (Table 1). Egg volume at day 0 did not influence embryo mortality (Table 1a), hatching time (Table 1c; Fig. 2a), or larval mortality (Table 1g) but was positively correlated with egg volume at 90 dpf (Table 1b), larval length at hatching (Table 1d; Fig 2b), larval yolk sac volume at hatching (Table 1e; Fig 2c) and larval length 21 days after hatching (Table 1f; Fig 2d).

Female (dam) and male (sire) identity significantly affected all response variables except for larval mortality where the dam effect was non-significant, and embryo mortality where the sire effect was not significant (Table 1). The dam x sire interaction had no significant effect on embryo or larval mortality and larval length after 21 days (Table 1a,f,g) but significantly affected the other offspring performance parameters (Table 1b-e).

Exposure to *PF* led to delayed hatching (Table 1c; Fig. 3a) of smaller larvae (Table 1d; Fig. 3b) with larger yolk sacs (Table 1e; Fig. 3c). Exposure to *PF* also induced higher larval mortality (Table 1f; Fig. 3e). Dam identity influenced the effects of *PF* on egg volume 90 dpf and on hatching time but not on the remaining variables (see D*t interactions in Table 1). Sire identity influenced the effects of *PF* on egg volume 90 dpf but not on any other response variables (see S*t interaction in Table 1).

Discussion

We wanted to test the 'bigger is not always better' hypothesis by exposing different-sized eggs to a mild pathogen. We predicted that large eggs with a larger surface area would experience stronger negative selection pressures because of pathogen exposure compared to smaller eggs. We found that 90 dpf, i.e. 69 days after exposure to *PF,* eggs had an overall reduction in volume. This reduction was slightly stronger for larger eggs. However, resistance to PF exposure seems to be driven by egg quality and not egg size. The exposure to *PF* during incubation influenced all the offspring performances at larval stage, including larval mortality. We finally demonstrated how in whitefish egg size determine larval size after hatching and 21 days after hatching.

The exposure to *PF* had the effect of reducing the volume of the eggs during incubation. According to our knowledge, this is the first time such a response to a stress has been recorded in incubating embryos. This effect was male specific indicating the presence of additive genetic variance

for resistance to treatment. In external fertilizers a male contributes only his DNA during fertilization. Thus, a significant sire x treatment (S*t) effect means that parental sib groups differ in their genetic quality and specific paternal DNA might confer a greater resistance to *PF* exposure, already at this embryonic stage. Maternal identity also appears to be important in explaining the effect of decreased egg volume during PF exposure (D*t). Dam effects could be genetic (additive genetic variance) or environmental (egg quality). Indeed, what might influence embryonic development is the quantity of substances stored by the mother during oogenesis (egg size) or the quality of those substances (Wilkins, et al. 2017). Considering the assumption that paternal and maternal genetic effects contribute equally on a specific offspring trait (Lynch and Walsh 1998), we can conclude that additive genetic variance, but mostly maternal environmental effect influence the response to the treatment. It is interesting to see how part of this environmental maternal effect is partially explained by the size of the egg. We found that larger eggs tend to lose more volume than smaller eggs (egg volume*t). Our results suggest that the severity of the response to the exposure to PF, partially depends on the area of the surface exposed to the water column i.e. more bacteria can adhere to a large surface thus generating a stronger response. This effect was, however, small and the absence of the same significant interaction on the other offspring performances, may indicate that it does not have a strong effect on offspring fitness at later life stages. To confirm this, we found that, for hatching time, there is a significant dam effect determining the response to PF exposure (D*t), but we did not find a significant interaction between sire effect or egg size on the resistance to PF. This confirms that the quality of eggs plays a major role than egg size in determining resistance to PF exposure. For instance, a female, in addition to nutrients, provides immune factors that protect the embryo in the developmental stages (Swain and Nayak 2009) and the quality of these substances may vary depending on the maternal status (de Guttry et al., in prep; chapter 1and 2). In our breeding design we also revealed the presence of non-additive genetic variance in offspring tolerance to the pathogen. This indicate that particular haplotype combinations of males and females explain a significant portion of the variance related to tolerance to PF, accordingly to what has been previously found on whitefish exposed to PF (Clark, et al. 2014).

Fish eggs contain a percentage of water that varies from 50 to 70% of the total composition (Fyhn, et al. 1999). However, a higher amount of water is needed to complete development and embryos create an osmotic gradient through the lysis of nutriment stocked in the egg (Dolomatov, et al. 2012). This creates a high concentration of solutes in the extracellular environment that let in the large amount of water they need for their final maturation. The exposure in our experiment appeared to slow development. It delayed hatching time, induced a reduced size of the larvae at hatching that had a larger volume of the yolk sac. These findings suggest that in response to the exposure to PF, the embryos reduced the consumption of nutrients from the yolk sac, thus reducing the amount of

incoming water. This may have led to the consumption of most the water already presents within the membrane and the consequent reduction in volume. Since large eggs have a faster metabolic rate (Regnier, et al. 2013) it is therefore possible that the consumption of the water contained inside the egg membrane is faster and hence the greater loss of volume.

This outcome reveals how, during incubation, larger eggs could be more affected by stressful conditions than smaller eggs. Einum et al. (2002) showed that larger eggs have higher survival in anoxic environment compared to small eggs. Having a larger surface area allowed large eggs to increase oxygen uptake from the water column. Our result is in line with theirs, suggesting that the pathogen effects slightly increase with an increasing surface area. Regnier et al. (2013), investigating the effect of increasing temperatures on different-sized brown trout (*Salmo trutta*) eggs, found that smaller eggs have higher survival rate compared to large eggs. In light of these results, we suggest that the selective pressure acting on different-sized eggs during incubation are dependent by the nature of the stressor. Further studies are now needed to better understand this dynamic. First, PF is considered a mild pathogen, so the response it generates can be interpreted as moderate (Clark, et al. 2013; Clark, et al. 2014). Using pathogens with stronger effects might help clarify whether we have strong size-based selectivity during incubation. Secondly, an experiment similar to ours, controlling for the quality of the eggs, could help to understand which factors contribute most to the response due to pathogen exposure.

Many studies that have been conducted on the effect of pathogens or chemicals on offspring performances (von Siebenthal, et al. 2009; Jonsson and Jonsson 2011; Clark, et al. 2013; Clark, et al. 2014; Wilkins, et al. 2015; Marques da Cunha, Maitre, et al. 2019; Marques da Cunha, Uppal, et al. 2019; Nusbaumer, et al. 2020; Selmoni, et al. 2020). However, the general focus was not on the effect of treatment on egg size before and after the exposure. In spite of this, Nusbaumer et al (2020) showed a reduction of the length at hatching of larger larvae from larger eggs exposed to S-metolachlor or diazinon. They did not test for a reduction in volume of the egg after the exposure, but the trend is the same as the one we obtained while exposing the offspring to PF. This suggests the occurrence of a similar dynamic during the incubation period in their experiment. The reduction in larval length is maybe due to a reduction in egg volume even when eggs are exposed to pesticides. These results show that it is necessary, in experiments designed to understand the effects of a stressor in early life stages, to include the size and quality of eggs in order to have a better understand on the effects of exposure.

The exposure to *PF* during incubation influenced all the offspring performances in early larval stages including larval mortality. This shows how the negative selection pressures experienced during ontogeny affected also post-hatching stages since, after hatching, the larvae were not exposed to PF. Interestingly, we found a sire but not dam effect on larval mortality. In contrast, we found the sole

influence of dam effects on embryo mortality. This confirms that maternal effects are prominent in early stages of development while paternal effects are prominent later in life (Clark, et al. 2014). By all means, we should be cautious in interpreting these results given the very low larval mortality rate. The effects of *PF* that we detected on hatching time and hatchlings size are coherent with the results on the offspring of another Alpine whitefish (*Coregonus palaea*) (Clark, et al. 2014). In our study, we did not find an effect of PF exposure on larval size 21 days after hatching. This might indicate the presence of compensatory growth of PF exposed embryos that slowed development during the embryonic phase being smaller but with a larger yolk sac at hatching. However, this result can be confounded with the effect that PF had on hatching time and should be interpreted with caution.

We then found that the size of the larvae is mostly determined by the size of the eggs. In our experiment larger eggs generate larger larvae than smaller eggs, independently by the treatment effect. The same trend is shared by most of the species in the salmonid's subfamilies (Roff 1992; Mousseau and Fox 1998; Einum and Fleming 1999; Leblanc, et al. 2016). This result demonstrates how the population phenotypic variability in the early life stages of salmonids is strongly dependent on egg size (Thorn and Morbey 2018). Being larger in the post-hatching period resulted in advantages such as low predation rate, higher motility and better feeding capabilities (Einum and Fleming 1999; Marshall and Keough 2008; Leblanc, et al. 2011). If we assume the presence of the same advantages for whitefish larger larvae and considering that large eggs might suffer stronger selection pressure during incubation, we suggest the presence of contrasting selection pressures acting on offspring size in early life stages of whitefish. However, to confirm our hypothesis a behavioral experiment is needed. Moreover, we did not expose the larvae to any treatment after hatching. Observing how they react to the exposure to different stressors even after hatching can help us understand how generalized our hypothesis could be.

A shortcoming in our experiment is that we used an average number of eggs per female as an estimate of post-fertilization egg size. Despite this, we have found a strong correlation with the mean egg volume per female 69 days after exposure. However, in a future experimental setting the same eggs incubated in the laboratory can be photographed after fertilization and after a period of exposure to a treatment. Given our high statistical power and the strong correlation of egg size in after fertilization and 90 dpf we do not expect this to change the observed results dramatically.

To conclude, egg size appears to have a smaller role than egg quality in determining the resistance to PF. Notwithstanding, to confirm our conclusion, studies of different pathogens present in natural populations are necessary and including specific measures of egg quality is required. We showed how the importance of environmental maternal effects play a key role in determining survival through the incubation period and in determines hatchlings phenotypes until 21 days after hatching.

This confirms their key role in population dynamics and in determining the evolutionary trajectories of a species.

Ethics

The experimental breeding and the raising of embryos in the laboratory were approved by the Fishery Inspectorate of the Aargau canton. Approvals by the veterinary offices of the involved cantons were not required because the fish were caught in a commercial fishing program, measurements and tissue samples were taken from dead fish, and all embryos and larvae were euthanized before the end of the yolk sac period.

Data availability

Data will be deposited on the Dryad depository upon acceptance of the manuscript.

Funding

The project was supported by the lottery funds of the Aargau canton and the Swiss National Science Foundation (31003A_182265).

Acknowledgements

We thank R. Stadelmann for catching the fish, J. Beninde, I. Castro, G. Fabre, L. Garaud, L. Marques da Cunha, K. Mobley, D. Nusbaumer, E. Seddon, and C. Vincent for assistance in the field and in the laboratory, D. Bittner for permits, and all the above for discussion.

References

Allen RM, Marshall D. 2014. Egg size effects across multiple life-history stages in the marine annelid *Hydroides diramphus*. PLoS One 9:e102253.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Bernardo J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. American Zoologist 36:216-236.

Clark ES, Pompini M, Marques da Cunha L, Wedekind C. 2014. Maternal and paternal contributions to pathogen resistance dependent on development stage in a whitefish (Salmonidae). Functional Ecology 28:714-723.

Clark ES, Stelkens RB, Wedekind C. 2013. Parental influences on pathogen resistance in brown trout embryos and effects of outcrossing within a river network. PLoS One 8:e57832.

Cogliati KM, Unrein JR, Stewart HA, Schreck CB, Noakes DL. 2018. Egg size and emergence timing affect morphology and behavior in juvenile chinook salmon, *Oncorhynchus tshawytscha*. Ecology and Evolution 8:778-789.

Core Team R. 2016. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Dolomatov S, Zukow W, Novikov NY, Muszkieta R, Bulatowicz I, Dzierzanowski M, Kazmierczak U, Strojek K. 2012. The regulation of osmotic and ionic balance in fish reproduction and in the early stages of ontogeny. Russian Journal of Marine Biology 38:365-374.

Einum S, Fleming IA. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): Norms of reaction to environmental quality. Proceedings of the Royal Society of London. Series B: Biological Sciences 266:2095-2100.

Einum S, Fleming IA. 2000. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). Evolution 54:628-639.

Einum S, Hendry AP, Fleming IA. 2002. Egg-size evolution in aquatic environments: does oxygen availability constrain size? Proceedings of the Royal Society B: Biological Sciences 269:2325-2330.

Fox CW, Mousseau TA. 1996. Larval host plant affects fitness consequences of egg size variation in the seed beetle Stator limbatus. Oecologia 107:541-548.

Fyhn HJ, Finn RN, Reith M, Norberg B. 1999. Yolk protein hydrolysis and oocyte free amino acids as key features in the adaptive evolution of teleost fishes to seawater. Sarsia 84:451-456.

Gatch AJ, Koenigbauer ST, Roseman EF, Hook TO. 2020. The effect of sediment cover and female characteristics on the hatching success of walleye. North American Journal of Fisheries Management 40:293-302.

Hendry AP, Day T. 2003. Revisiting the positive correlation between female size and egg size. Evolutionary Ecology Research 5:421-429.

Hendry AP, Day T, Cooper AB. 2001. Optimal size and number of propagules: Allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. American Naturalist 157:387-407.

Johnson DW, Grorud-Colvert K, Sponaugle S, Semmens BX. 2014. Phenotypic variation and selective mortality as major drivers of recruitment variability in fishes. Ecology Letters 17:743-755.

Jonsson B, Jonsson N. 2011. Maturation and spawning. In: Noakes DLG, editor. Ecology of Atlantic salmon and brown trout: habitat as a template for life histories. London: Springer. p. 327-414.

Kekäläinen J, Huuskonen H, Tuomaala M, Kortet R. 2010. Both male and female sexual ornaments reflect offspring performance in a fish. Evolution 64:3149-3157.

Kindsvater HK, Bonsall MB, Alonzo SH. 2011. Survival costs of reproduction predict age-dependent variation in maternal investment. Journal of Evolutionary Biology 24:2230-2240.

Kitano M. 1992. Updating of OECD guidelines for the testing of chemicals. Water Science and Technology 25:1-465.

Klemetsen A, Amundsen PA, Dempson JB, Jonsson B, Jonsson N, O'Connell MF, Mortensen E. 2003. Atlantic salmon *Salmo sala*r L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): A review of aspects of their life histories. Ecology of Freshwater Fish 12:1-59.

Kosman ET, Pernet B. 2011. Intraspecific variation in larval size and its effects on juvenile lophophore size in four bryozoans. Marine Ecology Progress Series 429:67-73.

Krogh A. 1941. The comparative physiology of respiratory mechanisms. Philadelphia, USA: University of Pennsylvania press.

Lasne E, Leblanc CA, Gillet C. 2018. Egg size versus number of offspring trade-off: Female age rather than size matters in a domesticated Arctic charr population. Evolutionary Biology 45:105-112.

Leblanc CAL, Benhaïm D, Hansen BR, Kristjánsson BK, Skúlason S. 2011. The importance of egg size and social effects for behaviour of Arctic charr juveniles. Ethology 117:664-674.

Leblanc CAL, Kristjánsson BK, Skúlason S. 2016. The importance of egg size and egg energy density for early size patterns and performance of Arctic charr *Salvelinus alpinus*. Aquaculture Research 47:1100-1111.

Lynch M, Walsh B. 1998. Genetics and analysis of quantitative traits. Sunderland, MA: Sinauer Associated Inc.

Mari L, Garaud L, Evanno G, Lasne E. 2016. Higher temperature exacerbates the impact of sediments on embryo performances in a salmonid. Biology Letters 12:20160745.

Marques da Cunha L, Maitre D, Wedekind C. 2019. Low adaptive potential for tolerance to ethynylestradiol, but also low toxicity, in a grayling population (*Thymallus thymallus*). BMC Evolutionary Biology 19:227.

Marques da Cunha L, Uppal A, Seddon E, Nusbaumer D, Vermeirssen ELM, Wedekind C. 2019. No additive genetic variance for tolerance to ethynylestradiol exposure in natural populations of brown trout (*Salmo trutta*). Evolutionary Applications 12:940-950.

Marshall DJ, Bonduriansky R, Bussière LF. 2008. Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. Ecology 89:2506-2517.

Marshall DJ, Keough MJ. 2008. The relationship between offspring size and performance in the sea. The American Naturalist 171:214-224.

Mousseau TA, Fox CW. 1998. Maternal effects as adaptations. New York, USA: Oxford University Press.

Nusbaumer D, Marques da Cunha L, Wedekind C. 2020. Testing for population differences in evolutionary responses to pesticide pollution in brown trout (*Salmo trutta*). Evolutionary Applications 00:1-14.

Nusslé S, Bornand CN, Wedekind C. 2009. Fishery-induced selection on an Alpine whitefish: quantifying genetic and environmental effects on individual growth rate. Evolutionary Applications 2:200-208.

Parker GA, Begon M. 1986. Optimal egg size and clutch size: Effects of environment and maternal phenotype. The American Naturalist 128:573-592.

Pompini M, Clark ES, Wedekind C. 2013. Pathogen-induced hatching and population-specific life-history response to waterborne cues in brown trout (*Salmo trutta*). Behavioral Ecology and Sociobiology 67:649-656.

Quinn TP, Hendry AP, Wetzel LA. 1995. The influence of life history trade-offs and the size of incubation gravels on egg size variation in sockeye salmon (*Oncorhynchus nerka*). Oikos 74:425-438.

Regnier 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 22:169-177.

Roff D. 1992. Evolution of life histories: theory and analysis. New York, USA: Chapman & Hall.

Rollinson N, Rowe L. 2018. Oxygen limitation at the larval stage and the evolution of maternal investment per offspring in aquatic environments. American Naturalist 191:604-619.

Rombough PJ. 2007. Oxygen as a constraining factor in egg size evolution in salmonids. Canadian Journal of Fisheries and Aquatic Sciences 64:692-699.

Sakai S, Harada Y. 2001. Why do large mothers produce large offspring? Theory and a test. The American Naturalist 157:348-359.

Schluter D, Price TD, Rowe L. 1991. Conflicting selection pressures and life history trade-offs. Proceedings of the Royal Society of London. Series B: Biological Sciences 246:11-17.

Selmoni OM, Maitre D, Roux J, Wilkins LGE, Marques da Cunha L, Vermeirssen ELM, Knorr S, Robinson-Rechavi M, Wedekind C. 2020. Sex-specific changes in gene expression in response to estrogen pollution around the onset of sex differentiation in grayling (*Salmonidae*). BMC Genomics 20:583.

Sifa L, Mathias J. 1987. The critical period of high mortality of larvae fish—a discussion based on current research. Chinese Journal of Oceanology and Limnology 5:80-96.

Smith CC, Fretwell SD. 1974. The optimal balance between size and number of offspring. The American Naturalist 108:499-506.

Stearns SC, Hendry AP. 2004. Evolution illuminated: Salmon and their relatives. Oxford, New York: Oxford University Press, Inc.

Sun Z, Hamel JF, Parrish CC, Mercier A. 2015. Complex offspring size effects: variations across life stages and between species. Ecology and Evolution 5:1117-1129.

Swain P, Nayak S. 2009. Role of maternally derived immunity in fish. Fish & Shellfish Immunology 27:89-99.

Thorn MW, Morbey YE. 2018. Egg size and the adaptive capacity of early life history traits in Chinook salmon (*Oncorhynchus tshawytscha*). Evolutionary Applications 11:205-219.

von Siebenthal BA, Jacob A, Wedekind C. 2009. Tolerance of whitefish embryos to *Pseudomonas fluorescens* linked to genetic and maternal effects, and reduced by previous exposure. Fish & Shellfish Immunology 26:531-535.

Wilkins LG, Marques da Cunha L, Glauser G, Vallat A, Wedekind C. 2017. Environmental stress linked to consumption of maternally derived carotenoids in brown trout embryos (Salmo trutta). Ecology and Evolution 7:5082-5093.

Wilkins LG, Rogivue A, Schütz F, Fumagalli L, Wedekind C. 2015. Increased diversity of egg-associated bacteria on brown trout (*Salmo trutta*) at elevated temperatures. Scientific Reports 5:17084.

Tables

Table 1. The effects of dam and sire identity, exposure to PF and egg volume at day 0 on (*a*) embryo mortality, (*b*) egg volume 90 dpf, (*c*) hatching time, (*d*) length at hatching, (*e*) yolk sac volume, (*f*) larval length 21 days after hatching, and (*g*) larval mortality. Likelihood ratio tests on mixed model regressions were used to compare a reference model (in bold) with models including or lacking the term of interest. Significant p-values are highlighted in bold.

Model	Effect tested	AIC	d.f.	χ^2	*P*
(a) Embryo mortality					
t + egg volume + S + D		707	5		
t + egg volume + S	D	738	4	32	**<0.001**
t + egg volume + D	S	705	4	0.05	0.82
t + egg volume + S + D + D*S	D*S	709	6	0	1
egg volume + S + D	t	707	4	707	0.18
t + egg volume + S + D + D*t	D*t	705	7	5.1	0.056
t + egg volume + S + D + S*t	S*t	710	7	0.6	0.72
t + S + D	egg volume	707	4	1.6	0.19
t + egg volume + S + D + egg volume*t	egg volume*t	708	6	0.9	0.34
(b) Egg volume 90 dpf (mm^3)					
t + egg volume + S + D		13476	6		
t + egg volume + S	D	13370	5	895	**<0.001**
t + egg volume + D	S	13578	5	104	**<0.001**
t + egg volume + S + D + D*S	D*S	13323	7	155	**<0.001**
egg volume + S + D	t	13918	5	443	**<0.001**
t + egg volume + S + D + D*t	D*t	13344	8	135	**<0.001**
t + egg volume + S + D + S*t	S*t	13468	8	12	**0.002**
t + S + D	egg volume	13523	5	48	**<0.001**
t + egg volume + S + D + egg volume*t	egg volume*t	13473	7	5.1	**0.02**
(c) Hatching time					
t + egg volume + S + D		36678	6		
t + egg volume + S	D	37708	5	1032	**<0.001**
t + egg volume + D	S	37570	5	894	**<0.001**
t + egg volume + S + D + D*S	D*S	36633	7	47	**<0.001**
egg volume + S + D	t	37522	5	846	**<0.001**
t + egg volume + S + D + D*t	D*t	36633	8	50	**<0.001**
t + egg volume + S + D + S*t	S*t	36678	8	3.8	0.14
t + S + D	egg volume	36676	5	0.02	0.87
t + egg volume + S + D + egg volume*t	egg volume*t	36679	7	1.2	0.25
(d) Length at hatching (mm)					
t + egg volume + S + D		9618	6		

t + egg volume + S	D	10111	5	494	**<0.001**
t + egg volume + D	S	9795	5	178	**<0.001**
t + egg volume + S + D + D*S	D*S	9612	7	8.7	**0.003**
egg volume + S + D	t	9637	5	20	**<0.001**
t + egg volume + S + D + D*t	D*t	9622	8	0.6	0.71
t + egg volume + S + D + S*t	S*t	9622	8	0	1
t + S + D	egg volume	9643	5	27	**<0.001**
t + egg volume + S + D + egg volume*t	egg volume*t	9620	7	0.2	0.61
(e) Yolk sac volume (mm^3)					
t + egg volume + S + D		-2209	6		
t + egg volume + S	D	-1033	5	1177	**<0.001**
t + egg volume + D	S	-2180	5	31	**<0.001**
t + egg volume + S + D + D*S	D*S	-2215	7	8.2	**0.004**
egg volume + S + D	t	-2202	5	8.6	**0.004**
t + egg volume + S + D + D*t	D*t	-2206	8	1.5	0.45
t + egg volume + S + D + S*t	S*t	-2209	8	4.1	0.12
t + S + D	egg volume	-2198	5	12	**<0.001**
t + egg volume + S + D + egg volume*t	egg volume*t	-2207	7	0.4	0.53
(f) Larval length 21 days after hatching (mm)					
t + egg volume + S + D		10026	6		
t + egg volume + S	D	11167	5	1143	**<0.001**
t + egg volume + D	S	10132	5	108	**<0.001**
t + egg volume + S + D + D*S	D*S	10026	7	2	0.15
egg volume + S + D	t	10024	5	0.3	0.61
t + egg volume + S + D + D*t	D*t	10027	8	2.5	0.28
t + egg volume + S + D + S*t	S*t	10029	8	0.4	0.82
t + S + D	egg volume	10042	5	18	**<0.001**
t + egg volume + S + D + egg volume*t	egg volume*t	10027	7	0.2	0.64
(g) Larval mortality					
t + egg volume + S + D		1447	5		
t + egg volume + S	D	1447	4	1.4	0.23
t + egg volume + D	S	1457	4	11	**<0.001**
t + egg volume + S + D + D*S	D*S	1449	6	0.6	0.44
egg volume + S + D	t	1517	4	71	**<0.001**
t + egg volume + S + D + D*t	D*t	1450	7	1	0.62
t + egg volume + S + D + S*t	S*t	1450	7	1.3	0.51
t + S + D	egg volume	1446	4	0.3	0.58
t + egg volume + S + D + egg volume*t	egg volume*t	1446	6	3.5	0.06

Fixed effect: treatment (t), egg volume at day 0 (egg volume). Random effects: sire (S) and dam (D)

Figures

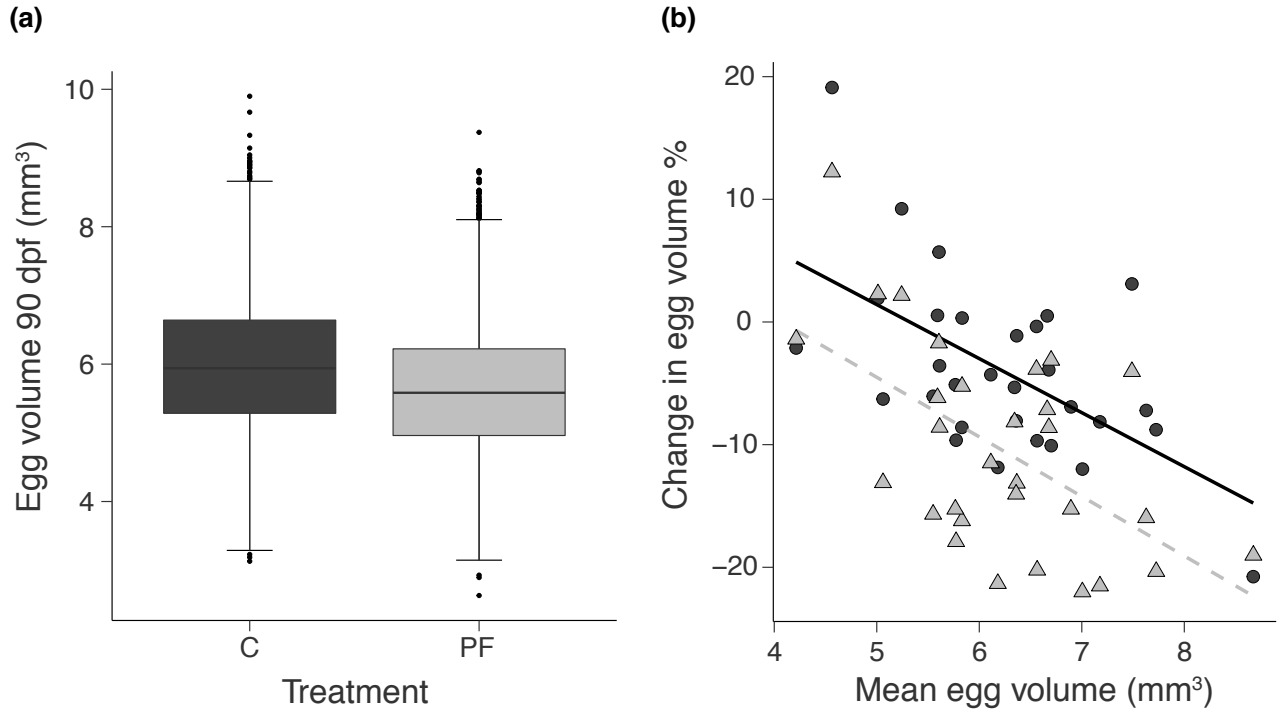


Figure 1. (a) The effect of the exposure to *Pseudomonas fluorescence* (PF) on egg volume shortly before hatching, i.e. 90 dpf (Tukey outlier boxplots with quartiles, whiskers, and outliers). (b) The combined effects of the exposure to PF and egg volume at day 0 (means per female) on the change in egg volume (in %) as observed 90 dpf, for PF-treated (light grey triangles, dotted regression line) and controls (black circles, continuous regression line). See Table 1b for statistics.

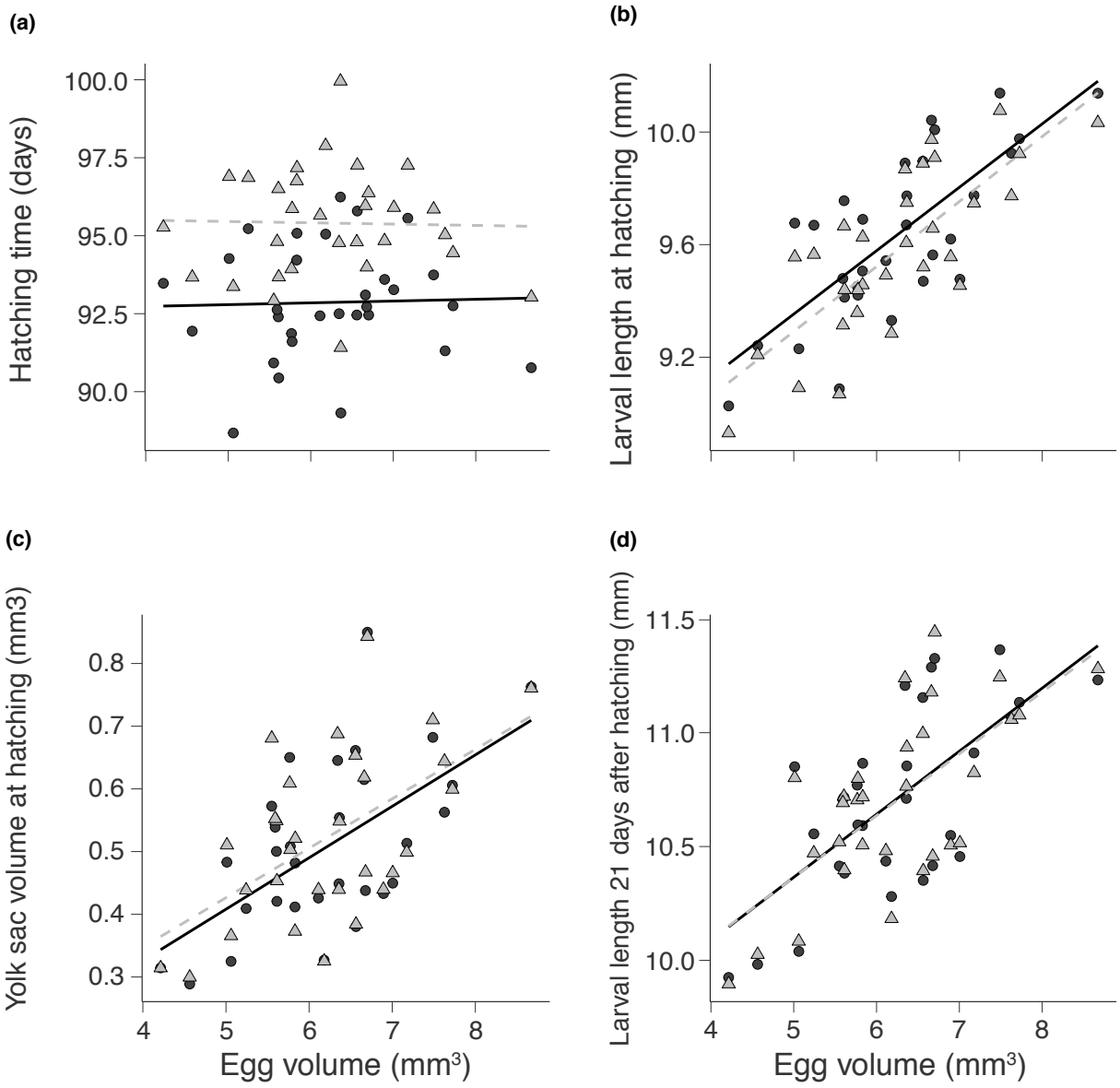


Figure 2. The effects of egg volume at day 0 (means per female) on (a) hatching time, (b) mean larval length at hatching, (c) mean yolk sac volume at hatching, and (d) mean larval length 21 days after hatching. PF-treated are shown with grey triangles and dotted regression line. Controls treated are shown with black circles and continuous regression line. See table 1 for statistics.

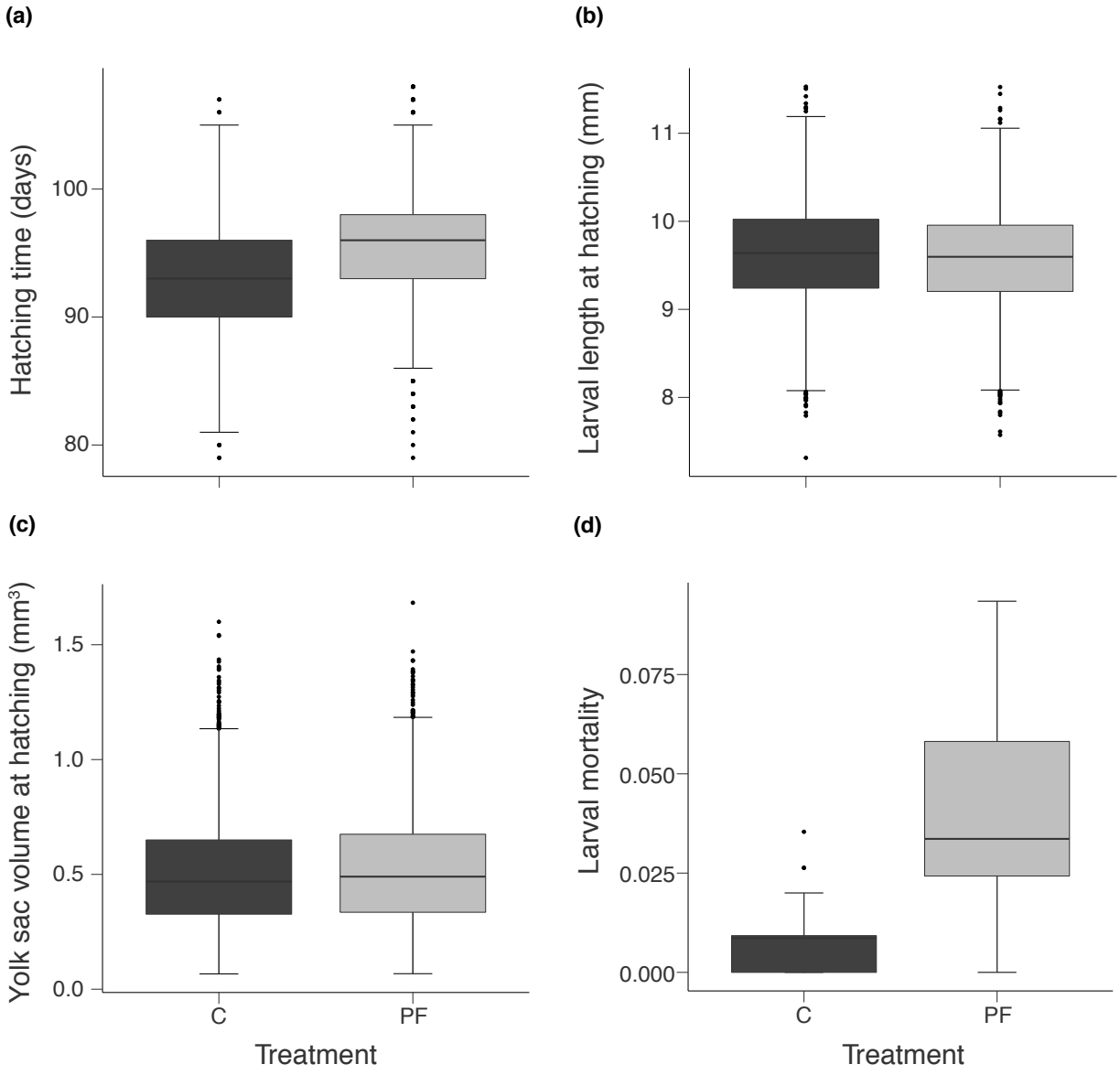


Figure 3. The effect of the exposure to *Pseudomonas fluorescence* (PF) on (a) larval hatching time, (b) larval length at hatching, (c) yolk sac volume at hatching, (d) larval mortality rate (Tukey outlier boxplots with quartiles, whiskers, and outliers). See table 1 for statistics.

Chapter 6 - The genetic architecture of *sdy* in whitefish give insights into salmonids sex determination and partly explain male gonad weigth

Christian de Guttry[1], Romain Feron[1,2], Claus Wedekind[1*] & Yann Guiguen[3*]

[1] Department of Ecology & Evolution, University of Lausanne, Lausanne, Switzerland

[2] Swiss Institute of Bioinformatics, University of Lausanne, Lausanne, Switzerland

[3] INRAE, LPGP, Rennes, France

[*] shared senior authors

Chapter status: unpublished

Authors contributions

CdG, YG and CW organized and conducted the field and laboratory work with the help of further members of the group. CdG took the tissue samples and dissected the gonads. CdG performed gDNA extraction and ddRADseq libraries preparation. CdG and RF processed and analyzed the genomic data. CdG and RF performed the statistical analysis and wrote the first draft of the manuscript.

Abstract

Most salmonids are characterized by a male heterogametic sex determination (SD) system driven by the presence on the Y chromosome of *sdy*, the master sex-determining (MSD) gene of this family. However, in some salmonid species, including several species of Coregoninae, *sdy* was found in both males and females. This observation led to two hypotheses: the presence of a dosage-dependent regulation of SD in salmonids, or the presence of an incomplete, non-functional *sdy* gene in females. To explore these hypotheses, we analyzed the genetic architecture of the *sdy* gene region in male and female whitefish. Using different next-generation sequencing techniques and a new reference genome from the study population, we found that both sexes carry multiple copies of some *sdy* exons, but males have a higher copy number than females, and exons 3 and 4 are missing from the female *sdy* copies. Furthermore, we found a positive correlation between the number of copies of *sdy* in males and the size of their testes. Our results currently support both hypotheses, and a study of the functionality of *sdy* in the two sexes is now needed. Finally, our results suggest the presence of a second role of *sdy* in the determination of male gonad size in male whitefish. Because gonad size can be an indicator of male reproductive strategy, it now needs to be tested whether copy number variation of the *sdy* is a useful predictor of variation in male life history.

Key words: Sex determination, *sdy*, master sex determining gene, ddRAD sequencing, pool-sequencing, whole genome re-sequencing, depth of coverage, gonad size.

Introduction

Sex determination is the process by which an individual acquires its sex during development, in species that reproduce sexually. This process involves a primary signal triggering a sex determination pathway, which eventually initiates sexual development. The pathway differs between the two sexes and the mechanisms leading to male or to female development are referred to as a sex-determination (SD) system. SD systems can be maintained unchanged over long evolutionary periods, as in the case of mammals and birds (Graves 2008), or they can be extremely labile and dynamic as in the case of teleost (Pan, et al. 2016; Pan, et al. 2019). In practice, the structure of the SD pathway is conserved between closely related – and even distant – species, but the primary signal can be highly variable in some clades (Bachtrog, et al. 2014), and consequently, dozens of SD systems have evolved independently and rapidly in many taxa (Bachtrog, et al. 2014). Most of these systems are sorted into two main categories based on the nature of the primary signal: genetic sex determination (GSD), where the primary signal is genetic, *i.e.*, a gene present only in one sex, (Beukeboom and Perrin 2014) and environmental sex determination (ESD), where the primary signal is external, *i.e.*, temperature during development (Bachtrog, et al. 2014). In most cases, GSD is controlled by a single master sex determining gene (MSD), and identifying the MSD is a crucial step in understanding the SD system and mechanisms in a species.

SD genes were first investigated in mammals, where male sex determination is initiated by the *Sry* gene (*sex-determining region of the Y chromosome)*(Sinclair, et al. 1990), in birds with the *dmrt1* gene (Stiglec, et al. 2007; Smith, et al. 2009), and in amphibians, carrying the *dm-W* gene (Yoshimoto and Ito 2011). Both *Sry* and *dmrt1* are highly conserved across mammals and birds, respectively, and *dm-W* is relatively conserved across amphibians (Ito 2019). In other clades, however, MSD genes are not conserved, for instance in teleost, where multiple MSD genes have recently been identified (Hattori, et al. 2012; Kamiya, et al. 2012; Myosho, et al. 2012; Feron, Zahm, et al. 2020; Wen, et al. 2020; Pan, et al. 2021) , revealing several instances of MSD gene and SD system turnover (Devlin and Nagahama 2002).The emergence of these different MSD has been hypothesized to be related to a gene duplication resulting in a change in function (Kikuchi and Hamaguchi 2013). This hypothesis is the most credited for salmonids, on the basis of the second duplication of the genome that they have undergone, after the radiation from the family of Esociformes 110 million years ago (Mya)(Macqueen and Johnston 2014).

Salmonids are characterized by a male-heterogametic SD system, *i.e.* males are XY and females are XX, where the *sdy* gene (*sexually dimorphic on the Y-chromosome*) was found to be the best MSD gene candidate (Yano, et al. 2012). *Sdy* was not found in the sister family Esociformes,

indicating that it likely originated after the second whole genome duplication specific to Salmonids (Yano, et al. 2013). Unlike the other MSD genes identified so far, *sdy* evolved from the duplication of a gene involved in the control of the immune response (Yano, et al. 2012; Yano, et al. 2013). *Sdy* was initially found in 15 salmonids species and its overexpression is expected to trigger testicular differentiation (Yano, et al. 2012). With these premises, *sdy* is supposed to regulate the sex determination in all the salmonids sub-families (Salmoninae, Thymallinae, Coregoninae). However, thanks to the development of faster and more accurate sequencing techniques, this hypothesis has since been tested in an increasing number of Salmonid species, which revealed multiple cases of discordance between phenotypic and genetic sex, *i.e.* presence/absence of the *sdy* gene (Larson, et al. 2016; Podlesnykh, et al. 2017; Ayllon, et al. 2020; Brown, et al. 2020). These results have been mostly attributed to a dosage-dependent regulation system, where multiple copies of the gene are required to activate the cascade for testis development (Yano, et al. 2013), or to the presence of 'incomplete', non-functional *sdy* gene copies in females (Eisbrenner, et al. 2014; Lubieniecki, et al. 2015; Brown, et al. 2020). Unveiling the exact mechanisms of sexual determination in salmonids would have many applications for aquaculture, which is of high cultural and economic interest in these species and would provide insights on the evolution of sex determination systems in teleost.

In this study, in order to explore these unresolved questions, we decided to work with one of the most emblematic species of the family, the whitefish (*Coregonus spp.*). This species represent a case of phenotypic/genotypic sex discordance in which the *sdy* region has been amplified in both males and females (Yano, et al. 2013). The lack of a good reference genome did not allow investigation of the genetic architecture of *sdy* in this species to date. Therefore, we first sequenced and assembled a chromosome level genome of a male Alpine whitefish (*Coregonus suidteri*) and used this assembly to investigate the MSD gene region using ddRAD sequencing, pooled sequencing, and re-sequencing of several individuals coming from the lake Hallwil (Switzerland). Our aim was to clarify the mechanism of sexual determination in whitefish by investigating whether it depends on a dosage dependent regulation system, explained by the presence of a higher *sdy* copy number in male than in females, or by the presence of differences in the gene structure between the two sexes with females presenting an incomplete *sdy* copy. Finally, we wanted to determine whether *sdy* also plays a role in determining the size of the male gonads since its expression lead to testis development.

Materials and Methods

Sample collection

Sexually mature male (N = 138) and female (N = 73) whitefish were collected with gillnets from lake Hallwil (Switzerland; 47.2772° N, 8.2173° E) during their spawning season (December 2016 / January 2017). After being euthanized, males were carefully stripped for their sperm and females for their eggs that were subsequently used in a separate experiment. Once the weight and standard length were recorded, male gonads were dissected and stored in Falcon tubes (Corning, Gurgaon, India) previously filled with 25 ml of 70% ethanol to be later weighted with a high precision scale. The gonadosomatic index (GSI) was estimated as the proportion of the gonad mass over the entire body mass [(gonad weight / male weight)*100]. Before returning the fish to local fishermen, a tissue sample from the anal fin was taken and stored in ethanol 70% for genetic analysis.

DNA extraction

To obtain genomic DNA, anal fin tissue samples were processed with the DNAeasy Blood & Tissue kit (Quiagen, Venlo, Netherland). Briefly, a 20 mg tissue sample was left digesting with proteinase K (Thermo Scientific™, England) for 12h at 56C°. The resulting solution underwent 5 washes to remove cellular residues, and the DNA was finally eluted in 200 µl of saline buffer solution. DNA integrity was checked by electrophoresis on a 1% agarose gel and the final concentration determined using a Qubit™ dsDNA HS Assay Kit (Invitrogen, England).

Detection of the sdy sequence in the newly assembled whitefish genome

The *sdy* gene sequence was extracted from the rainbow trout genome (GenBank assembly accession: GCA_002163495.1). This sequence was then searched in the newly assembled male whitefish genome belonging to the lake Hallwil population (GenBank assembly accession: XXX) using *blastn* with all settings to default (Altschul, et al. 1990) and Minimap2 (Li 2018). The best hit of the *sdy* gene was identified by both programs on the HiC_scaffold_48.

Double digested Rad sequencing (ddRADseq)

The DNA concentration of the 211 individuals was normalized to 20 ng/µl. A total of six libraries were prepared following the Brelsford et al. (2016) protocol adapted from Parchman et al. (2012). In each library, individuals were identifiable with a different 5 bp barcode designed to bind the restriction sites of EcoRI-HF (New England Biolab, Ipswich, Massachusetts, United States). MspI (New England

Biolab, Ipswich, Massachusetts, United States) was used as the second restriction enzyme. After digestion and ligation, fragments were size-selected on agarose gels using the range of 400-600 bp. Once the DNA was purified, single-end genotyping was done on an Illumina HiSeq 2500 with fragments of 125/150 bp length at the Lausanne Genomic Technologies Facility (University of Lausanne).

The quality of the sequenced reads was checked using FASTQC v0.11.7 (Andrews 2010) and reads were trimmed to 90 bp using Trimmomatic v. 0.36 (Bolger, et al. 2014) to ensure consistent base-pair quality along and between reads (>20 Phred score). The raw sequences were then demultiplexed with *process_radtags* from stacks v. 1.48 (Catchen, et al. 2013) using default parameters. To search for a sex-specific signal, reads were analyzed using SexGenomicsToolkit/radsex: version 1.1.2 (Feron, Pan, et al. 2020). First, a table of marker depth for the entire dataset was created using the *process* command setting the minimum depth to 1 (--min-depth 1). Second, the distribution of marker between males and females was computed from the depth table using the *distrib* command, retaining only markers with a minimum depth of 5 (--min-depth 5). The figure showing the results of *distrib* was generated with the *radsex_distrib()* function of *sgtr* (10.5281/zenodo.3773063).

Pooled sequencing

Two sex specific pooled libraries were created using the DNA from 30 females and 30 males previously normalized to 20 ng/ul. Libraries were generated using Truseq nano kit (Illumina, FC-121–4001) following the manufacturer's instructions. Pool-seq libraries were subsequently prepared using the Illumina TruSeq Nano DNA HT Library Prep Kit (Illumina, SanDiego, CA) following the manufacturer's protocol. Briefly, 200 ng of DNA from each pool were shortly sonicated using a Bioruptor sonication device (Diagenode, Liege, Belgium). The DNA was subsequently end-repaired and fragments of 550 bp were selected using magnetic beads. The Illumina adapters and indexes were then ligated to the previously A-tailed fragments. The quality of the 2 libraries was checked using a fragment analyzer (Advanced Analytical Technologies, Inc., Ankeny, IA) and quantified using the Kapa Library Quantification Kit (qPCR) (Roche Diagnostics Corp, Indianapolis, IN). The paired-end sequencing was performed on a NovaSeq S4 lane (Illumina, San Diego, CA) at the GeT-PlaGe core facility of INRA Toulouse, France (http://get.genotoul.fr/en/).

To search for a sex-specific signal the PSASS workflow was used (https://github.com/SexGenomicsToolkit/PSASS-workflow). Briefly, the resulting reads from the 2 pools were aligned to our new chromosome-level genome assembly (GenBank assembly accession:

XXX) of a male whitefish (*Coregonus suidteri*) using Bwa v. 0.7.17 (Li and Durbin 2010) with the algorithm for short reads *mem.* The resulting alignment files were sorted, and PCR duplicates were removed using the *sort* and *rmdup* functions from Samtools v 1.10 respectively (Li, et al. 2009). Then, a file with nucleotide counts for each genomic position was generated with the *pileup* function from PSASS (version 3.0.1b, 10.5281/zenodo.3702337). This file was used as input for the *analyze* function from PSASS to compute the female and male absolute and relative depth with a non-overlapping 50 kb sliding window along the HiC_scaffold_48 with the following parameters: window-size 50,000, output-resolution 1000, freq-het 0.5, range-het 0.15, freq-hom 1, range-hom 0.05, min-depth 1, and group-snps. The pool-seq coverage figure was obtained with *sgtr* v. 1.1.1 (10.5281/zenodo.3773063).

Whole genome re-sequencing

A total of 37 Illumina TruSeq PCR-free libraries were prepared, following the manufacturer's protocol, using the DNA of 34 males and 3 females. Briefly, 1µg of DNA per individual was sonicated using a Covaris sonication device (Woburn, Massachusetts, USA). The ends of the fragments were repaired and fragments of 550 bp were selected using purification beads. After the adenylation of the 3' ends, a unique index adapter was added to each individual and the library quality was checked using KAPA library quantification kit (Roche Diagnostics Corp, Indianapolis, IN) and using a fragment analyzer (Advanced Analytical Technologies, Inc., Ankeny, IA). Individuals were then pooled on 20 lanes of Illumina HISeq 4000 at the Lausanne Genomic Technologies Facility of University of Lausanne (https://wp.unil.ch/gtf/).

The resulting raw reads were checked for sequencing quality using FastQC v. 0.11.7 (Andrews 2010). To ensure consistent quality across lanes and between individuals, the reads were cut from their index adapters and their length was reduced to 120 bp using Trimmomatic v. 0.36 (Bolger, et al. 2014). The resulting paired-end reads were aligned to our new chromosome-level genome assembly (GenBank assembly accession: XXX) of a male whitefish (*Coregonus suidteri*) using Bwa v. 0.7.17 (Li and Durbin 2010) with the algorithm for short reads *mem*. The bam files preprocessing was done following the GATK best practices (Van der Auwera, et al. 2013). Briefly, read groups were added using the Picard tools function *addReadGroups* (http://broadinstitute.github.io/picard). Using the same software, the bam files were cleaned (*CleanSam*), paired-ends were fixed (*fixmate*), and duplicates were removed (*MarkDuplicates*). To complete the preprocessing, Samtools v. 1.8 (Li, et al. 2009) was used to sort the bam files (*sort*), to fix the paired ends (*fixmate*), and to remove secondary alignment (*view -bh -F 256*).

From the resulting bam files, reads aligned to the HiC_scaffold_48 containing the *sdy* gene were extracted and the read depth for each position was estimated using Samtools *depth* v. 1.8 (Li, et al. 2009). Only reads with the highest mapping quality (*-Q 60*) were retained. The same procedure was repeated for the longest chromosome (HiC_scaffold_01) and the resulting depth values were used to normalize the depth estimates in the HiC_scaffold_48 region. The *sdy* gene model was subsequently investigated thanks to our genome annotation. The coordinates of the four distinctive exons of the salmonids *sdy* gene were identified (Genomic positions on the HiC_scaffold_48 = exon1: 118858 to 119011; exon2: 116771 to 117133; exon3: 115738 to 115838; exon4: 113417 to 113684). The relative depth of coverage for each exon was estimated for females and males using a custom made python script.

The genotype of each individual was called by first generating a GVCF file using the GenomeAnalysisTK's function *HaplotypeCaller* and then converting the GVCF to VCF using the function *GenotypeGVCFs*. The resulting single nucleotides polymorphisms (SNPs) for the HiC_scaffold_48 underwent a technical filtering step using parameter values recommended by GATK (-filter *"QD<2.0"; "QUAL<30.0"; "SOR>3.0"; "FS>60.0"; "MQ<40.0";"MQRankSum<-12.5"; "ReadPosRankSum<-8.0"*). No other filters were applied to the VCF.

Statistical analysis

To determine the possible correlation of depth variation in exon 3 and 4 of the *sdy* gene on male GSI, a multiple regression was used.

Results

The whitefish sdy gene

The *sdy* sequence extracted from the rainbow trout (*Oncorhynchus mykiss*) genome (GenBank assembly accession: GCA_002163495.1) was approximately 10 kb in length. The best hit of the *sdy* sequence on the whitefish genome (*Coregonus suidteri*), for both Blast (e-value < 1e-50) and Minimap2, was located on HiC_scaffold_48. This scaffold could not be placed in any of the 40 chromosomes and has a length of about 463 kb. The whitefish *sdy* sequence begins at position 113,417 and end at position 119,011 based on the new genome annotation (Fig.3).

Double digested Rad sequencing (ddRADseq)

After demultiplexing, we obtained an average of 4,147,723 reads per individual. In total, 739 markers were found to be significantly associated with male phenotype or female phenotype (chi-square test, $p > 0.05$ after Bonferroni correction). However, no markers were present only in males and absent in females as expected in a male-heterogametic sex determination system (Fig 1).

Pooled sequencing

Comparison of pooled sequencing of males and females revealed up to three times higher depth of coverage in males than in females in a region spanning from 111 kb to 119 kb on HiC_scaffold_48, where the sex-determining gene *sdy* is located (Fig. 2B). This coverage difference suggests a male-specific duplication of the region, which could indicate a dosage dependent regulation system as was previously suspected in whitefish (Yano, et al. 2013).

Whole genome re-sequencing

After the pre-processing of bam files for the HiC_scaffold_48, a median depth of coverage of 29X was obtained. The absolute depth differed between sexes along the *sdy* region with males having a higher depth of coverage compared to females (Fig. 2C). Considering gene model (Fig. 2A) it can be observed that exons 3 and 4 are present only in males.

By further investigating the depth of coverage for each exon in the different sexes we observed the presence of multiple copies of exon 1 and exon 2 in females and males, with males generally having a higher number of copies than females (Fig. 3; Table 1). Interestingly, we reported the presence of multiple copies of exon 3 and exon 4 just in males. Females have a depth of coverage approximately 0 for these 2 regions (Fig. 3; Table 1).

We obtained 2,183 SNPs in the HiC_scaffold_48 region with a mean coverage of 47.1 X. Within the *sdy* region we found 18 SNPs of which 2 (biallelic) presents in all males but in none of the three females after the end of exon 4.

Phenotypes characteristics and link to exon depth of coverage variation

The mean (±sd) weight of males was 206.6 ± 26.5 g, and their mean (±sd) gonad weight was 4.8 ± 1.8 g. The mean (±sd) weight of females was 212.4 ± 24.4 g.

The effect of the depth of coverage for each male and exon 3 and 4, the ones present just in males, was plotted against the individual GSI and a significant positive correlation between the depth of coverage and GSI was found ($F_{1,31}$= 4.4, p = 0.02, r^2 = 0.22; Fig. 4a)

Discussion

After *sdy* was first discovered to be the MSD gene in salmonids, further studies investigated this region in multiple salmonid species, and the results painted a complex picture of the mechanism of sex determination in this important family (Yano, et al. 2013; Bertho, et al. 2018). For instance, in whitefish, the sequence of *sdy* was amplified in both males and females (Yano, et al. 2013), suggesting that sex determination in this species diverges from canonical mechanisms of sex determination in mammals and birds. Its peculiarities continued to emerge as molecular biology and sequencing techniques advanced, revealing, in several salmonid species, a discordance between phenotypic and genotypic sex (Eisbrenner, et al. 2014; Cavileer, et al. 2015; Ayllon, et al. 2020; Brown, et al. 2020). In order to understand how *sdy* contributes to sex determination in whitefish, we investigated its genetic architecture. After assembling the genome of a male *Coregonus suidteri*, we found the presence of *sdy* in both sexes, confirming no male specific sex-linkage. However, males are characterized by the presence of multiple copies of all the 4 *sdy* exons, while females were found to carry only exons 1 and 2, suggesting the presence of an incomplete *sdy* sequence in this sex.

Through the use of the RADsex pipeline we have attempted to identify non-polymorphic markers that segregate between males and females. Despite the large number of individuals used, and the considerable number of markers significantly associated with one of the two sexes, we did not find markers associated with phenotypic sex present only in males or in females. An absence of sex-linked markers from RAD-Seq data is not uncommon in fishes (Feron, Pan, et al. 2020), and has recently been documented in two species in the Esociformes, a sister family to salmonids, where it was linked to the reduced size of the sex locus (Pan, et al. 2021). Our results suggest that the sexual determining region in whitefish is also small as in the other salmonids (Woram, et al. 2003). Indeed, the small size of the *sdy* region allowed it to transpose or translocate to different chromosomes in several species of salmonids(Woram, et al. 2003; Guiguen, et al. 2018) but also in different chromosomes of individuals within the same species (Eisbrenner, et al. 2014; Gabian and Moran 2019).

To overcome the low resolution of RAD-Sequencing, we used two complementary whole genome sequencing approaches in order to identify and study the likely characteristics of the sex determining region in the two sexes. The analysis of sequences generated by pooled sequencing showed a difference in coverage between males and females in a small region on scaffold 48, where

the *sdy* gene was found. The difference in relative coverage in this region is nearly three times in favor of males , which suggests a variation in *sdy* copy numbers between males and females whitefish. Multiple copies of *sdy* were recently identified in a quantitative study on Atlantic salmon (*Salmo salar*) (Ayllon, et al. 2020) and *sdy* copy number was found to be higher in males than in females of the Tasmanian strain of the same species (Eisbrenner, et al. 2014; Brown, et al. 2020). The same pattern emerged from our analysis of whole genome re-sequencing data of 37 whitefish individuals. Results from this analysis confirmed the presence of a higher number of copies of *sdy* in males than in females, which might indicate a dosage dependent regulation of sex determination in whitefish, as initially suggested by Yano et al. (2013) and as proposed recently for Atlantic salmon by Brown et al. (2020). Such a system requires that the expression of *sdy* exceeds a specific threshold to trigger the cascade that leads to the development of male gonads. To further explore this hypothesis, the first step would be to perform a functional study quantifying *sdy* expression in males and females during sexual development.

By investigating in more detail the genetic architecture of *sdy*, we found that the observed difference in depth between males and females varies between the four exons of *sdy*. In males, we found all four exons of *sdy,* but the copy number of each exon varies among individuals, and exons 1 and 2 are present at higher copy numbers than exons 3 and 4 within an individual. In females, we found only exons 1 and 2 which have a lower copies number variation than in males, and exons 3 and 4 were not detected. The absence of *sdy* specific exons in females of a salmonid population was recently identified using quantitative approaches such as qPCR (Ayllon, et al. 2020; Brown, et al. 2020), but some of the observed patterns, such as the presence of only some exons in only part of the females, were reported as possible errors due to the quality of the gDNA and the limitations of qPCR (Brown, et al. 2020). Using whole genome re-sequencing, we have shown a consistent pattern regarding the presence/absence of the 4 exons in the 2 sexes, and we can exclude technical artifacts due to the detection technique. In our study, we can therefore confirm the presence of an 'incomplete *sdy* sequences in females' and a complete *sdy* sequence in males. The presence of incomplete copies is not uncommon in salmonids (Eisbrenner, et al. 2014) and has been imputed to the instability of the sequence due to the high motility of this gene within the genome (Faber-Hammond, et al. 2015; Lubieniecki, et al. 2015). However, additional functional studies are required to assess the functional significance of incomplete *sdy* copies in females.

We found that the copy number of exons 3 and 4, the exons specific to males, seems to contribute to the determination of testes size, and the correlation between coverage for an exon and testes size was significant for both absolute depth and relative depth, *i.e.,* after correcting the exons depth by the genome-wide depth for each individual. If this effect is confirmed, it would represent the

first occurrence of another possible role of the *sdy* gene. A similar effect has already been documented in drosophila, where the dosage of specific sex-linked genes has been linked to male body size and specific tissues size (Mathews, et al. 2017). Our result can be interpreted in the light of the putative dosage-dependent sex determination system of *sdy*: having more active copies of *sdy* would lead to an increase of testis size in the short period of time during development in which it is expressed. An alternate hypothesis is that *sdy* is still expressed after gonadal differentiation and involved in testis growth in whitefish. Functional experiments will be necessary to unravel the mechanistic details of the correlation between *sdy* copy number and testes size. Gonad size has also been linked to males reproductive strategy in salmonids (Rudolfsen, et al. 2008) and future studies needs to test whether copy number variation of different the *sdy* exons can be a predictor of variation in male life history.

The results of a genomic approach to better understand the genomic architecture and role of *sdy* as an MSD gene in whitefish opened very interesting scenarios. Prior to our study, two mechanisms by which *sdy* acts as the MSD gene in salmonids despite its presence in females were suggested: 1) a dosage dependent regulation system, and 2) the presence of an incomplete gene sequence in females (Yano, et al. 2012; Yano, et al. 2013; Guiguen, et al. 2018). Here, we found that in lake Hallwill whitefish, males have higher *sdy* copy numbers than females, suggesting the presence of a dosage dependent regulation system, but *sdy* copies of females are incomplete. Our results thus support both hypotheses and show that they are not mutually exclusive. To proceed further, a study of gene expression can help to finally understand how this happens in our whitefish population. However, given the characteristics of this gene, we do not know how far this result can be extended to other salmonid species. We suggest that a genomic approach, where technical limitations are minimized, should be used to better understand the fascinating history of sexual determination in salmonids.

Ethics approval

The samples collection was approved by the Fishery Inspectorate of the Aargau canton. Approvals by the Veterinary Offices of the involved cantons were not required because the fish were caught in a commercial fishing program.

Data availability

Data will be deposited on the Dryad depository upon acceptance of the manuscript.

Funding

The project was supported by the Swiss National Science Foundation (31003A_182265), the *Swisslos-Fonds* of the Aargau canton and Agence Nationale de la Recherche (ANR-13-ISV7-0005)

Acknowledgments

We thank R. Stadelmann for catching the fish and D. Bittner for permits, J.Beninde, N. Caloz, I. Castro, G. Fabre, L. Garaud, A. Jimenez, E. Longange, L. Marquez da Cunha, K. Mobley, D. Nusbaumer, G. Perroud, E. Seddon, N. Sironi and C. Vincent for assistance on the field, and D.L. Jeffries for discussion.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403-410.

Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Ayllon F, Solberg MF, Besnier F, Fjelldal PG, Hansen TJ, Wargelius A, Edvardsen RB, Glover KA. 2020. Autosomal sdY pseudogenes explain discordances between phenotypic sex and DNA marker for Sex identification in Atlantic salmon. Frontiers in Genetics 11:544207.

Bachtrog D, Mank JE, Peichel CL, Kirkpatrick M, Otto SP, Ashman T-L, Hahn MW, Kitano J, Mayrose I, Ming R. 2014. Sex determination: Why so many ways of doing it? PLoS Biology 12:e1001899.

Bertho S, Herpin A, Branthonne A, Jouanno E, Yano A, Nicol B, Muller T, Pannetier M, Pailhoux E, Miwa M, et al. 2018. The unusual rainbow trout sex determination gene hijacked the canonical vertebrate gonadal differentiation pathway. Proceedings of the National Academy of Sciences of the United States of America 115:12781-12786.

Beukeboom LW, Perrin N. 2014. The evolution of sex determination. New York, USA: Oxford University Press.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120.

Brelsford A, Dufresnes C, Perrin N. 2016. High-density sex-specific linkage maps of a European tree frog (*Hyla arborea*) identify the sex chromosome without information on offspring sex. Heredity 116:177-181.

Brown MS, Evans BS, Afonso LOB. 2020. Discordance for genotypic sex in phenotypic female Atlantic salmon (*Salmo salar*) is related to a reduced sdY copy number Scientific Reports 10:9651.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. 2013. Stacks: An analysis tool set for population genomics. Molecular Ecology 22:3124-3140.

Cavileer TD, Hunter SS, Olsen J, Wenburg J, Nagler JJ. 2015. A sex-determining gene (*sdY*) assay shows discordance between phenotypic and genotypic sex in wild populations of chinook salmon. Transactions of the American Fisheries Society 144:423-430.

Devlin RH, Nagahama Y. 2002. Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. Aquaculture 208:191-364.

Eisbrenner WS, Botwright N, Cook M, Davidson EA, Dominik S, Elliott NG, Henshall J, Jones SL, Kube PD, Lubieniecki KP, et al. 2014. Evidence for multiple sex-determining loci in Tasmanian Atlantic salmon (*Salmo salar*). Heredity 113:86-92.

Faber-Hammond JJ, Phillips RB, Brown KH. 2015. Comparative analysis of the shared sex-determination region (SDR) among salmonid fishes. Genome Biology and Evolution 7:1972-1987.

Feron R, Pan Q, Wen M, Imarazene B, Jouanno E, Anderson J, Herpin A, Journot L, Parrinello H, Klopp C. 2020. RADSex: a computational workflow to study sex determination using Restriction Site-Associated DNA Sequencing data. Molecular Ecology Resources 1755-0998:13360.

Feron R, Zahm M, Cabau C, Klopp C, Roques C, Bouchez O, Eché C, Valière S, Donnadieu C, Haffray P. 2020. Characterization of a Y-specific duplication/insertion of the anti-Mullerian hormone type II receptor gene based on a chromosome-scale genome assembly of yellow perch, *Perca flavescens*. Molecular Ecology Resources 20:531-543.

Gabian M, Moran P. 2019. A genetic tool for evaluating male-mediated stock introgression in Atlantic salmon. Aquatic Conservation-Marine and Freshwater Ecosystems 29:142-147.

Graves JAM. 2008. Weird animal genomes and the evolution of vertebrate sex and sex chromosomes. Annual Review of Genetics 42:565-586.

Guiguen Y, Bertho S, Herpin A, Fostier A. 2018. Sex determination and sex control in Salmonidae. In: Wang HP, Piferrer F, Chen SL, Shen ZG, editors. Sex Control in Aquaculture: Wiley Online Library. p. 249-280.

Hattori RS, Murai Y, Oura M, Masuda S, Majhi SK, Sakamoto T, Fernandino JI, Somoza GM, Yokota M, Strüssmann CA. 2012. A Y-linked anti-Müllerian hormone duplication takes over a critical role in sex determination. Proceedings of the National Academy of Sciences 109:2955-2959.

Ito M. 2019. Instability of Sex-Determining Systems in Frogs. In: Chen Y-C, editor. Gene Expression and Phenotypic Traits: IntechOpen.

Kamiya T, Kai W, Tasumi S, Oka A, Matsunaga T, Mizuno N, Fujita M, Suetake H, Suzuki S, Hosoya S. 2012. A trans-species missense SNP in Amhr2 is associated with sex determination in the tiger pufferfish, *Takifugu rubripes* (fugu). PLoS Genetics 8:e1002798.

Kikuchi K, Hamaguchi S. 2013. Novel sex-determining genes in fish and sex chromosome evolution. Developmental Dynamics 242:339-353.

Larson WA, McKinney GJ, Seeb JE, Seeb LW. 2016. Identification and characterization of sex-associated Loci in sockeye salmon using genotyping-by-sequencing and comparison with a sex-determining assay based on the sdY gene. Journal of Heredity 107:559-566.

Li H. 2018. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 34:3094-3100.

Li H, Durbin R. 2010. Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics 26:589-595.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078-2079.

Lubieniecki KP, Lin S, Cabana EI, Li JY, Lai YYY, Davidson WS. 2015. Genomic instability of the sex-determining locus in Atlantic salmon (*Salmo salar*). G3-Genes Genomes Genetics 5:2513-2522.

Macqueen DJ, Johnston IA. 2014. A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification. Proceedings of the Royal Society B: Biological Sciences 281:20132881.

Mathews KW, Cavegn M, Zwicky M. 2017. Sexual dimorphism of body size is controlled by dosage of the X-chromosomal gene Myc and by the sex-determining gene tra in Drosophila. Genetics 205:1215-1228.

Myosho T, Otake H, Masuyama H, Matsuda M, Kuroki Y, Fujiyama A, Naruse K, Hamaguchi S, Sakaizumi M. 2012. Tracing the emergence of a novel sex-determining gene in medaka, *Oryzias luzonensis*. Genetics 191:163-170.

Pan QW, Anderson J, Bertho S, Herpin A, Wilson C, Postlethwait JH, Schartl M, Guiguen Y. 2016. Vertebrate sex-determining genes play musical chairs. Comptes Rendus Biologies 339:258-262.

Pan QW, Feron R, Jouanno E, Darras H, Herpin A, Koop B, Rondeau E, Goetz FW, Larson WA, Bernatchez L, et al. 2021. The rise and fall of the ancient northern pike master sex-determining gene. eLife 10:e62858.

Pan QW, Feron R, Yano A, Guyomard R, Jouanno E, Vigouroux E, Wen M, Busnel JM, Bobe J, Concordet JP, et al. 2019. Identification of the master sex determining gene in Northern pike (*Esox lucius*) reveals restricted sex chromosome differentiation. PLoS Genetics 15:1008013.

Parchman TL, Gompert Z, Mudge J, Schilkey FD, Benkman CW, Buerkle CA. 2012. Genome-wide association genetics of an adaptive trait in lodgepole pine. Molecular Ecology 21:2991-3005.

Podlesnykh AV, Brykov VA, Kukhlevsky AD. 2017. Unstable Linkage of Molecular Markers with Sex Determination Gene in Pacific Salmon (*Oncorhynchus spp*.). Journal of Heredity 108:328-333.

Rudolfsen G, Müller R, Urbach D, Wedekind C. 2008. Predicting the mating system from phenotypic correlations between life-history and sperm quality traits in the Alpine whitefish *Coregonus zugensis*. Behavioral Ecology and Sociobiology 62:561-567.

Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischauf A-M, Lovell-Badge R, Goodfellow PN. 1990. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346:240-244.

Smith CA, Roeszler KN, Ohnesorg T, Cummins DM, Farlie PG, Doran TJ, Sinclair AH. 2009. The avian Z-linked gene DMRT1 is required for male sex determination in the chicken. Nature 461:267-271.

Stiglec R, Ezaz T, Graves JM. 2007. A new look at the evolution of avian sex chromosomes. Cytogenetic and Genome Research 117:103-109.

Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, Jordan T, Shakir K, Roazen D, Thibault J. 2013. From FastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. Current Protocols in Bioinformatics 43:11.10. 11-11.10. 33.

Wen M, Feron R, Pan QW, Guguin J, Jouanno E, Herpin A, Klopp C, Cabau C, Zahm M, Parrinello H, et al. 2020. Sex chromosome and sex locus characterization in goldfish, *Carassius auratus* (Linnaeus, 1758). BMC Genomics 21:552.

Woram RA, Gharbi K, Sakamoto T, Hoyheim B, Holm L-E, Naish K, McGowan C, Ferguson MM, Phillips RB, Stein J. 2003. Comparative genome analysis of the primary sex-determining locus in salmonid fishes. Genome research 13:272-280.

Yano A, Guyomard R, Nicol B, Jouanno E, Quillet E, Klopp C, Cabau C, Bouchez O, Fostier A, Guiguen Y. 2012. An immune-related gene evolved into the master sex-determining gene in rainbow trout, *Oncorhynchus mykiss*. Current Biology 22:1423-1428.

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 6:486-496.

Yoshimoto S, Ito M. 2011. A ZZ/ZW-type sex determination in *Xenopus laevis*. The FEBS Journal 278:1020-1026.

Table

Table 1. The mean (±SD) relative depth of coverage for females and males in the 4 exons of the *sdy* region.

Sex	Exon 1	Exon 2	Exon 3	Exon 4
Males	3.60 ± 2.15	3.84 ± 2.15	1.58 ± 1.33	1.56 ± 1.18
Females	0.79 ± 0.70	1.19 ± 1.06	0.13 ± 0.12	0.07 ± 0.08

Figures

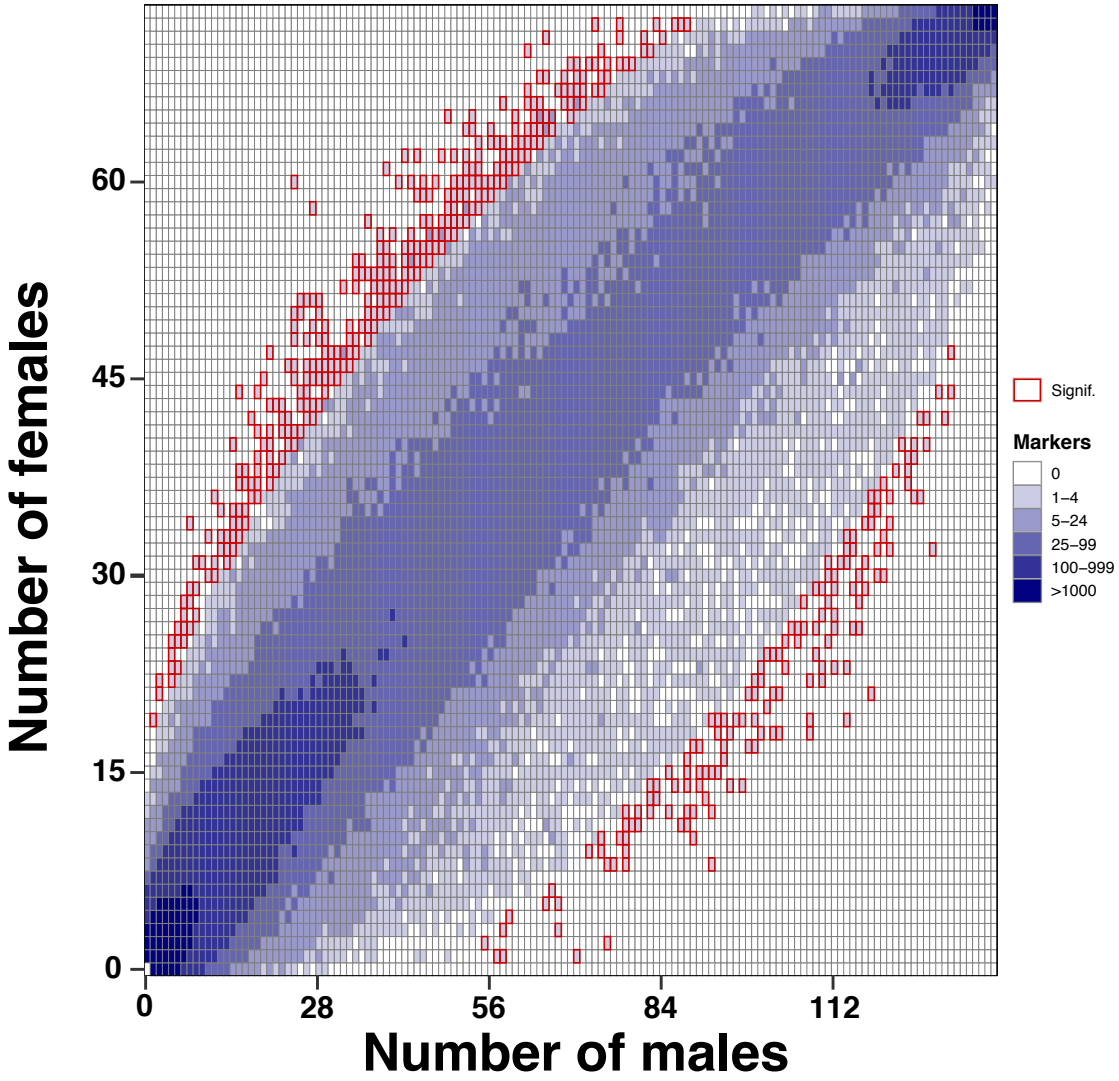


Figure 1. Distribution of ddRAD sequencing markers obtained with RADSex (Feron, Pan, et al. 2020) between females (N = 73) and males (N = 138) whitefish. The color of a tile indicate the number of markers found in a number of males (x axis) and a number of females (y axis). Only markers with a minimum depth of 5 in at least one individual were retained. Markers significantly associated with sex ($p<0.05$, chi-squared test with Bonferroni correction) are highlighted with a red border; in this case, many markers were associated with sex because of the large sample size.

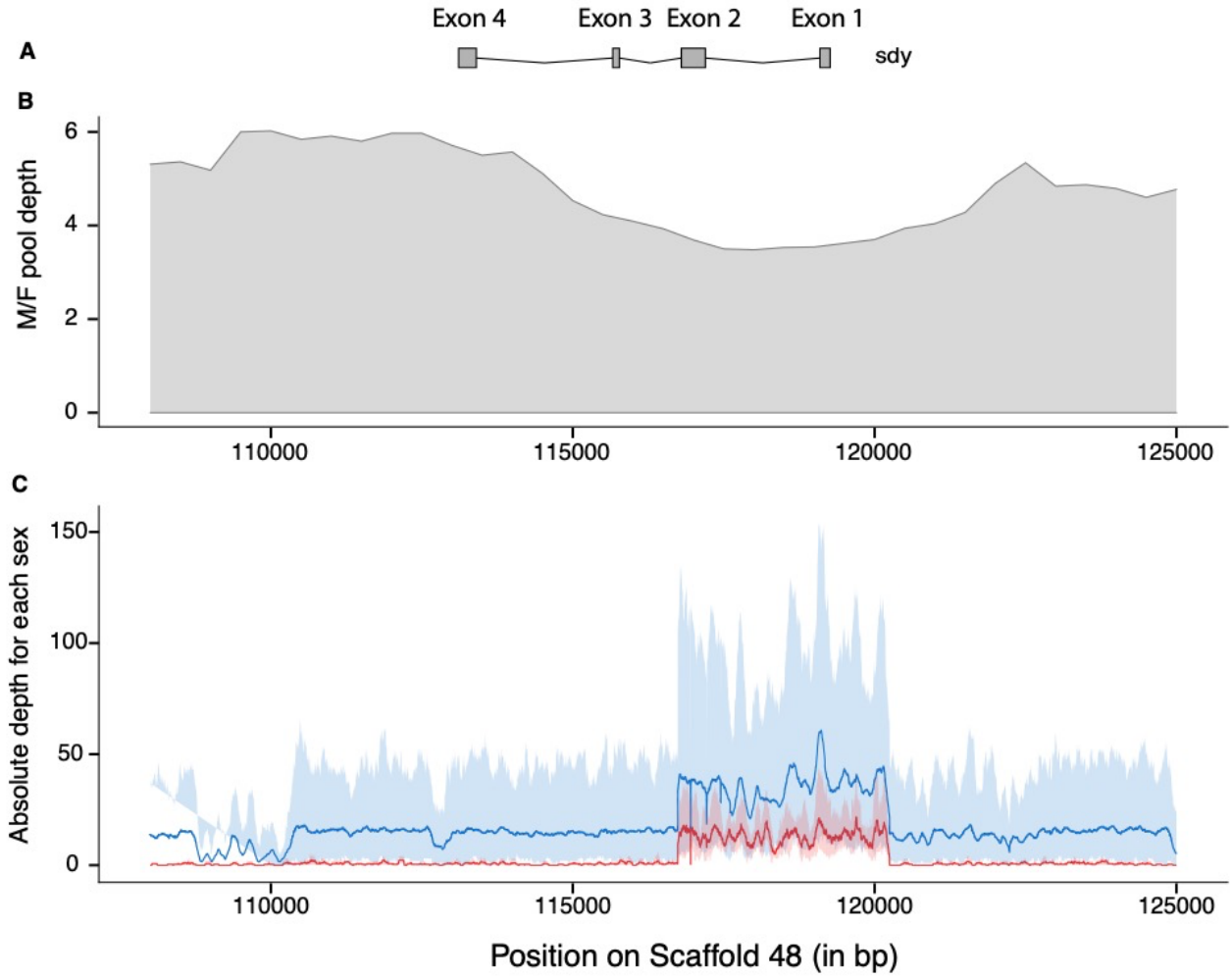


Figure 2. The *sdy* gene model (the region of the genome which transcribe into mRNA) with the corresponding exons. (B) Ratio of absolute depth of coverage between males and females in the *sdy* gene for 30 females and 30 males. (C) Absolute depth of coverage of the *sdy* region on HiC_scaffold_48 for three females (red) and 34 males (blue). The lines represent the mean, and the interval shows minimum and maximum values.

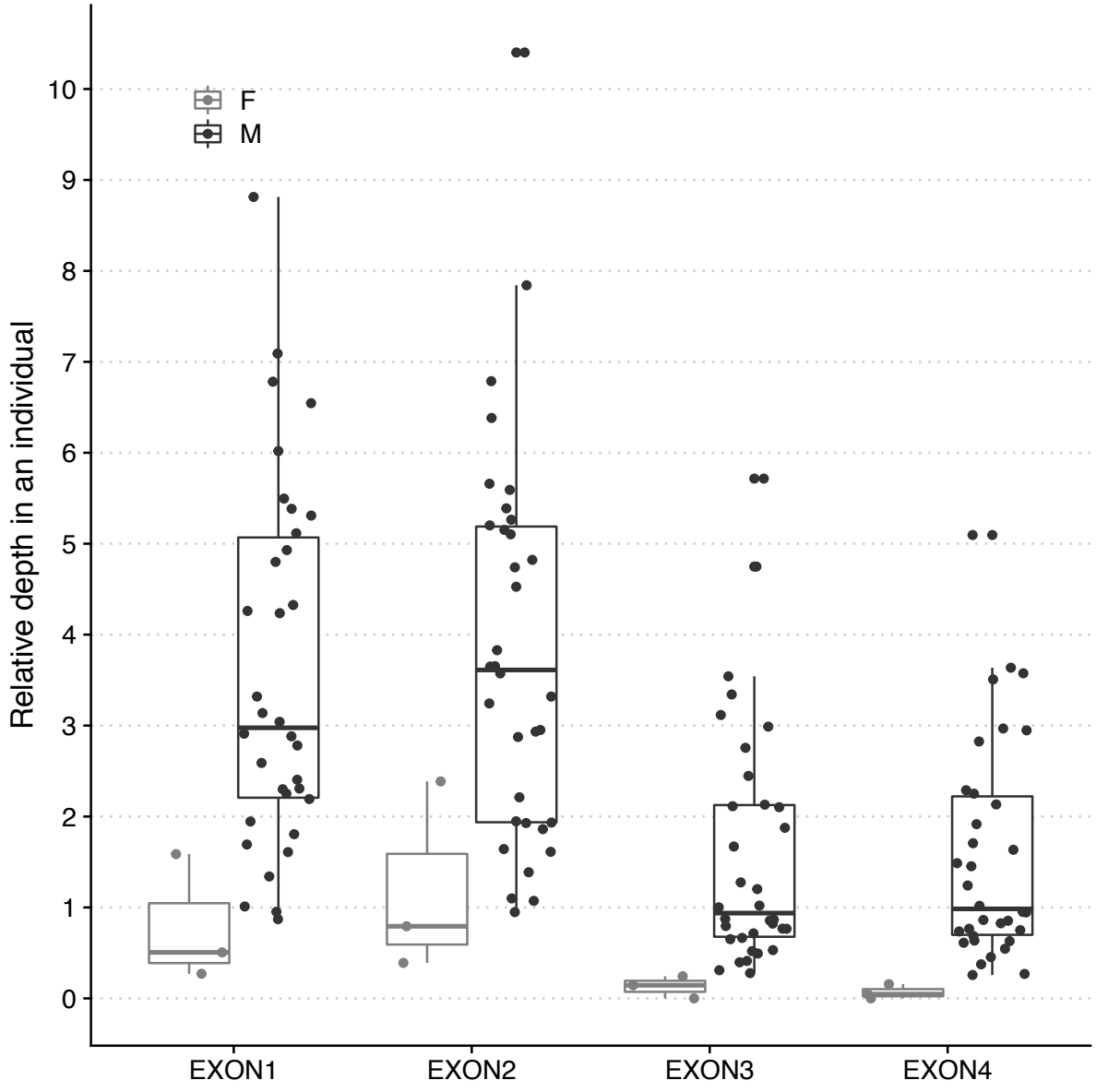


Figure 3. The depth of coverage of the four *sdy* exons normalized (relative) using the average depth of chromosome 1 (HiC_scaffold_1). Boxplot with quartiles, whiskers and data points are plotted.

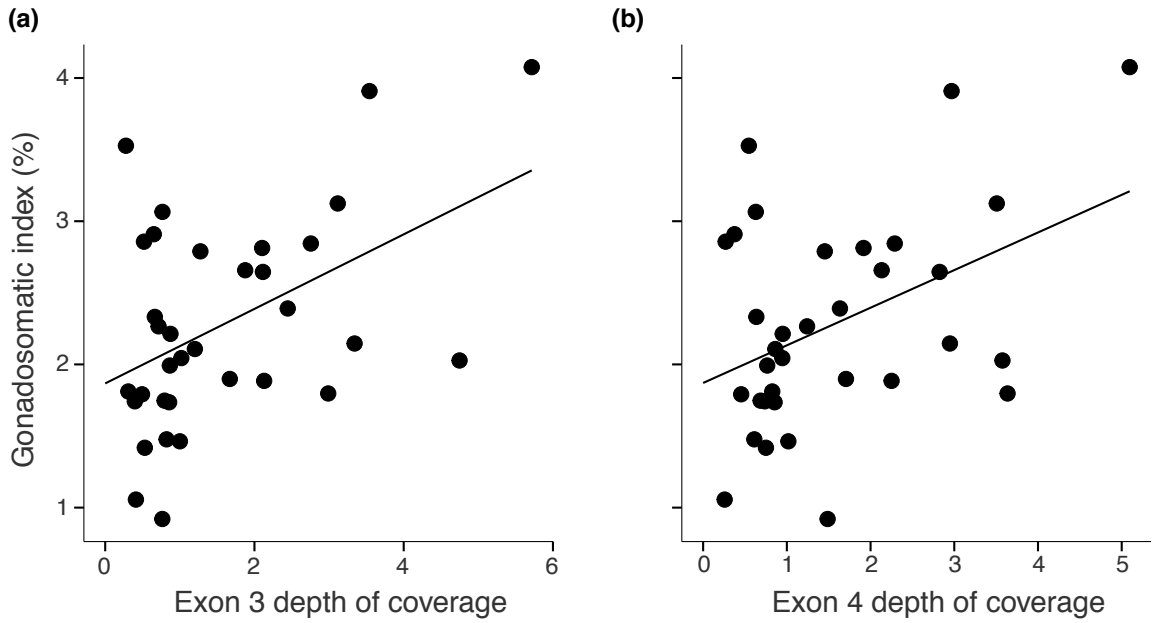


Figure 4. The significant correlation between males gonadosomatic index (GSI) and the relative depth of coverage of (a) exon 3 and (b) exon 4 of the *sdy*. The lines give the regressions. See text for statistics.

Chapter 7 - Heritability of inbreeding in a large whitefish population

Christian de Guttry & Claus Wedekind*

[1] Department of Ecology & Evolution, University of Lausanne, Lausanne, Switzerland

* correspondence: claus.wedekind@unil.ch

Chapter status: unpublished

Author contributions

CdG and CW planned the study and conducted the field work with the help of further members of the group. CdG took the tissue samples, extracted the DNA, prepared the ddRADseq libraries, and processed and analyzed the genomic data. CdG and CW did the statistical analyses and wrote the manuscript.

Abstract

Individual inbreeding coefficients give the probability of two alleles at any locus being identical by decent from common ancestors. These coefficients are often assumed to show no significant heritability in large natural populations. We used samples of a large population of whitefish to quantify the link between inbreeding coefficients of parents and their progeny. Inbreeding coefficients of in total 201 parents were determined based on 18,380 SNPs. The expected mean inbreeding coefficients of their expected progeny were calculating from the expected outcome of multiple random crosses. We found that such a mating scenario would reduce the average inbreeding coefficient of the population. However, the expected inbreeding coefficients in the progeny would be significantly linked to the parental inbreeding coefficients, i.e. inbreeding coefficients showed significant heritability under random mating despite a large census size (that is suggested by the average fishing yield of around 49,000 adults per year over the last decade).

Keywords: Inbreeding coefficient, kinship, ddRAD sequencing, heritability

Introduction

Mating is rarely random in nature, and there are many forms of mate preferences (Andersson 1994). Among the most common ones is inbreeding avoidance, i.e. avoiding to mate with a close relative to avoid high inbreeding coefficients and hence inbreeding depression in the common offspring (recent examples include Hu, et al. 2017; Shirane, et al. 2019; Leedale, et al. 2020; Schultz, et al. 2020). There are cases where inbreeding avoidance could not be found, but such a lack is usually seen as surprising (e.g. Billing, et al. 2012; Vuarin, et al. 2018; Zhou, et al. 2020). Possible mechanisms of inbreeding avoidance include, for example, the so-called "Westermarck effect" (Rantala and Marcinkowska 2011) or preferences based on odors that reveal information about the major histocompatibility complex (MHC), a set of immune genes that can be useful markers of kin in small or structured populations (Ruff, et al. 2012). In large populations, inbreeding coefficients themselves are often assumed to be non-heritable, for example, when induced outbreeding is used to estimate a population's mean inbreeding from hybrid vigor (Saccheri, et al. 1998; Clark, et al. 2013). However, inbreeding coefficients can show significant heritability in small and structured populations (Neff and Pitcher 2008; Nietlisbach, et al. 2016). This led Neff and Pitcher (2008) to suggest that the heritability of inbreeding coefficients could solve the so-called "lek paradox" (Kirkpatrick and Ryan 1991).

In lekking species, males typically aggregate at breeding locations and are then visited by females who prefer males with elaborate sexual displays. In these species, males usually provide no parental care, but females may potentially receive "good genes" from well-ornamented males if the ornament reveals health and vigor (Johnstone 1997). However, natural and sexual selection is expected to deplete additive genetic variance for fitness and hence the benefits of female choice. Various hypotheses have been suggested to possibly solve this paradox, including mate choice for non-additive genetic benefits (Neff and Pitcher 2005; Reid 2007) that include inbreeding coefficients (Neff and Pitcher 2008).

In natural populations, the realized heritability of inbreeding coefficients is expected to depend on the mechanisms and the strength of sexual selection. In species with preferences for condition-dependent sexual ornaments, as may typically be the case in lek-breeding species (Andersson 1994; Johnstone 1997), the sexual ornamentation is likely to be affected by inbreeding depression and would hence be expected to reveal individual inbreeding coefficients. In order to better understand the relevance of inbreeding for solving the so-called "lek paradox", it is necessary to quantify the heritability of inbreeding coefficients in natural populations under different mating scenarios that include random mating.

Here we use fishery monitoring data to describe the size and fluctuations of a whitefish population (*Coregonus suidteri*) of a typical lake of the pre-Alpine region. We then use RADseq genotypes of a large number of males and females of that population that had been sampled over the course of one breeding season, and whose gametes were used for block-wise full-factorial breeding in the context of other studies (chapters 1, 4 and 5 of the present thesis), to describe the variance in individual inbreeding coefficients and to test whether expected average inbreeding coefficients of offspring correlate with each of the parental inbreeding coefficients, i.e. whether inbreeding coefficients show significant heritability under random mating.

Material and Methods

Fishery statistics and yearly measurements of total phosphorus concentration of the water of Lake Hallwil (Switzerland) were provided from the administration of the Aargau canton (Environment Department). Here they are plotted to illustrate the development of the total yearly fishery catches (as an indicator of population size) relative to cultural eutrophication and re-oligotrophication of the lake.

During spawning season between December 2016 and January 2017, a total of 138 adult male and 73 adult female whitefish had been sampled at 3 different times (Dec 22nd, Jan 9th, and Jan 11th) from multiple places along the western half of the lake. Their gametes were collected for block-wise *in vitro* fertilization experiments in the course of studies on the evolution of female ornaments (chapter 1 of this thesis), the evolutionary potential of adaptation to changes in pH (chapter 4 of this thesis), and the significance of egg size for the virulence of a pathogen (chapter 5 of this thesis). The breeding blocks varied in size, but each female was mated with at least 10 males to produce at least 10 different maternal sib groups, and each male was mated with at least 5 females to produce at least 5 different paternal sib groups.

Tissue samples were taken from anal fins and stored in 70% ethanol until further use. Genomic DNA was extracted from the anal fin using the DNAeasy Blood & Tissue kit (Quiagen, Venlo, Netherland). DNA integrity and concentration were measured respectively on agarose gel and with Qubit 2.0. The samples were then normalized to a concentration of 20 ng/µl. Libraries with unique 5bp ECORI barcodes for each individuum were prepared based on the protocol of Brelsford et al. (2016) as follow: 120 ng of DNA of each individual were digested with the enzymes EcoRI-HF and MspI (New England Biolab). After PCR amplification, fragment between 400-550bp were selected. The single-end genotyping was done with an Illumina Hiseq 2500 at the Lausanne Genomic Technologies Facility (University of Lausanne, Switzerland). Quality control was done on the fastq files using FASTQC v0.11.7 (Andrews 2010). Given the insufficient per-base quality (< 20 Phred) between 95bp

and 125 bp reads were trimmed to 90bp using Trimmomatic (Bolger, et al. 2014). After the demultiplexing performed with *process_radtags* in Stacks version 1.48 (Catchen, et al. 2013), reads were mapped using BWA with the MEM algorithm (Li and Durbin 2010) to the whitefish genome (De-Kayne, et al. 2020). The retained reads were then processed with the Stacks reference-aligned pipeline (Stacks v. 1.48) as follow: Pstacks was done using the bounded SNP model with the default parameters to possibly distinguish between actual heterozygous sites and genotyping errors. The minimum depth to create a stack was set at 5 (*-m* 5). The catalogue of loci was built using Cstacks with 2 mismatches (*-n* 2) allowed between loci. Using Stacks' Populations, loci were filtered for a minimum depth of 10 (*-m* 10), presence in 80% of the individuals and a heterozygosity filter at 0.5 to avoid possible genotyping errors (Hohenlohe, et al. 2011) and to remove heterozygous loci resulting from a possible hidden paralogy. The VCF obtained at the end of the Stacks pipeline has been filtered using *VCFTOOLS* v0.1.15 (Danecek, et al. 2011). Here, loci were filtered for a minimum mean depth of 15 X in order to decrease type I errors and for a maximum mean depth of 50 X to discard possible paralogs still retained after the previous steps. The latter was applied after a visual inspection of the data where the distribution of coverage among the SNPs was plotted. Only bi-allelic loci and loci that significantly deviate from Hardy-Weinberg equilibrium with a threshold of $P < 0.05$ were retained. No filter for minor allele frequencies was applied. Consider the entire allele frequency spectrum, including rare variants, is suggested for more accurate inbreeding coefficient estimates (Goudet, et al. 2018). After filtering, a total of 18,380 SNPs had been kept with a mean presence of 87% across 201 individuals and a mean coverage of 29.3 X. Three females (4.1%) and 7 males (5.1%) were excluded from further analysis due to low genotyping rate caused by low DNA quality or technical artefacts during library preparation or sequencing (O'Leary, et al. 2018).

The kinship, the expected average inbreeding coefficient of the embryos ($F_{\beta.\text{offspring}}$), per full-sib family that had been produced in the block-wise breeding, were estimated using the genotypes of respective males and females and the function *beta.dosage* from the package *Hierfstat* (Goudet 2005). This function allows to estimate kinship and F_β based on small sample size (Goudet, et al. 2018). Compared to the other estimators, no need of prior information about the allele frequency in the population is needed. Females inbreeding coefficient ($F_{\beta.dam}$) and males inbreeding coefficient ($F_{\beta.sire}$) were extracted from the diagonal of the kinship matrix obtained using *beta.dosage* (dam and sire indicate respectively maternal and paternal identity). The mean expected kinship per individual was calculated using all the possible mates of the experimental design.

Results

Yearly fishing yield varied from 0.1 t whitefish in 1969 to 76.9 t whitefish in 1997 (Fig. 1). Over the last decade, fishing yield was on average 9.8 t ± 2.1 (SD) that corresponds to an average of about 49,000 whitefish/year (± 10,400; assuming an average weight of 200 g per fish). Figure 1 also shows the yearly average phosphorus content in the lake water and the period of artificial oxygenation of the lake to illustrate fishery yield relative to re-oligotrophication and oxygenation.

The average (± SD) F_β of the 201 adults that could be genotyped was 0.005 ± 0.037 and ranged from -0.158 to 0.144 (Fig. 2). Two females and 2 males had F_β that were > 3 SD smaller than the overall mean, and 1 male had an F_β that was > 3 SD larger than the overall mean (marked in Fig. 2). These 5 fish (2.5% of the total sample) were therefore considered outliers and removed from further analyses. There was no sex difference in mean F_β ($t_{194} = 0.60$, $p = 0.55$) nor the variance of F_β (Brown-Forsythe's $F_{199} = 0.15$, $p = 0.70$). However, fish caught at the beginning of the spawning season had on average higher F_β than fish caught at one of the two sampling days towards the end of the spawning season ($F_{2,198} = 10.2$, $p < 0.0001$; Fig. 2).

The mating regime that was used for the calculations of average $F_{\beta.\text{offspring}}$ would reduce the mean inbreeding coefficient of the population (paired t-test between $F_{\beta.\text{sire}}$ and $F_{\beta.\text{offspring}}$: $t_{127} = -4.4$, $p < 0.0001$; between $F_{\beta.dam}$ and $F_{\beta.\text{offspring}}$: $t_{67} = -2.3$, $p = 0.026$). The expected average $F_{\beta.\text{offspring}}$ could be predicted from both, $F_{\beta.dam}$ and $F_{\beta.\text{sire}}$ (Fig. 3a, b). The amount of variance in the expected $F_{\beta.\text{offspring}}$ that was explained by $F_{\beta.dam}$ and $F_{\beta.\text{sire}}$ was 20.7% and 12.1%, respectively. This link did not seem to differ between sampling dates (multiple regression on $F_{\beta.\text{offspring}}$; effect of sampling date: $F_2 = 0.9$, $p = 0.42$); effect of parental F_β: $F = 27.0$, $p < 0.0001$; interaction between sampling date and parental F_β: $F = 1.1$, $p = 0.32$).

Discussion

The yearly fishing yields reveal strong fluctuations of population size over time. Much of this variation seems to be due to the cultural eutrophication that was most severe during the 1970s and 1980s. The local authorities reacted to this ecological crisis with various measures that also included artificial oxygenation of the lake, starting in 1985 and still ongoing. These measures led to a marked re-oligotrophication and seemed to first create strong positive effects on the whitefish population (see also Enz 2000).

Whether and to what extend the eutrophication crisis caused a genetic bottleneck in the whitefish population remains unclear, because fishery statistics cannot provide an exact representation of the population size over time. However, yearly yield was never below 0.1 t that corresponds to about

500 adult fish. Wedekind et al. (2001) sampled adults from this population in 1999, used their gametes in a full-factorial breeding experiment, and found significant genetic variation of fitness-relevant traits. In parallel, Binz et al. (2001) used the same sample of adults to study the diversity on three different exons of the MHC. They found this diversity to be very high, with up to 20 different alleles per locus in only 15 individuals. Wedekind et al. (2004) then focused on the variation in MHC genotypes within several full-sib families and found it to affect embryo susceptibility to a bacterial infection. Taken together, these observations suggest that the population did not experience a significant genetic bottleneck during the eutrophication crisis. Over the last 10 years, phosphorus concentrations varied between 10-20 mg/m^3 while fishery yield varied around 49,000 adults per year, i.e. more recent genetic bottlenecks seem unlikely.

Interestingly, mean F_β varied over the sampling dates within one spawning season, with individuals sampled early from the spawning place being on average more inbred than individuals sampled towards the end of the spawning season. This apparent season effect remains unexplained here but could potentially reveal condition-depend reproductive strategies, with low-quality (more inbred) individuals spawning earlier in the season that high quality individuals, for example, to avoid intra-sexual competition during optimal spawning times. If so, such condition-dependent strategies seem to be similar in males and females.

The fact that mean F_β varied over sampling dates needs to be considered when calculating the expected mean $F_{\beta.\text{offspring}}$ from random mating with many mates, because late spawning individuals may not be available to early spawning ones and vice versa. When we did this and then correlated the maternal and paternal F_β to the expected mean $F_{\beta.\text{offspring}}$, we found that parental F_β explained much of the variance in the expected mean $F_{\beta.\text{offspring}}$, i.e. inbreeding coefficients showed significant heritability under random mating. These heritabilities could be observed on both, the maternal and the paternal sides.

It remains to be shown whether and to what extent correlations between paternal F_β are linked to the fluctuations in population size over the previous generations. Moreover, to the best of our knowledge it is currently not known whether there is inbreeding avoidance on the natural spawning place. If there were inbreeding avoidance, mate choice would be expected to reduce the heritabilities of F_β. Supportive breeding in hatcheries is largely based on random mating would hence cancel such effects of mate choice. Post-mating sexual selection that is still allowed for in the routine protocols of supportive breeding of whitefish (Wedekind, et al. 2007) may not solve the problem: Wedekind et al. (2004) searched for evidence of non-random gamete fusion in whitefish and found none, i.e. there is currently no evidence of inbreeding avoidance at the level of gametes in whitefish. Random mating in

hatcheries may therefore increase the heritability of inbreeding coefficients as compared to natural mating. Such negative effects of hatchery breeding would be additional to any potential negative effects of supportive breeding on the genetically effective population size due to, for example, an induced increase in the variance of reproductive success within individuals of a population (Ryman, et al. 1995).

Ethics

The sampling of the fish was approved by the Fishery Inspectorate of the Aargau canton.

Data availability

Data will be deposited on the dryad depository upon acceptance of the manuscript.

Funding

The project was supported by the lottery funds of the Aargau canton and the Swiss National Science Foundation (31003A_182265).

Acknowledgements

We thank R. Stadelmann for catching the fish, J. Beninde, I. Castro, G. Fabre, L. Garaud, L. Marques da Cunha, K. Mobley, D. Nusbaumer, E. Seddon, R. Sermier, and C. Vincent for assistance in the field and in the laboratory, DL Jeffries for comments, D. Bittner for permits, and all the above for discussion.

References

Andersson MB. 1994. Sexual selection. Princeton, New Jersey: Princeton University Press.

Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Billing AM, Lee AM, Skjelseth S, Borg AA, Hale MC, Slate J, Parn H, Ringsby TH, Saether BE, Jensen H. 2012. Evidence of inbreeding depression but not inbreeding avoidance in a natural house sparrow population. Molecular Ecology 21:1487-1499.

Binz T, Largiadèr C, Müller R, Wedekind C. 2001. Sequence diversity of MHC genes in lake whitefish. Journal of Fish Biology 58:359-373.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120.

Brelsford A, Dufresnes C, Perrin N. 2016. High-density sex-specific linkage maps of a European tree frog (*Hyla arborea*) identify the sex chromosome without information on offspring sex. Heredity 116:177-181.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. 2013. Stacks: An analysis tool set for population genomics. Molecular Ecology 22:3124-3140.

Clark ES, Stelkens RB, Wedekind C. 2013. Parental influences on pathogen resistance in brown trout embryos and effects of outcrossing within a river network. PLoS One 8:e57832.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, et al. 2011. The variant call format and VCFtools. Bioinformatics 27:2156-2158.

De-Kayne R, Zoller S, Feulner PGD. 2020. A de novo chromosome-level genome assembly of *Coregonus* sp. "*Balchen*": One representative of the Swiss Alpine whitefish radiation. Molecular Ecology Resources 20:1093-1109.

Enz CA. 2000. Population dynamics of whitefish (*Coregonus suidteri* Fatio) in artificially oxygenated Lake Hallwil, with special emphasison larval mortality and sustainable management. Zürich, Switzerland: ETH.

Goudet J. 2005. HIERFSTAT, a package for R to compute and test hierarchical F-statistics. Molecular Ecology Notes 5:184-186.

Goudet J, Kay T, Weir BS. 2018. How to estimate kinship. Molecular Ecology 27:4121-4135.

Hohenlohe PA, Amish SJ, Catchen JM, Allendorf FW, Luikart G. 2011. Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. Molecular Ecology Resources 11:117-122.

Hu YB, Nie YG, Wei W, Ma TX, Van Horn R, Zheng XG, Swaisgood RR, Zhou ZX, Zhou WL, Yan L, et al. 2017. Inbreeding and inbreeding avoidance in wild giant pandas. Molecular Ecology 26:5793-5806.

Johnstone RA. 1997. The evolution of animal signals. In: Krebs JR, Davies NB, editors. Behavioural ecology: an evolutionary approach. Fourth edition. p. 155-178.

Kirkpatrick M, Ryan MJ. 1991. The evolution of mating preferences and the paradox of the lek. Nature 350:33-38.

Leedale AE, Simeoni M, Sharp SP, Green JP, Slate J, Lachlan RF, Robinson EJH, Hatchwell B. 2020. Cost, risk, and avoidance of inbreeding in a cooperatively breeding bird. Proceedings of the National Academy of Sciences of the United States of America 117:15724-15730.

Li H, Durbin R. 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 26:589-595.

Neff BD, Pitcher TE. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Molecular Ecology 14:19-38.

Neff BD, Pitcher TE. 2008. Mate choice for non-additive genetic benefits: A resolution to the lek paradox. Journal of Theoretical Biology 254:147-155.

Nietlisbach P, Keller LF, Postma E. 2016. Genetic variance components and heritability of multiallelic heterozygosity under inbreeding. Heredity 116:1-11.

O'Leary SJ, Puritz JB, Willis SC, Hollenbeck CM, Portnoy DS. 2018. These aren't the loci you'e looking for: Principles of effective SNP filtering for molecular ecologists. Molecular Ecology 27:3193 - 3206.

Rantala MJ, Marcinkowska UM. 2011. The role of sexual imprinting and the Westermarck effect in mate choice in humans. Behavioral Ecology and Sociobiology 65:859-873.

Reid JM. 2007. Secondary sexual ornamentation and non-additive genetic benefits of female mate choice. Proceedings of the Royal Society B-Biological Sciences 274:1395-1402.

Ruff JS, Nelson AC, Kubinak JL, Potts WK. 2012. MHC signaling during social communication. Advances in Experimental Medicine and Biology 738:290-313.

Ryman N, Jorde PE, Laikre L. 1995. Supportive breeding and variance effective population size. Conservation Biology 9:1619-1628.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392:491-494.

Schultz AJ, Cristescu RH, Hanger J, Loader J, de Villiers D, Frere CH. 2020. Inbreeding and disease avoidance in a free-ranging koala population. Molecular Ecology 29:2416-2430.

Shirane Y, Shimozuru M, Yamanaka M, Tsuruga H, Nakanishi M, Ishinazaka T, Nose T, Kasai S, Shirayanagi M, Masuda Y, et al. 2019. Sex-biased dispersal and inbreeding avoidance in Hokkaido brown bears. Journal of Mammalogy 100:1317-1326.

Vuarin P, Bouchard A, Lesobre L, Leveque G, Chalah T, Jalme MS, Lacroix F, Hingrat Y, Sorci G. 2018. No evidence for prezygotic postcopulatory avoidance of kin despite high inbreeding depression. Molecular Ecology 27:5252-5262.

Wedekind C, Müller R, Spicher H. 2001. Potential genetic benefits of mate selection in whitefish. Journal of Evolutionary Biology 14:980-986.

Wedekind C, Rudolfsen G, Jacob A, Urbach D, Müller R. 2007. The genetic consequences of hatchery-induced sperm competition in a salmonid. Biological Conservation 137:180-188.

Wedekind C, Walker M, Portmann J, Cenni B, Müller R, Binz T. 2004. MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in whitefish. Journal of Evolutionary Biology 17:11-18.

Zhou P, He XZ, Chen C, Wang Q. 2020. No evidence for inbreeding depression and inbreeding avoidance in a haplodiploid mite *Tetranychus ludeni* Zacher. Systematic and Applied Acarology 25:1723-1728.

Figures

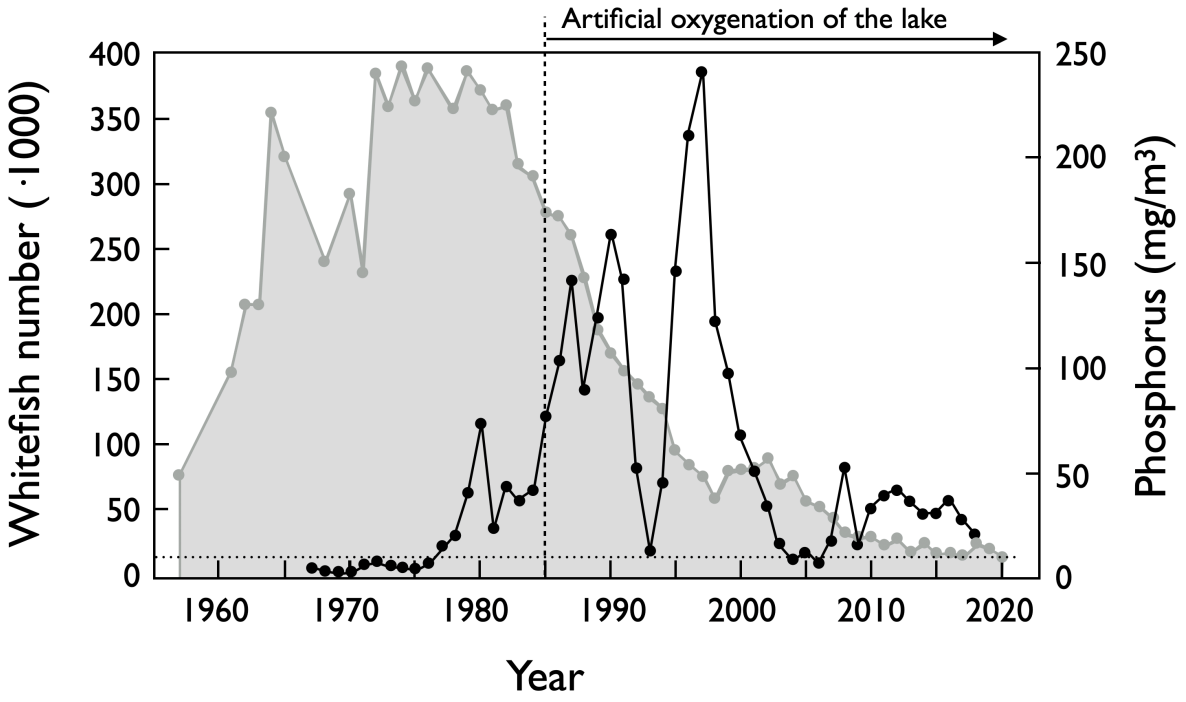


Figure 1. Number of whitefish caught each year class (black symbols), and phosphorus content in the lake water (grey symbols). Artificial oxygenation of the lake started in 1985 with the aim of a phosphorus concentration of < 10 mg/m3 (dotted line).

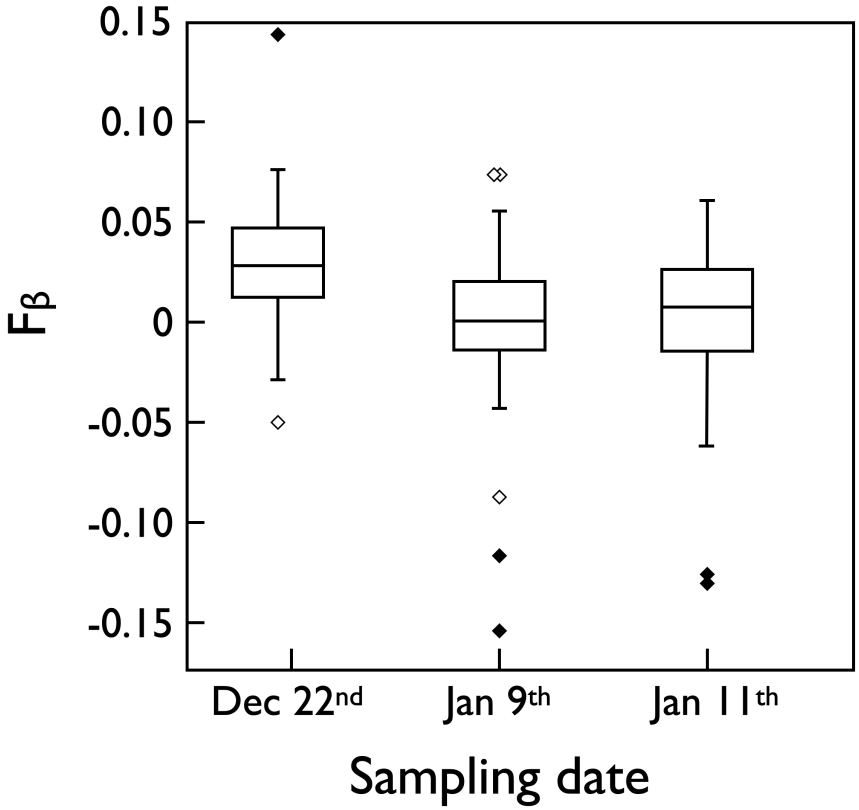


Figure 2. Inbreeding coefficients F_β of whitefish caught early (Dec 22nd) or late (Jan 9th and 11th) in the spawning season. Tukey outlier boxplots with quartiles, whiskers, and outliers. Extreme values (> 3 SD away from the total mean) are indicated with filled symbols. See text for statistics.

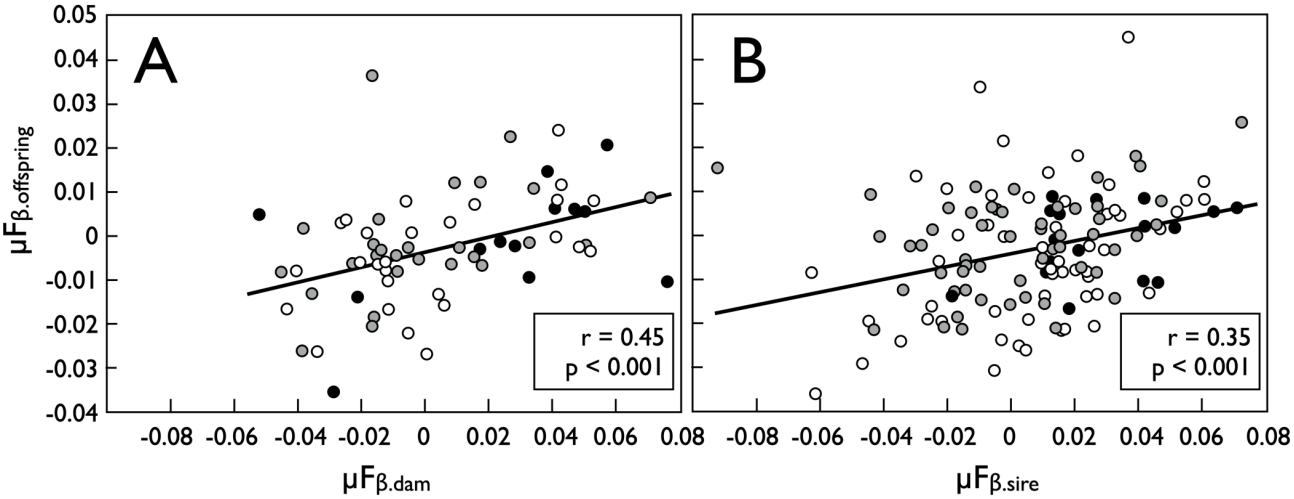


Figure 3. Expected average inbreeding coefficient of embryos ($\mu F_{\beta.offspring}$) predicted by (A) their mother's inbreeding coefficient ($F_{\beta.dam}$; r = 0.44, n = 71, p = 0.002) and (B) their father's inbreeding coefficient ($F_{\beta.sire}$; r = 0.32, n = 135, p < 0.001) from the first (black), second (grey), and third (open symbols) sampling day. The lines give the regressions.

General discussion

The interest in maternal effects has increased exponentially over the last decades. Maternal effects can shape offspring phenotypes (Einum and Fleming 1999), behavior (Leblanc, et al. 2011) and also determine life history strategies (Cogliati, et al. 2018). Maternal effects are therefore a strong determinant in evolutionary trajectories but are themselves influenced by other population processes such as inbreeding. Inbreeding has always been a fundamental field of study for evolutionary biologists (Charlesworth and Charlesworth 1987; Keller and Waller 2002). Recently with the advent of the sixth mass extinction, species worldwide are experiencing loss of genetic diversity (Lehnert, et al. 2019; Manel, et al. 2020). Consequently, studies that seek to understand the effect of inbreeding on wild population are growing. The main goal of this thesis was to experimentally investigate how maternal effects and inbreeding influence offspring performances and population dynamics. To address these questions we used the whitefish population from Lake Hallwil. Approximately 250 adults and 20000 larvae were used in total. These were divided into the 7 main chapters and allowed to reveal interesting aspects that had been difficult for evolutionary biologists to explain so far.

The effect of inbreeding on females reproductive traits

Through the first two chapters of this thesis, we shown evidence that inbreeding influences females' reproductive traits. Individuals with a higher inbreeding coefficient produced fewer eggs (chapter 1) and have a larger variation in the size of the eggs produced (chapter 2) compare to those with a lower inbreeding coefficient. The first result is not a novelty since a similar trend was reported for rainbow trout (*Oncorhynchus mykiss*) (Su, et al. 1996) and for Coho salmon (*Oncorhynchus kisutch*) (Gallardo, et al. 2004) using pedigree to estimate individual *F*. However, thanks to the use of genomics, we were able to estimate the effect of inbreeding in a wild population without the use of a pedigree. It is interesting to observe how even in the absence of extreme values of consanguinity, inbreeding depression is present and negatively affects life-history traits of the individuals. This result suggests that the ability to detect inbreeding depression on polygenic traits depends on the nature and number of genetic markers used (David 1998; Kardos, et al. 2015; Galla, et al. 2020). The second result was rather unexpected. Among females of the same population, consistency in offspring size is predicted to optimize offspring survival and performance (Smith and Fretwell 1974). Despite this, a large variation in offspring size within population and within brood is commonly reported (Einum and Fleming 2004; Marshall, et al. 2008). Our result highlights that in addition to environmental (Philippi and Seger 1989) and physiological factors (Burton, et al. 2013) the genetics of the female should also be considered when trying to predict the evolution of offspring size within a brood or a population.

We suggested that a high level of inbreeding reduces a female ability to interpret environmental stimuli inducing the production of different-sized eggs to maximize its fitness, mechanism usually adopted in an unpredictable environment (Crean and Marshall 2009; Kvalnes, et al. 2018; Song, et al. 2020).

In addition to traits directly related to reproduction, we found that inbreeding also negatively influences secondary sexual characteristics in females (chapter 1). Females with a lower inbreeding coefficient produce larger breeding tubercles (sexual ornamentation). Quantitative genetic studies have already associated female breeding tubercles to signal of genetic quality (Kekäläinen, et al. 2010), however which kind of quality they express was still unknown. We have evidence that breeding tubercles size depends largely on the inbreeding status (or heterozygosity) of the mother and discussed the findings in the context of sexual selection in a mutually ornamented species. Our results shows how inbreeding affects the dynamics of sexual selection and might have driven the evolution of secondary sexual characters in female whitefish. We acknowledge that observational studies are needed to understand whether the preference of males toward females with larger breeding tubercles and vice versa. However, ours is a starting point that suggests that the inbreeding level of females is expressed through their secondary sexual characters. Our findings should give a new incentive to the investigation of theories of sexual selection on females, since several dynamics have already been shown to be similar between sexes (Hare and Simmons 2019).

The intergenerational effect of maternal inbreeding on embryos

In chapters 1 and 2 we found that the inbreeding coefficient of a female influences the growth of the embryos and larvae. To date, few studies investigated the presence of intergenerational effects of inbreeding (Mattey, et al. 2013). In addition, those studies focused on plants (Del Castillo 1998) or species with parental cares (Reid, et al. 2003; Szulkin, et al. 2007). We show evidence that this also occurs in external fertilizer without parental care such as salmonids. We discussed this finding in the context of reduced egg quality produced by more inbred females. In this framework, the maternal inbreeding coefficient becomes to all intents and purposes a maternal effect since its effect on fitness is indirect (Wolf and Wade 2009). The biggest implications of this result are the consequent underestimation of the cost of inbreeding and the attribution of its effects to different factors. This result, together with the finding that, in the population we studied, inbreeding can have a level of heritability not negligible (Chapter 7) shows us that even today, after decades of study on its effects, there are still important aspects and dynamics that deserve to be investigated and understood more thoroughly.

Despite these findings, our approach had its limitations. We worked with a wild species so the size of our samples depended on the catches of the fishermen and on the effort required to create full factorial breed designs. Typically they consist of several families and several members per family. In the first chapter the results were obtained by analyzing 13 females collected at the beginning of the breeding season and a larger sample is needed to confirm our findings. Furthermore, in chapters one and two, we attributed the reduced embryonic growth to the reduced quality of eggs produced by inbred females, but we did not analyze their content. To solve these two problems in December 2020 we went to the field and collected about 100 females at the beginning of the breeding season. Their sexual ornamentations have been analyzed with 3D scanner, their DNA has been collected, the number of eggs produced has been recorded and the content of about 30 eggs per female will be analyzed.

The correlation of maternal traits

In chapter three we demonstrated how female age rather than size determines the size of offspring in early life stages. Theories on the evolution of offspring size have predicted that age and size of the mother may have separate effects on offspring (Hendry, et al. 2001; Kindsvater, et al. 2011). The difficulty is isolating their effects because of their strong correlation i.e. large females are usually older (Marshall, et al. 2010). We were able, through the selectivity of the fishing net, to decrease the variability in body size and obtain females of different age classes. The increase of egg size with age seems to be a common feature of the salmonid family (Jonsson and Jonsson 1999; Johnston, et al. 2012; Johnston 2018). We discussed this result in the light of the terminal investment hypothesis (Williams 1966) and under the light of more recent optimality theories (Kindsvater, et al. 2011). We suggest that females' investment in reproduction is age-dependent in whitefish. However, we only examined two age group in our study. It would be worth investigating whether in species with undetermined growth, senility affects maternal investment in reproduction (Barks and Laird 2020). Our study shows how the influence of a maternal trait on offspring can be easily confounded with the influence of another in species with indetermined growth. Therefore it is necessary, in further experimental studies, to separate not only the effect of age and size but also the condition of the female which has already been shown to have an influence on offspring performances (Rollinson and Rowe 2016).

The importance of egg size larval phenotypic variation

In chapters four and five we investigated various aspects related to one of the most important maternal traits: egg size. We demonstrated how egg size is the major determinant of larval size and yolk sac

volume in whitefish. The observed correlation between egg size and larval size is common among salmonids (Hutchings 1991; Einum and Fleming 1999; Einum 2003; Klemetsen, et al. 2003; Leblanc, et al. 2016). Indeed, salmonids do not provide parental care for their offspring after fertilization, so egg size is a good approximation for the energy the mother has invested in each individual. Consequently, this family has been used as model organism in the main theories of offspring size evolution (Einum 2003; Stearns and Hendry 2004). In our experiments we followed the embryos approximately to the end of endogenous feeding. Despite the fact that maternal effects are strongest in the early stages of life, it has recently been shown that they can also persist over time (Goos, et al. 2019; Reichert, et al. 2020). It would be interesting in future studies to determine whether the effect of egg size also influence phenotypic and life-history traits of individuals later in life. In an ongoing study, to try to elucidate this dynamic in whitefish, we allowed some of the individuals obtained from the families of chapters 2 and 4 to grow in semi-natural conditions for 6 months. Once recaptured, we recorded their phenotypic characteristics and sequenced their DNA using ddRAD sequencing. After assigning them to the corresponding families, we will determine whether individuals generated from females producing larger eggs are still larger six months after hatching. In addition to this experiment, tracking individuals throughout their entire life cycle, might enable to investigate whether the large variability of whitefish ecotypes is determined at this stage of life as it has been observed for other salmonids (Leblanc, et al. 2011; Cogliati, et al. 2018). Tracking females through different reproductive seasons could also confirm the age-dependent investment in reproduction that we suggested in chapter 3.

The size of the eggs appears to be more important than the genetic characteristics of the embryo in determining its size at hatching. We used full factorial breeding design where the eggs of a female were fertilized with gametes from different males of the same population. Therefore, the genetic composition of the offspring varied based on the parental combination. Although males and females were different in their genetic quality, what determined the size of the larvae at hatching, and during endogenous feeding, is not its genetic background of the embryo but the size of the egg where it incubated. This suggest that egg size, as maternal effect, can act as a buffer in the early stages of life, against negative genetic conditions. This effect has already been demonstrated in species with parental care (Pilakouta and Smiseth 2016), but it still lack experimental evidence in species without parental care. Further experiments capable of controlling for maternal environmental effects and for the realized inbreeding of the offspring are needed to test this hypothesis.

Conflicting selection stages in salmonids

Most of the studies that have been conducted on salmonids demonstrated that larger eggs generate larger larvae. In the same environmental conditions, larger larvae have a higher growth rate and survival (Roff 1992; Mousseau and Fox 1998), better swimming performances (Leblanc, et al. 2011) and a better ability to forage for food (Auer, et al. 2018) compared to smaller larvae. In light of this, we expect evolutionary trajectories of offspring size to go toward the production of large offspring that will increase maternal and individual fitness. However, we found that extrinsic factors (chapters 4 and smaller effect in chapter 5) influence large eggs during incubation. For instance, when exposed to an acidic environment during ontogeny, larger eggs suffer stronger selective pressures than smaller eggs. These results were interpreted in the context of the 'bigger is not always better' hypothesis (Krogh 1941; Sargent, et al. 1987). Large eggs are predicted to suffer more stressful condition during incubation than small eggs. This suggest that individuals that will be more competitive at the larval stage due to their size, suffer stronger selective pressures during incubation. Our results therefore confirmed the presence of conflicting selective pressures (Schluter, et al. 1991) acting on offspring size during incubation and post-hatching in whitefish. This result may also help explain the large variability in size within a brood that is often reported. A female produces eggs of different sizes to balance the selective forces present in the two life stages of the offspring. We recognize that we have no behavioral data on larvae after hatching and we assumed that whitefish behave like the other salmonids. Our results are also consistent with theoretical models about the different per-offspring allocation under different environmental conditions (Parker and Begon 1986; Hendry, et al. 2001; Bashey 2008). Those models predict that when the female perceives environmentally stressful conditions egg size is decreased to increase the likelihood of offspring survival. This is a typical case of adaptive maternal effects where the female modify the size of the offspring to increase their and its own fitness (Mousseau and Fox 1998). Nevertheless, in the literature many empirical studies found that larger eggs survive better under stressful conditions than smaller eggs (Johnston and Leggett 2002; Taborsky 2006; Rollinson and Hutchings 2013). We suggest that these results depend largely on the factor generating the selective pressure. A larger egg has a larger exchange surface with the external environment compared to a smaller egg (Einum, et al. 2002; Rombough 2007). This favors large egg in low oxygen conditions (Einum, et al. 2002; Jonsson and Jonsson 2009) but is a disadvantage in presence of acidification or, with less important consequences, in presence of a common pathogen as we observed. Therefore, based on the stressors used in this thesis, a female is predicted to increase its fitness from producing smaller eggs. There are two important points to consider for future research. Firstly, few models on the evolution of offspring size consider the presence of opposing selection

pressures acting during incubation and post-hatching (Hendry, et al. 2001). We suggest that to correctly predict the evolution of offspring size in species with a complex life cycle, the different selection pressures acting on a trait at each life stage must be considered. Secondly, most studies focus on the role of oxygen deficiency as a major factor limiting egg size evolution concluding that the production of bigger eggs results in higher fitness. We suggest that the investigation of the effect of different stress factors present in the water column is important to better understand the evolution of the size of the progeny in aquatic environment. To date, the effect of oxygen deprivation (Einum, et al. 2002) and temperature (Regnier, et al. 2013) have been investigated. We will contribute to the literature by adding the effect of acidification. A step forward in understanding the natural dynamics could also be given by the study of the effects of their interaction.

Conclusions

This thesis covered some of the most important evolutionary dynamics present on wild populations such as maternal effects, inbreeding and sexual selection. We were able to determine the influence of maternal effects through the use of quantitative genetics and in particular full factorial breeding designs. The reproductive biology of the salmonids allowed the crossing of multiple females and multiple males in order to recognize maternal and paternal contribution on offspring performances. The ease of measuring their eggs and larvae allowed us to understand the importance that egg size has in determining phenotype and survival in early life stages. With these results we have demonstrated the prominent role of maternal effects, and specifically egg size, in the early life stages dynamics of salmonids. Genomics allowed us to obtain precise estimates of parental inbreeding coefficient and to understand how these directly influence their fitness related traits and indirectly influence offspring growth. In addition, thanks to new sequencing technologies we have been able to clarify, at least partly, how sex is determined in this species (chapter 6), where sexual determination has been very enigmatic since the first advances in this field. The detection of the biological signals has been possible thanks to the combination of a quantitative genetics and genomics approaches. Our results call for a change in some important evolutionary models and especially open up fields of research that are still underexplored today. Sexual selection from a female perspective, the intergenerational effect of female inbreeding, and the study of stressors affecting offspring size evolution are issues to be explored. These problems are more relevant than ever given the global loss of genetic diversity that is occurring, the rapid environmental changes that are taking place and the continuous introduction of harmful substances of anthropogenic origin in natural systems. I know that this thesis cannot change much but

maybe this can be a small spark that will one day lead to a better understanding and protection of our natural world.

General discussion references

Auer SK, Anderson GJ, McKelvey S, Bassar RD, McLennan D, Armstrong JD, Nislow KH, Downie HK, McKelvey L, Morgan TAJ, et al. 2018. Nutrients from salmon parents alter selection pressures on their offspring. Ecology Letters 21:287-295.

Barks PM, Laird RA. 2020. Parental age effects and the evolution of senescence. American Naturalist 195:886-898.

Bashey F. 2008. Competition as a selective mechanism for larger offspring size in guppies. Oikos 117:104-113.

Brown JL. 1997. A theory of mate choice based on heterozygosity. Behavioral Ecology 8:60-65.

Burton T, Hoogenboom MO, Beevers ND, Armstrong JD, Metcalfe NB. 2013. Among-sibling differences in the phenotypes of juvenile fish depend on their location within the egg mass and maternal dominance rank. Proceedings of the Royal Society B-Biological Sciences 280:20122441.

Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237-268.

Cogliati KM, Unrein JR, Stewart HA, Schreck CB, Noakes DL. 2018. Egg size and emergence timing affect morphology and behavior in juvenile chinook salmon, *Oncorhynchus tshawytscha*. Ecology and Evolution 8:778-789.

Crean AJ, Marshall DJ. 2009. Coping with environmental uncertainty: Dynamic bet hedging as a maternal effect. Philosophical Transactions of the Royal Society B: Biological Sciences 364:1087-1096.

David P. 1998. Heterozygosity–fitness correlations: new perspectives on old problems. Heredity 80:531–537.

Del Castillo RF. 1998. Fitness consequences of maternal and nonmaternal components of inbreeding in the gynodioecious *Phacelia dubia*. Evolution 52:44-60.

Einum S. 2003. Atlantic salmon growth in strongly food-limited environments: Effects of egg size and paternal phenotype? Environmental Biology of Fishes 67:263-268.

Einum S, Fleming IA. 2004. Environmental unpredictability and offspring size: Conservative versus diversified bet-hedging. Evolutionary Ecology Research 6:443-455.

Einum S, Fleming IA. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): Norms of reaction to environmental quality. Proceedings of the Royal Society of London. Series B: Biological Sciences 266:2095-2100.

Einum S, Hendry AP, Fleming IA. 2002. Egg-size evolution in aquatic environments: does oxygen availability constrain size? Proceedings of the Royal Society B: Biological Sciences 269:2325-2330.

Galla SJ, Moraga R, Brown L, Cleland S, Hoeppner MP, Maloney RF, Richardson A, Slater L, Santure AW, Steeves TE. 2020. A comparison of pedigree, genetic and genomic estimates of relatedness for informing pairing decisions in two critically endangered birds: Implications for conservation breeding programmes worldwide. Evolutionary Applications 13:991-1008.

Gallardo JA, Garcia X, Lhorente JP, Neira R. 2004. Inbreeding and inbreeding depression of female reproductive traits in two populations of coho salmon selected using BLUP predictors of breeding values. Aquaculture 234:111-122.

Goos JM, Swain CJ, Munch SB, Walsh MR. 2019. Maternal diet and age alter direct and indirect relationships between life-history traits across multiple generations. Functional Ecology 33:491-502.

Hare RM, Simmons LW. 2019. Sexual selection and its evolutionary consequences in female animals. Biological Reviews 94:929-956.

Hendry AP, Day T, Cooper AB. 2001. Optimal size and number of propagules: Allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. American Naturalist 157:387-407.

Hutchings JA. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. Evolution 45:1162-1168.

Johnston TA. 2018. Egg size and lipid content of lake trout (*Salvelinus namaycush*) in the wild and in captivity. Canadian Journal of Fisheries and Aquatic Sciences 75:2123-2135.

Johnston TA, Leggett W. 2002. Maternal and environmental gradients in the egg size of an iteroparous fish. Ecology 83:1777-1791.

Johnston TA, Wong DMM, Moles MD, Wiegand MD, Casselman JM, Leggett WC. 2012. Reproductive allocation in exploited lake whitefish (*Coregonus clupeaformis*) and walleye (*Sander vitreus*) populations. Fisheries Research 125:225-234.

Jonsson B, Jonsson N. 2009. A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. Journal of Fish Biology 75:2381-2447.

Jonsson N, Jonsson B. 1999. Trade-off between egg mass and egg number in brown trout. Journal of Fish Biology 55:767-783.

Kardos M, Luikart G, Allendorf FW. 2015. Measuring individual inbreeding in the age of genomics: Marker-based measures are better than pedigrees. Heredity 115:63-72.

Kekäläinen J, Huuskonen H, Tuomaala M, Kortet R. 2010. Both male and female sexual ornaments reflect offspring performance in a fish. Evolution 64:3149-3157.

Keller LF, Waller DM. 2002. Inbreeding effects in wild populations. Trends in Ecology & Evolution 17:230-241.

Kindsvater HK, Bonsall MB, Alonzo SH. 2011. Survival costs of reproduction predict age-dependent variation in maternal investment. Journal of Evolutionary Biology 24:2230-2240.

Klemetsen A, Amundsen PA, Dempson JB, Jonsson B, Jonsson N, O'Connell MF, Mortensen E. 2003. Atlantic salmon *Salmo sala*r L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): A review of aspects of their life histories. Ecology of Freshwater Fish 12:1-59.

Krogh A. 1941. The comparative physiology of respiratory mechanisms. Philadelphia, USA: University of Pennsylvania press.

Kvalnes T, Roberg AA, Jensen H, Holand H, Parn H, Saether BE, Ringsby TH. 2018. Offspring fitness and the optimal propagule size in a fluctuating environment. Journal of Avian Biology 49:e01786.

Leblanc CAL, Benhaïm D, Hansen BR, Kristjánsson BK, Skúlason S. 2011. The importance of egg size and social effects for behaviour of Arctic charr juveniles. Ethology 117:664-674.

Leblanc CAL, Kristjansson BK, Skulason S. 2016. The importance of egg size and egg energy density for early size patterns and performance of Arctic charr *Salvelinus alpinus*. Aquaculture Research 47:1100-1111.

Lehnert SJ, Kess T, Bentzen P, Kent MP, Lien S, Gilbey J, Clément M, Jeffery N, Waples RS, Bradbury IR. 2019. Genomic signatures and correlates of widespread population declines in salmon. Nature Communications 10:1-10.

Manel S, Guerin P-E, Mouillot D, Blanchet S, Velez L, Albouy C, Pellissier L. 2020. Global determinants of freshwater and marine fish genetic diversity. Nature Communications 11:1-9.

Marshall DJ, Bonduriansky R, Bussière LF. 2008. Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. Ecology 89:2506-2517.

Marshall DJ, Heppell SS, Munch SB, Warner RR. 2010. The relationship between maternal phenotype and offspring quality: Do older mothers really produce the best offspring? Ecology 91:2862-2873.

Mattey S, Strutt L, Smiseth P. 2013. Intergenerational effects of inbreeding in *Nicrophorus vespilloides*: Offspring suffer fitness costs when either they or their parents are inbred. Journal of Evolutionary Biology 26:843-853.

Mousseau TA, Fox CW. 1998. Maternal effects as adaptations. New York, USA: Oxford University Press.

Parker GA, Begon M. 1986. Optimal egg size and clutch size: Effects of environment and maternal phenotype. The American Naturalist 128:573-592.

Philippi T, Seger J. 1989. Hedging one's evolutionary bets, revisited. Trends in Ecology & Evolution 4:41-44.

Pilakouta N, Smiseth PT. 2016. Maternal effects alter the severity of inbreeding depression in the offspring. Proceedings of the Royal Society B: Biological Sciences 283:20161023.

Regnier 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 22:169-177.

Reichert S, Berger V, Jackson J, Chapman SN, Htut W, Mar KU, Lummaa V. 2020. Maternal age at birth shapes offspring life-history trajectory across generations in long-lived Asian elephants. Journal of Animal Ecology 89:996-1007.

Reid JM, Arcese P, Keller LF. 2003. Inbreeding depresses immune response in song sparrows (*Melospiza melodia*): Direct and inter–generational effects. Proceedings of the Royal Society of London. Series B: Biological Sciences 270:2151-2157.

Roff D. 1992. Evolution of life histories: theory and analysis. New York, USA: Chapman & Hall.

Rollinson N, Hutchings JA. 2013. Environmental quality predicts optimal egg size in the wild. American Naturalist 182:76-90.

Rollinson N, Rowe L. 2016. The positive correlation between maternal size and offspring size: fitting pieces of a life-history puzzle. Biological Reviews 91:1134-1148.

Rombough PJ. 2007. Oxygen as a constraining factor in egg size evolution in salmonids. Canadian Journal of Fisheries and Aquatic Sciences 64:692-699.

Sargent RC, Taylor PD, Gross MR. 1987. Parental care and the evolution of egg size in fishes. The American Naturalist 129:32-46.

Schluter D, Price TD, Rowe L. 1991. Conflicting selection pressures and life history trade-offs. Proceedings of the Royal Society of London. Series B: Biological Sciences 246:11-17.

Smith CC, Fretwell SD. 1974. The optimal balance between size and number of offspring. The American Naturalist 108:499-506.

Song ZT, Lin X, Que PJ, Halimubieke N, Huang Q, Zhang ZW, Szekely T, Liu Y. 2020. The allocation between egg size and clutch size depends on local nest survival rate in a mean of bet-hedging in a shorebird. Avian Research 11:40.

Stearns SC, Hendry AP. 2004. Evolution illuminated: Salmon and their relatives. Oxford, New York: Oxford University Press, Inc.

Su GS, Liljedahl LE, Gall GAE. 1996. Effects of inbreeding on growth and reproductive traits in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 142:139-148.

Szulkin M, Garant D, McCleery R, Sheldon B. 2007. Inbreeding depression along a life-history continuum in the Great tit. Journal of Evolutionary Biology 20:1531-1543.

Taborsky B. 2006. Mothers determine offspring size in response to own juvenile growth conditions. Biology Letters 2:225-228.

Williams GC. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. The American Naturalist 100:687-690.

Wolf JB, Wade MJ. 2009. What are maternal effects (and what are they not)? Philosophical Transactions of the Royal Society B: Biological Sciences 364:1107-1115.

Appendix 1 - Impact of long runs of homozygosity on life history traits in whitefish

Audrey Atherton[1], Christian de Guttry[1] and Claus Wedekind[1]

[1]Department of Ecology and Evolution, Biophore, University of Lausanne, Lausanne, Switzerland

Master thesis project

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole de Biologie

Impact of long runs of homozygosity on life history traits in whitefish

Travail de Maîtrise universitaire ès Sciences en Comportement, Evolution et Conservation, spécialisation "Ecologie et Evolution Computationnelles"

Master Thesis of Science in Behaviour, Evolution and Conservation, specialisation "Computational Ecology and Evolution

by

Audrey ATHERTON

Director : Prof. Claus Wedekind

Supervisor : Christian de Guttry

Expert : Anonymous

Department of Ecology and Evolution

January 2020

Abstract

Inbreeding increases the likelihood of extinction of at-risk populations by diminishing fitness through an accumulation of deleterious mutations. Its effects can be more pronounced in endangered populations where genetic drift, recent bottlenecks and increased consanguineous mating may cause a smaller effective population size and decreased genetic diversity. As such, measuring inbreeding and its impact is of great interest for conservation efforts. For fish populations, pedigree-based methods are usually impossible to implement due to the cryptic nature of the individuals and the difficulty of determining parentage. The use of long runs of homozygosity (ROHs) is an intriguing alternative to calculate individuals inbreeding coefficient (F), for it has been shown to be an accurate representation of inbreeding. For this study, gametes were used in a full factorial breeding design from 27 Swiss whitefish *(Coregonus suidteri)* from lake Hallwil to obtain 4368 offspring that were then singly raised. We then checked for the presence of long ROHs in paternal genomes through whole-genome resequencing and tested whether individual F_{ROH} affected life history traits of both the fathers and their progeny. We found that ROHs seem to have an influence on adult body size but not on embryo performance. We conclude that sires' long ROHs influence individual characteristics, highlighting the effect of inbreeding depression given the possibility of consanguineous matings during restocking.

Abstrait

La consanguinité accroît le risque d'extinction des populations à risque en accumulant les mutations délétaires. Ses effets peuvent être plus prononcés dans les populations menacées, chez lesquelles la dérive génétique, les goulots d'étranglements récents et une augmentation des accouplements consanguins amènent à un effectif efficace moindre et une diminution de la diversité génétique. De ce fait, il est important de pouvoir mesurer la consanguinité et son impact pour les efforts de conservation. Pour les populations de poissons, les méthodes basées sur les pédigrés sont impossibles à implémenter du fait de la nature cryptique de leurs individus et de la difficulté à déterminer les liens de parenté. L'utilisation de longs segments d'homozygocité continus (ROHs) est une alternative intéressante au calcul du coefficient de consanguinité individuel (F), car ils été prouvé de bien représenter la consanguinité. Pour cette étude, des gamètes ont été mises dans un design de reproduction entièrement factoriel composé de 27 féras suisses *(Coregonus suidteri)* provenants de la population du lac de Hallwil pour obtenir 4368 descendants élevés individuellement. Nous avons ensuite testé les génomes paternels pour la présence de longs segments d'homozygocité continus en reséquançant entièrement leur génome. Nous avons également testé si le coefficient individuel F_{ROH} affecte les traits d'historique de vie des pères et de leurs progénie. Nous avons trouvé que les longs segments d'homozygocité continus

semblent avoir une influence sure la taille des adultes mais pas sur la performence des embryons. Nous concluons que les longs segments d'homozygocité continus des mâles inflence les caractéristiques des individus, mettant en évidence l'effet de la dépression de consanguinité étant donné la possibilité de d'accouplements consanguins lors de restockages.

Contents

List of Figures

List of Tables

1 Introduction

Inbreeding is a natural phenomenon of increased autozygosity that occurs when related individuals mate and produce offspring. To distinguish possibly harmful levels of inbreeding from the background inbreeding that stems from distant common ancestry, measures and estimators are commonly calculated against the background of a broader population (Wright, 1949; Beaumont, 2005). Inbreeding depression occurs when the increase in homozygous loci causes loss of fitness in the offspring (Charlesworth and Willis, 2009) through an increase of deleterious mutations. This increment can arise after bottlenecks, genetic drift, consanguineous mating or a combination of all the above. The loss of fitness can take many forms, including causing higher mortality (Ralls et al., 1979; Wang et al., 2002), lower fertility (Dickerson, 1973; Su et al., 1996), diminished adaptability (Reed et al., 2003) and cause an increase in rare diseases within the population (Alvarez et al., 2011; Leroy and Baumung, 2011).

Small populations, like those of endangered species, are particularly likely to experience increased inbreeding. Habitat loss (Newbold et al., 2015; Gibbons et al., 2000), changes in habitat (Laidre et al., 2008; Brook et al., 2008), invasive species (Gibbons et al., 2000) and even epistatic effects (Goodnight, 1987) tend to have a stronger impact on them than on larger populations. Inbreeding and its effects on fitness are thus valuable parameters to quantify for conservation efforts, to ensure that endangered species can recover healthily and avoid a population crash further down the line.

Before the advent of widespread genomics, inbreeding was calculated based on pedigrees (MacCluer et al., 1983; Calboli et al., 2008). F_P, the inbreeding coefficient as defined by pedigrees, is calculated based on meticulously documented genealogical trees to determine the inbreeding of individuals on lower branches of the tree (Ballou, 1983). F_P relies on assumptions, such as the initial progenitors being in no way related and each generation systematically passing on half of its genetic data to the one following, a quarter to the one after, following a power law. The high variation that recombination brings in the passing of maternal and paternal loci brings inaccuracy to pedigree measurements of inbreeding (Stam, 1980; Leutenegger et al., 2003).

In wild populations, pedigrees are often impossible to obtain, so genetic methods are a very appealing approach to estimating the inbreeding coefficient. This is particularly true for fish, who are often impossible to track underwater to build a pedigree. The rise of high throughput sequencing has brought with it marker-based estimators of inbreeding based on high numbers of markers. These methods can now surpass pedigrees in accuracy when it comes to estimation of inbreeding depression (Wang, 2016). Nowadays, the most common

estimator to rely on genomic data is multi-locus heterozygosity, MLH. It describes the proportion of genotyped SNPs that are heterozygous (Slate et al., 2004). When this measure is correlated with fitness traits, we get a measure called the Heterozygosity-Fitness Correlation (HFC), which can be used to estimate the extent of inbreeding depression (David, 1998; Forstmeier et al., 2012).

A newly introduced tool to measure inbreeding in wild populations are runs of homozygosity (ROHs). These are continous regions of the genome where an individual is homozygous over all sites. Due to their continuous nature, these stretches oh homozygous loci are likely to come from individuals that share a common ancestor, and can also be called identical by descent. They have been widely studied on human (McQuillan et al., 2008; Pemberton et al., 2012) and cattle (Curik et al., 2014; Xie et al., 2019) but are a relatively new method of measuring inbreeding when it comes to wild populations. Kardos et al. (2018) were the first to use ROHs on a wild species, namely the grey wolf (*Canis lupus*). The inbreeding coefficient calculated with ROHs, F_{ROH} corresponds to the total length of all ROHs over the size of the genome, representing the proportion of the genome covered in ROHs. F_{ROH} can provide both an accurate estimate of inbreeding load (Keller et al., 2011; McQuillan et al., 2012) and an estimate of how long ago the inbreeding occurred (Browning and Browning, 2012; Thompson, 2013). Short ROHs indicate a consanguineous mating that dates many generations back whereas long ROHs suggest a more recent inbreeding event, with ROHs above 1MB reflecting inbreeding within 5 to 6 generations (Ceballos et al., 2018). Finally, they can be used to find genes under selection in different species (Xie et al., 2019). This versatility makes F_{ROH} a very interesting measure to use in conservation efforts. We focused on ROHs because they represent a promising new technique for conservation, offering information on both inbreeding level and time to different inbreeding events.

Switzerland has a high diversity of freshwater environments but large eutrophication events starting in the 1950's and 1970's have caused a crash in many populations of fish by causing anoxic conditions in many Swiss lakes. Since then, efforts have been made by the government to restore and maintain fish populations (Laurent, 1972; Enz et al., 2001). Whitefish (*Coregonus spp.*) are both keystone species and sources of protein and recreation to humans, making them economically and ecologically valuable. Whitefish are present in many Swiss lakes, sometimes in two "species" that inhabit different levels of the water column. Although the population size has increased thanks to restocking efforts, there have been worries about hybridization between different morphs (Bittner et al., 2010) or a diminished effective population size (Wedekind and Müller, 2004). The first would result in outbreeding depression and the latter to a potential inbreeding depression through reduction of genetic diversity and genetic drift. The whitefish population of lake Hallwil is one such population

that has been maintained partially through artificial stocking (Enz et al., 2001).Another major concern is the protocol used for the restocking, where the genetics of the fish is often poorly considered, which in turn leads to a possible increase of consanguineous matings. Simultaneously, these populations are subject to a strong fishing pressure made by the use of size selective gillnets. This complex dynamic makes genetic monitoring an interesting prospect to maintain optimal population health as well as to sustain the genetic diversity of the managed population.

The purpose of this paper is to expand our understanding of ROHs as indicators of inbreeding depression and their impact on a wild whitefish population that is partially artificially sustained in a lake under high fishing pressure. Using the inbreeding coefficient F_{ROH} calculated from long ROH of 14 male whitefish and a fully factorial breeding design with 13 females, we were able to model the effect of inbreeding depression on life history traits of both the sires and their offspring. We studied body, sperm and breeding tubercles in the sires and early life history traits in the offspring.

2 Materials and Methods

2.1 Phenotypical data measurements

2.1.1 Sampling and experimental design

Adult whitefish were caught in their spawning ground in lake Hallwil (47.2772°N, 8.2173°E) with gill nets by the fishermen of "Fischerei Hallwil" at the beginning of the breeding season in December 2016. In total, 13 females and 14 males were randomly sampled from the fishermen's catch.

Plaster casts were then taken following the method of Wedekind et al. (2001) to the characteristics of breeding tubercles, a secondary sexual ornament present in both male and female whitefish. Tissue samples from the anal fin were taken for genetic analysis and preserved in 70% ethanol. Fish were weighed and their pictures were taken for morphometric analysis using a photo-box in standardized conditions. The exact age of the fish could not be ascertained.

In vitro fertilization was done with gametes stripped from the euthanized male and female fish. It followed a full-factorial breeding design containing one breeding block (14x13) for a total of 182 sib-groups. Standardized water was used during the fertilization and the rearing of the embryos, according to the OECD guideline No. 203 (Kitano, 1992). Two

hours post-fertilization, 24 embryos of each sib-group (total 4368) were transported to a climate chamber at 4 $°C$ in a 12-12 hour day-night cycle at University of Lausanne. They were washed with flowing lake water ($4L/min$) for $30s$ and distributed into 24-well plates (Greiner bio-one, Germany) filled with $1.8ml$ of autoclaved standardized water following von Siebenthal et al. (2009).

The 24-well plates were left undisturbed for 15 days post-fertilization, after which embryos were individually controlled for fertilization success and early mortality. Each sib-group was divided into two groups of 12 individuals : a control and a treatment. Twenty-one days post-fertilization, the control group had 200 μl of nutrient broth, identical to that used for bacteria described in (Clark et al., 2013) added to their wells and the treatment group had a solution containing 106 $cells/\mu l$ of the bacteria *Pseudomonas fluorescens* (PS2) were added to their wells. This strain of bacteria was chosen because it has been shown to be only mildly virulent and thus create variability in early embryo performance without increasing mortality (Clark et al., 2014).

Plates were then checked on a daily basis to keep track of mortality and hatching time. Eggs were photographed 90 days after fertilization, before hatching, with a Canon 70D in a photo-box with standardized light conditions to test for a possible effect of the treatment on egg volume. Pictures were taken in the same manner upon hatching and 21 days post-hatching. Hatchlings were placed in a new 24-well plate with a drop of water to avoid distortions in the pictures. Egg volume (mm^3), hatchling length (mm) and yolk sac volume (mm^3) were measured from the resulting pictures with ImageJ v2.0.0 (https://imagej.net).

2.2 3D scanner measurements

The characteristics of breeding tubercles, a secondary sexual ornament, were measured from a plaster cast with a 3D macroscope scanner VR-3200 (Keyence International, Mechelen, Belgium). The scanner granted repeatability on the magnitude of $0.5\mu m$ on all three axes (x, y and z). A motorized table and stitching software increased the scanning surface to 200x100x10mm, which corresponded to the entire adult cast. Each plaster was scanned with the stitching function using standard settings. The function "set curved surface" was used to correct irregularities at the edges of the plaster cast shape. The function "waveform removal" further normalized the surface by flattening the area surrounding the breeding tubercles. The breeding tubercles, by then the only concavities, were measured with volumetric software for volume (mm^3), cross section area (mm^2), surface area (mm^2), surface area over cross section area (no unit), area ratio (%), average depth (mm), maximum depth (mm), perimeter (mm), horizontal feret (mm), vertical feret (mm), circle equivalent diame-

ter (mm) and circularity. The output files were individual breeding tubercles, each identified with a unique number. The final version of the images, like the ones shown in Figure 1, was cleaned of artifacts due to irregularities in the plaster cast. The same protocol with the same parameters was used for all the parents' plaster casts.

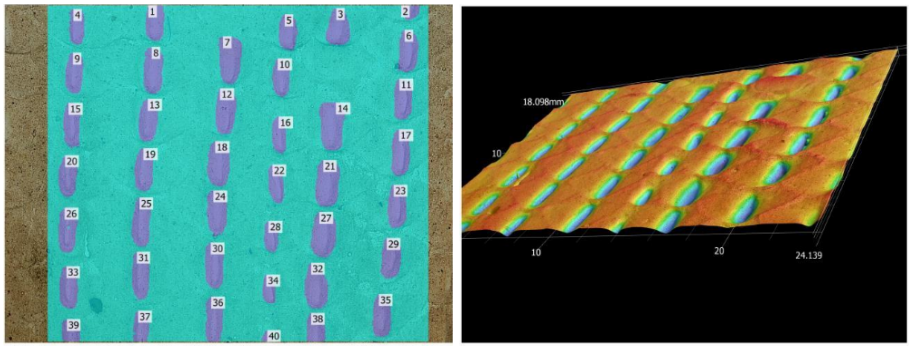


Figure 1: Pictures of breeding tubercles. On the left, pictures of the breeding tubercles, classification obtained after 3D image processing. On the right, picture of the 3D image of the same part of the skin

2.3 Sperm traits measurements

On the field, fresh sperm was diluted at 10% in Storfish (IMV Technologies, France) and activated once in the University of Lausanne with Actifish in a final dilution ratio of 1:500. Then, 2 μl of activated semen were analysed in a 4-well chamber slide (Leja, IMV Technologies, France) on a cooling stage set at 6.5$\mathscr{C}$. Motility characteristics were analysed with the CASA Qualisperm® software (Biophos AG, Switzerland).

2.4 DNA extraction and library preparation

To insure good DNA quality, genomic DNA extraction from the anal fin was done using the DNAeasy Blood & Tissue kit (Quiagen) in the days following the sampling. DNA quality and concentration were measured respectively on agarose gel and with Qubit 2.0. The samples were normalized to a concentration of 20 $ng/\mu l$.The whole genome resequencing libraries were prepared following the Illumina TruSeq PCR-free protocol by the University of Lausanne genomic center facility. The sequencing was done on the Illumina HiSeq 4000 system on 16 different lanes. In order to avoid a possible lane effect in the analysis, the

individuals were multiplexed using individual-specific barcodes. Each individual's DNA was run through 16 lanes for sequencing, then each individual's sequence was separated via their barcode. This was done in order to increase the coverage and the quality of the homozygosity estimation per site.

2.5 Genetic data analysis

2.5.1 Data Preprocessing

The quality of the resulting raw data fastq files was tested using FastQC 0.11.7 (Andrews et al., 2012). All 16 lanes yielded good results with a pair base quality higher than 30 (phred score) along all the length of the sequences (150bp). We proceeded with the data preprocessing following GATK best practices (Van der Auwera et al., 2013). Fastq files were mapped to the newly published whitefish genome (De-Kayne et al., 2019) using the Burrows-Wheeler aligner version 0.7.17 (Li, 2013) on pair-ended reads using the *BWA-MEM* algorithm. The choice for the algorithm was done following the recommendations on the Burrows-Wheeler website (http://bio-bwa.sourceforge.net/) as *BWA-MEM* is recommended for Illumina sequences and we did not expect any large gaps in the sequences.

We continued data pre-processing using Samtools 1.8 (Li et al., 2009), Picard-tools 2.18.11 (`http://broadinstitute.github.io/picard/`) and GenomeAnalysisTK 4.1.3.0 (McKenna et al., 2010). The data was cleaned with Picard-Tools *CleanSam* function, which removed alignments that went past the end of the reference sequence and marked unmapped reads as having $MAPQ = 0$. Read groups were added using Picard-tools *AddOrReplaceReadGroups*, adding a header to the bam files containing the names of the run group (*RGID*), the library (*RGLB*) which we kept at the default lib1, the sequencing platform (*RGPL*), the run name (*RGPU*) and the subject name (*RGSM*). This gave information for later steps on which individual sequence data was in which file. Samtools *fixmate* was used to fix paired-ends and remove the "reads paired" flag in the files. Secondary alignments were removed samtools *view* with the option *-bh* to keep the header and use a binary file as an input and *-F 256* to keep only the primary alignment. Each sequence can only belong in one position of the reference genome, and the secondary alignments represent less likely placement than the primary alignment. Files were sorted by position using samtools *sort*. Duplicate sequences were marked using picard-tools *MarkDuplicates*, very few variants were lost due to the PCR-free protocol when extracting the DNA. GATK requires specific reference files, so our reference genome was referenced using picard-tools *CreateSequenceDictionary*, followed by samtools *faidx*. Files were reordered using picard-tools *ReorderSam* to insure

they were compatible with GATK during SNP calling. Index files were generated using samtools *index*. Preprocessed files were validated using picard-tools *ValidateSamFile*, checking each files for errors.

Before SNP calling, all lanes of a single individual were merged using picard-tools MergeSamFiles so that one file remained for every individual. This insured that different lanes of the same individual would not be considered different individuals by GATK. Single nucleotide polymorphisms (SNPs) were called into a GVCF file using GATK's *HaplotypeCaller* with the options *–emit-ref-confidence GVCF* and *–genotyping-mode DISCOVERY* on 4 chromosomes at a time for every individual. Dividing the files into fewer chromosomes allowed for faster computation . Chromosomes of the same individual were then merged together using GATK's *CombineGVCFs*. Individual level GVCF files were then merged using the same function to make a final GVCF, which was turned into VCF using GATK's *GenotypeGVCFs*.

The VCFs went through several filtering steps to maintain only the highest quality SNPs. We first used GATK's *VariantFiltration* for a set of technical filters. We normalized variant quality (*QD<2.0*), checked for sample quality (*QUAL<30.0*), reduced strand bias (*SOR>3.0* and *FS>60.0*), insured mapping quality (*MQ<40.0*), compared mapping quality between reference and alternate alleles (*MQRankSum<-12.5*) and checked the similarity in position between reference and alternate alleles (*ReadPosRankSum<-8.0*). These parameters were suggested by Auwera (2013) for datasets of SNPs such as ours. GATK's *VariantSelection* was then run to retain only the SNPs that passed the hard filter. Further filtering was done with vcftools (Danecek et al., 2011). First, variants out of Hardy-Weinberg equilibrium (*–hwe 0.05*) were weeded out, removing variants that are likely to be under selection. Rare alleles (*–maf 0.05*) were removed, in a small sample size like ours this would constitute any allele present in less that 0.7 individuals so it would remove errors and artifacts but no actual allele. The VCFs were then filtered low coverage (*–min-meanDP 10*) to remove variants that are less well supported and less reliable. This helps avoid type I errors in heterozygosity calling per site. Similary, variants with coverage higher than twice mean coverage (*–max-meanDP 60*) were removed to reduce the possible presence of paralogs. Variant of low genomic quality (*–minGQ 20*) were removed to avoid errors being maintained in the final VCFs. We removed variants with more than 10% missing data (*–max-missing 0.9*) so the genotypes are as complete as possible. To keep only SNPs, indels (*–remove-indels*) and multi-allelic sites (*–max-alleles 2*) were filtered out. Finally, a filter for excess heterozygotes (*–max_obs_het 0.80*) was done using Stacks 1.48 (Catchen et al., 2013) population function.

2.5.2 ROH calling and classification

The final filtered vcf was converted to a bed file using Plink 1.90 (Purcell et al., 2007) –*make-bed* function. Calling the ROHs was done using the –*homozyg* function of Plink, set for 40 chromosomes (–*chr-set 40*) instead of the human default of 23 chromosomes. Plink was run with the following parameters : a minimum of 100 SNPs in any ROH (–*homozyg-snp 100*), ROHs with a minimum size of 100Kb (–*homozyg-kb 100*), 100 SNPs in the sliding window (–*homozyg-window-snp 100*), a maximum of 10 heterozygotes per ROH (–*homozyg-window-het 10*), a maximum of 25 missing genotypes per ROH (–*homozyg-window-missing 25*), a threshold of SNP inclusion in the ROH of 0.05 (–*homozyg-window-threshold 0.05*). We set the parameters for minimum SNP density and gap between SNPs (–*homozyg-density* and –*homozyg-gap*, respectively) to 5000 to exclude them. This was done because, due to previous research with RAD-seq by de Guttry et al. (N.D.), we expected our individuals to be highly inbred and thus show both low SNP density and large gaps between SNPs. We decided against pruning for linkage disequilibrium for it can be the results of some types of inbreeding Szulkin et al. (2010). Testing ROH-calling with and without pruning showed no significant difference in the results.

There does not seem to be a consensus as to what constitutes a short, medium or long ROH yet (McQuillan et al., 2008; Kardos et al., 2018; Grossen et al., 2018; Abdellaoui et al., 2015). We classified ROHs in the following way : small ROHs between 0.1 and 0.5MB, medium ROHs between 0.5 and 1MB and long ROH for anything above 1MB. We chose 1MB for our threshold for a long ROH because Nietlisbach et al. (2018) showed through simulations that ROHs equal or above 1MB best predict inbreeding depression, which is the main focus of this paper. Furthermore, ROHs above 1MB represent inbreeding in the last 5-6 generations (Ceballos et al., 2018), which is consistent with recent inbreeding that we expect to see in our population due to . The results presented consider only long ROH, but results containing both medium and long ROH can be found in the Appendix figures A3 to A7 and do not differ significantly from results. The analyses were not done on short ROHs because they represent inbreeding events further in the population history and are not representative of recent inbreeding.

F_{ROH} was calculated following the formula explicited in McQuillan et al. (2008) but considering only ROHs that are 1MB or longer to maximize relevancy and accuracy :

$$F_{ROH} = \frac{L_{ROH}}{L_{GEN}}$$

Where L_{ROH} is the total additive length of ROHs, here only those above 1MB, and L_{GEN} is the length of the genome. F_{ROH} thus represents the proportion of the genome covered in ROHs.

2.5.3 Statistical analysis

The data was analyzed using three types of statistical models, depending on what was more appropriate for the response and explanatory variables. All analyses were run using R version 3.6.1 (R Core Team, 2018). The package lme4 (Bates et al., 2014) was used for linear mixed models (LMMs). The package vcfR (Knaus and Grünwald, 2017) was used to visualize the data of the final VCF file. The packages ggplot2 (Wickham, 2016) and tidyverse (Wickham et al., 2019) were used for graphing the data

The effect of F_{ROH} (long ROHs) on the sires' life history traits was measured through simple linear models (LMs). The response variable considered for body (standard length, weight), sperm (speed, number of immotiles) and breeding tubercle (mean volume, maximum depth) characteristics were continuous numerical variables, as was F_{ROH}. We made an assumption that these characteristics would be normally distributed within the population and respond in a linear fashion to inbreeding depression as described by F_{ROH}.

The effect of paternal long runs of homozygosity on offspring performance was measured with linear mixed models (LMMs). We tested the effects on egg volume before hatching, hatching time, yolk sack volume, length at day 0, length at day 21 and growth rate between day 0 and day 21. All of these responses were continuous variables and seemed appropriate for an LMM. The explanatory variables used in every model were : dam and sire effects as random effect, and the effect of the treatment and of the sire's inbreeding coefficient F_{ROH} as fixed effects.

The impact of long ROHs on sires and on their offspring were tested separately, using two different datasets. The two sets were considered independent in calculating the correction for multiple testing because they applied to two different sets of individuals. Correction for multiple testing was done using the false discovery rate (FDR) method as described in Benjamini and Hochberg (1995) and Storey (2011). The method involves ordering in increasing order the p-values obtained through each model and, for $q = 0.05$, calculating the p-value that would be required for significance following $p^* = 0.05 \cdot \frac{i}{n}$ with i the rank of the p-value and n the total number of models. This method is less stringent than a Bonferroni correction (Bland and Altman, 1995) but seemed more appropriate for our small sample size of genotyped individuals. The outliers on the embryo response variables, that is observations that diverge from the overall pattern, were identified using the grubbs.test function in R. This test calculates the outlier score "G" (outlier minus mean and divided by standard deviation) and compares it to the appropriate critical values.

Both the genetic processing pipeline and code for the data analysis can be found at `https://github.com/audreyeatherton/MasterThesis`

3 Results

3.1 SNP and ROH distribution over the genome

We found a total of 9'931'674 SNPs divided on the whitefish genome of 40 chromosomes. Of those chromosomes, 21 are between 50 and 90MB, 18 are between 33 and 50MB and chromosome 40 is the smallest at barely 1MB. Figure 2 shows the distribution of SNPs over each chromosome. The number of SNPs comprises SNPs of all individuals taken together, hence the number of SNPs often exceeding one thousand per MB. The majority of chromosomes have between 4000 and 5000 SNPs, which corresponds to an average of between 280 and 360 SNPs per individual per MB of chromosome. Chromosomes 32 (6181$SNPs/MB$), 37 (6040$SNPs/MB$), 38 (7105$SNPs/MB$), 39 (5784$SNPs/MB$) and especially chromosome 22 (9598$SNPs/MB$) have a larger number of SNPs per MB of chromosome. The shortest, 40, has the fewest SNPs with barely 2932$SNPs/MB$.

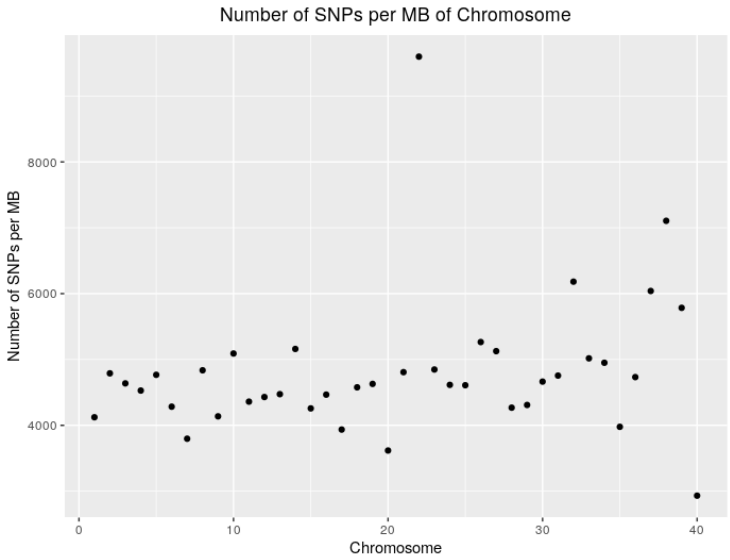


Figure 2: Number of SNPs per MB of each chromosome, comprising the SNPs for all of genotyped individual

Figure 3 shows the distribution of all ROHs, of length between 0.1MB and 8.1MB, over each chromosome with colors differentiating the individuals. There seems to be the expected slight downward trend, with smaller chromosomes generally having fewer ROHs than longer chromosomes. There was a total of 33'942 ROHs with 31'267 small, 2'020

medium and 655 long ROHs. The chromosomes that showed having more SNPs in Figure 2, chromosomes 22, 32 and 38, also have fewer ROHs than other chromosomes of similar size, likely because the added SNPs divided ROHs. A table with the exact number of SNPs present on every chromosome as well as one with the inbreeding coefficient and number of ROHs for each individual are available in the Appendix, tables A2 and A3 respectively.

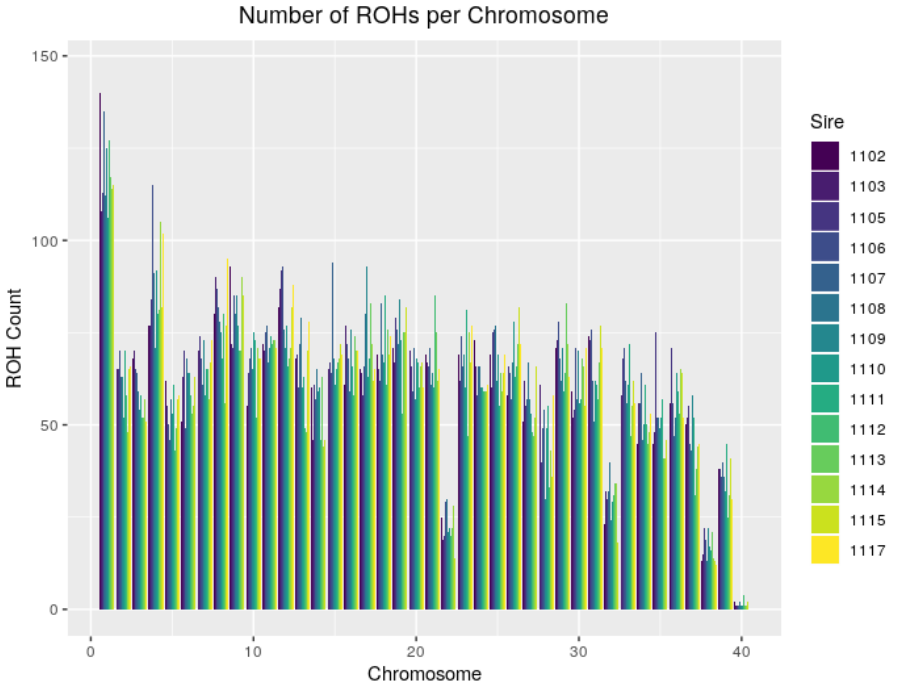


Figure 3: Number of ROH per chromosome as averaged over all 14 genotyped individuals, ROHs measured between 0.1MB and 8.1MB, each colour represents one of the genotyped individuals

Figure 4 shows the proportion of each chromosome covered in ROHs for every genotyped individual. Most chromosomes seem to be made of between $20 - 30\%$ of ROHs. Chromosomes 22, 32 and 38 show much lower ROH coverage than other chromosomes whereas chromosome 40 is nearly fully covered in ROHs for individuals 1109 (94.51%), 1112 (93.56%), 1113 (78.15%) and 1114 (94.51%).

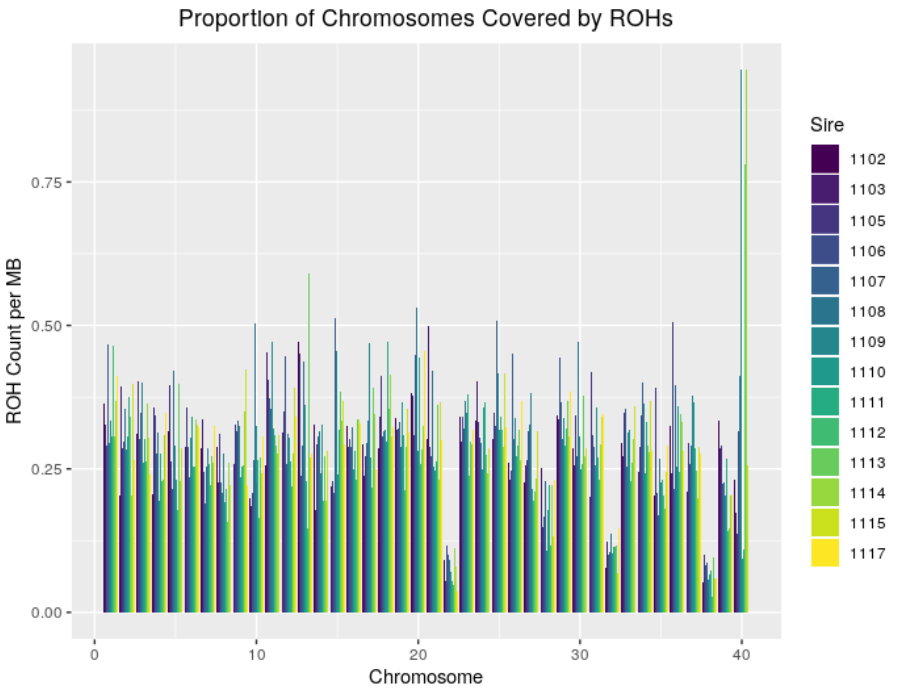


Figure 4: Proportion of chromosome covered by ROHs for every genotyped individual, ROHs measured between 0.1MB and 8.1MB, each colour represents one of the genotyped individuals

Figure 5 focuses on long ROHs, the ones we used in the following data analysis. The number of long ROHs on each chromosome is much more variable from individual to individual than the overall number of ROHs in Figure 4. Chromosomes 32 and 38 have no long ROH in any of the genotyped individuals. Each individual has between 23 and 64 long ROHs, for individuals 1112 and 1108 respectively. The mean number of long ROHs, all individuals considered was 46.78571.

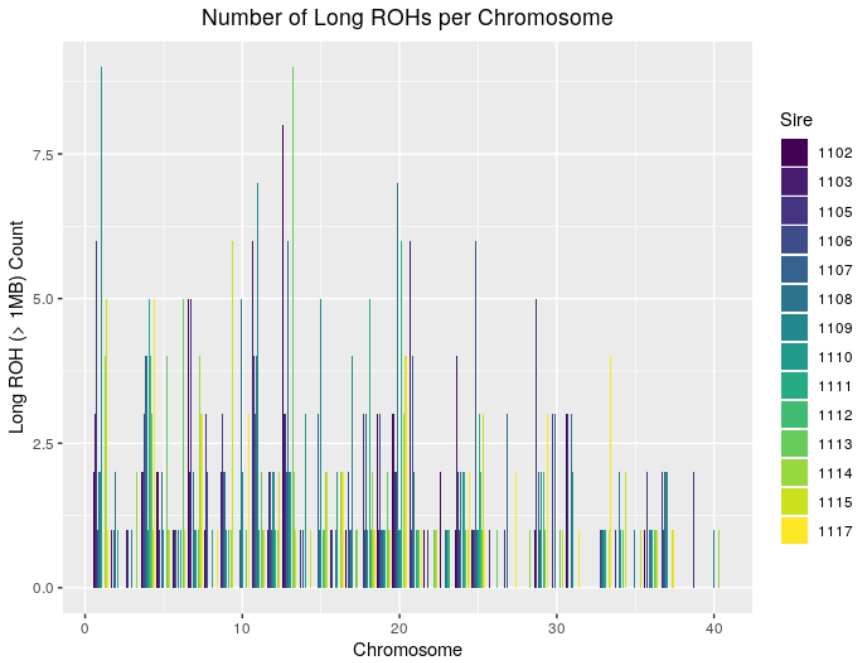


Figure 5: Number of long ROHs per chromosome, ROHs measured between 1MB and 8.1MB, each colour represents one of the genotyped individuals

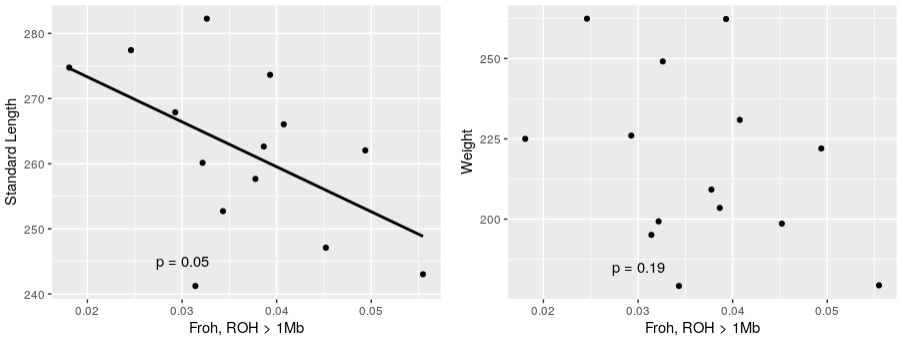
3.2 Effect of inbreeding on sire body, sperm and breeding tubercles

We used the FDR method to determine the level of significance of the p-values for the six models that were run on sire data. The smallest p-values were all linked to body characteristics, followed by sperm characteristics and the highest p-values were linked to breeding tubercles. A table with the ordered p-values for both sire and offspring models and the p-values required for significance can be found at the beginning of the Appendix, table A1.

3.3 Body Characteristics

Two body characteristics of the sires were studied, the results of their correlation with F_{ROH} are in Figure 6. Figure 6a shows the relationship between standard length (mm) and F_{ROH}. It shows a negative trend that shows increasing F_{ROH} leads in a decrease in body length (p = 0.05). This was the smallest p-value and not sufficient to be significant even before FDR

analysis. Figure 6b shows the relationship between body weight (g) and F_{ROH}. It shows a negative trend that suggests increasing F_{ROH} causes a decrease in body weight (p = 0.19).



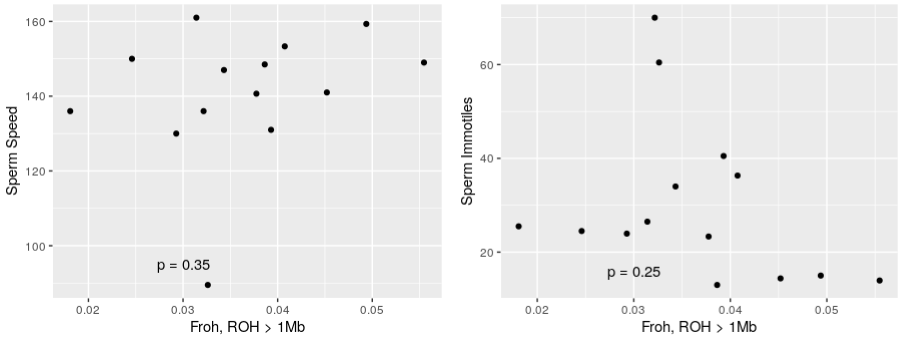
(a) Standard Length (mm)

(b) Weight (g)

Figure 6: Body characteristics of sires in relation with F_{ROH} for ROH$\geq$1MB, trend line obtained through a LM

3.3.1 Sperm Characteristics

Two different sperm characteristics were studied in correlation with F_{ROH}, the results can be found in Figure 7. Figure 7a shows the relationship between sperm speed ($\mu m/s$) and $FROH$. It shows a positive trend that is not significant (p = 0.35). Figure 7b shows the relationship between the number of immotile sperm cells and F_{ROH}. It shows a slight downward trend (p = 0.25), but is not significant. The data does not seem to match the assumption of linearity, further work should be done to elucidate the relationship between inbreeding and sperm characteristics.

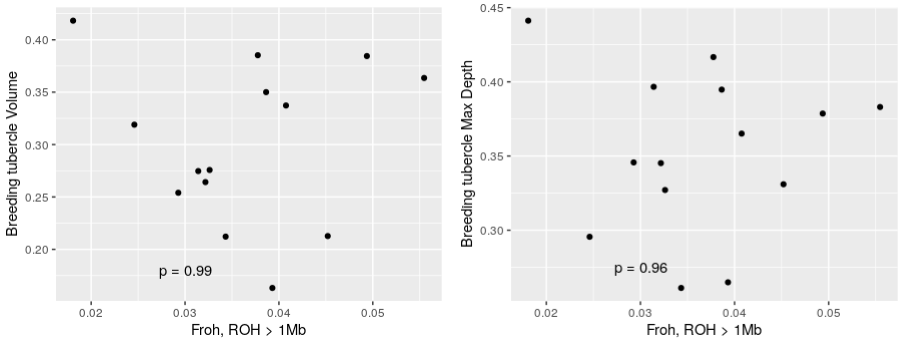


(a) Speed ($\mu m/s$) **(b)** Immotiles (cells)

Figure 7: Sperm characteristics of sires in relation with F_{ROH} for ROH≥1MB, trend line obtained through a LM

3.3.2 Breeding Tubercle Characteristics

Two aspects of breeding tubercles, secondary sexual ornaments, were studied as to their correlation with long ROHs. The results can be found in Figure 8. Figure 8a shows the relationship between breeding tubercule volume and F_{ROH}. It shows no trend (p = 0.99). Figure 8b shows the relationship between the maximum depth of the breeding tubercles and F_{ROH}. It shows no trend (p = 0.96).



(a) Volume (mm^3) **(b)** Maximum Depth (mm)

Figure 8: Breeding tubercle characteristics of sires in relation with F_{ROH} for ROH≥1MB, trend line obtained through a LM

3.4 Effect of pathogens and paternal inbreeding on offspring early life history traits

We tested the effect of F_{ROH} on 6 different offspring characteristics (Table 1). We did not use mortality as response variable given that less than 1% of the embryos died before hatching and less than 2% of the total larvae that hatched died in the first 21 days of life. Treatment with PF had a significant effect on all characteristics. The interaction between treatment and F_{ROH} was never significant, meaning that long paternal ROHs had no influence on pathogen resistance. Globally no effect of the inbreeding coefficient F_{ROH} was found on the embryo response variables but sire effect was always significant, highlighting the presence genetic variability between males. Strong dam effect was always found.

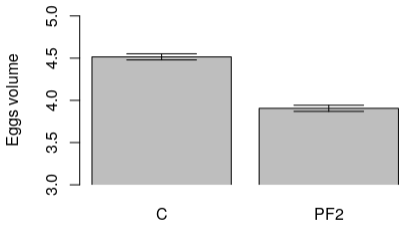
Table 1: The effect of treatment (exposure to PF), sire F_{ROH} (long ROH, ROH $\geq$ 1MB), sire and dam identity on : **(A)** Egg volume before hatching, **(B)** Hatching time, **(C)** Yolk sac volume, **(D)** Length on hatching day, **(E)** Length on day 21 and **(F)** Growth rate between hatching day and day 21.Likelihood ratio tests on mixed model regressions were used to compare a reference model (in bold and italic) with models including or lacking the term of interest. Significant effects are highlighted in bold.

Model	Effect Tested	AIC	d.f.	χ^2	P-value
(A) Egg Volume Before Hatching					
Ref : F_{ROH} ***+ Treatment + Sire + Dam***		7983	6		
F_{ROH} + Treatment + Sire	Dam	9174	5	1193	**<0.001**
F_{ROH} + Treatment + Dam	Sire	7987	5	6.5	**0.01**
F_{ROH} + Sire + Dam	Treatment	8555	5	574	**<0.001**
Treatment + Sire + Dam	F_{ROH}	7983	5	2	0.15
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	7984	7	0.7	0.39
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	7992	8	0	1
(B) Hatching Time					
Ref : F_{ROH} ***+ Treatment + Sire + Dam***		19253	6		
F_{ROH} + Treatment + Sire	Dam	20135	5	1193	**<0.001**
F_{ROH} + Treatment + Dam	Sire	19815	5	564	**<0.001**
F_{ROH} + Sire + Dam	Treatment	19356	5	105	**<0.001**
Treatment + Sire + Dam	F_{ROH}	19254	5	2.3	0.12
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	19225	7	0.5	0.46
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	19256	8	0.8	0.63
(C) Yolk Sac Volume					
Ref : F_{ROH} ***+ Treatment + Sire + Dam***		82	6		
F_{ROH} + Treatment + Sire	Dam	696	5	615	**<0.001**
F_{ROH} + Treatment + Dam	Sire	126	5	46	**<0.001**

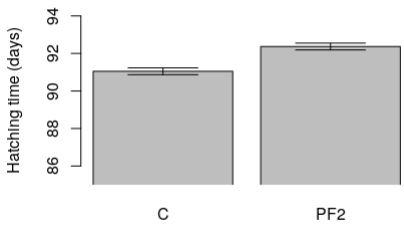
Table 1 Continued : The effect of treatment (exposure to PF), sire F_{ROH}, sire and dam identity on : **(A)** Egg volume before hatching, **(B)** Hatching time, **(C)** Yolk sac volume, **(D)** Length on hatching day, **(E)** Length on day 21 and **(F)** Growth rate between hatching day and day 21.Likelihood ratio tests on mixed model regressions were used to compare a reference model (in italic) with models including or lacking the term of interest. Significant effects are highlighted in bold.

F_{ROH} + Sire + Dam	Treatment	86	5	6.2	**0.01**
Treatment + Sire + Dam	F_{ROH}	80	5	0.21	0.64
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	84	7	0.16	0.68
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	86	8	0.09	0.95
(D) Length at Hatching Day					
Ref :* F_{ROH} *+ Treatment + Sire + Dam		4582	6		
F_{ROH} + Treatment + Sire	Dam	5232	5	651	**<0.001**
F_{ROH} + Treatment + Dam	Sire	4669	5	88	**<0.001**
F_{ROH} + Sire + Dam	Treatment	4632	5	51	**<0.001**
Treatment + Sire + Dam	F_{ROH}	4580	5	0.2	0.61
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	4583	7	1.1	0.28
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	4585	8	0.6	0.72
(E) Length at Day 21					
Ref :* F_{ROH} *+ Treatment + Sire + Dam		4148	6		
F_{ROH} + Treatment + Sire	Dam	5477	5	1331	**<0.001**
F_{ROH} + Treatment + Dam	Sire	4209	5	62	**<0.001**
F_{ROH} + Sire + Dam	Treatment	4152	5	5.8	**0.01**
Treatment + Sire + Dam	F_{ROH}	4146	5	0.4	0.51
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	4147	7	3.1	0.07
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	4151	8	1	0.60
(F) Growth Rate Until Day 21					
Ref :* F_{ROH} *+ Treatment + Sire + Dam		-9155	6		
F_{ROH} + Treatment + Sire	Dam	-9050	5	107	**<0.001**
F_{ROH} + Treatment + Dam	Sire	-9152	5	4.7	**0.03**
F_{ROH} + Sire + Dam	Treatment	-9137	5	19.6	**<0.001**
Treatment + Sire + Dam	F_{ROH}	-9154	5	2.1	0.13
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	-9153	7	0.03	0.84
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	-9151	8	0	0.99

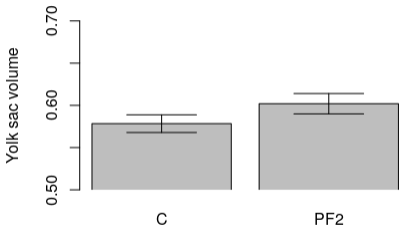
Previous research by de Guttry et al. (N.D.) showed that PF influenced all the response variables, its effects can be found in Figure 9. Embryo put in contact with the mild pathogen had a smaller egg size (9a), hatched later (9b) and were smaller than control embryo (9d, 9e) but had a larger yolk sac (9c) grew more quickly (9f).



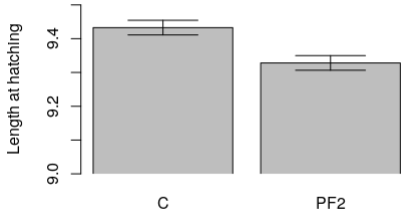
(a) Egg volume (mm^3)



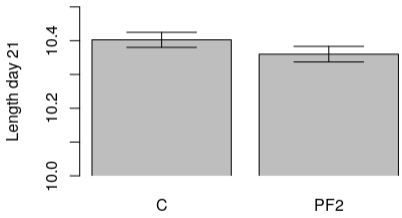
(b) Hatching time ($days$)



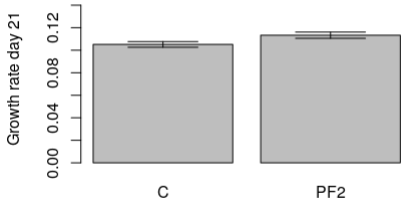
(c) Yolk sac volume (mm^3)



(d) Length at D0 (mm)



(e) Length at D21 (mm)



(f) Growth rate

Figure 9: Offspring life history characteristics in relation to treatment (PF) from de Guttry et al. (N.D.), boxplots of the mean characteristic for each group, error bars represent the standard deviation

4 Discussion

In this study, we aimed to better understand how long ROHs predict inbreeding depression, with a focus on parental traits and embryo performance in a wild population of whitefish. For the embryo, this included testing the difference of early life history traits in response to the presence or absence of a common non-lethal pathogen in the water they grew in. The whitefish population of lake Hallwil, which are suspected to have gone through a genetic bottleneck due to eutrophication events, is now partially artificially sustained. These features make it an ideal study system to investigate the possible presence of inbreeding. Using ROHs to estimate inbreeding and inbreeding depression is a new method when it comes to wild populations. Studying if long ROHs are present in the genome and how they correlate with fitness traits in both sires and offspring can help determine if it may be useful for whitefish management and conservation. A genetic method to monitor inbreeding might be very useful to maintain a healthy population of this commercially and ecologically important fish.

To achieve this, we used whole genome resequencing of 14 male whitefish to detect the presence of ROHs, with a special on long ROHs which are an indicator of recent inbreeding. Every sire was found to have short, medium and long ROHs, some to the point of having a chromosome nearly fully in homozygosity. We found that long ROHs seem to affect sire body size but neither sperm nor breeding tubercles. This could indicate a slower growth for more inbred males, those with a higher F_{ROH}, without having a significant impact on their reproductive success. It may even give them an advantage in a lake with a high fishing pressure such as Hallwil. Fishing regulations require fish measure a minimum of 24cm before being caught, which is normally just large enough to go through a single breeding season. Smaller males may in this case be able to go through two breeding seasons. The slower growth on inbred individuals added to an environment where gillet fishing targets larger individuals leads to an accelerated loss in genetic diversity for the whitefish population in lake Hallwil .

In their breeding experiment, Su et al. (1996) studied the effect of inbreeding depression described by pedigree on various fitness traits in male and female rainbow trout. They found an effect of inbreeding on adult size and weight, as well as a decrease in egg number in females. This is first finding is congruent with our findings and adds support to our hypothesis that the smaller size might add a breeding season to the inbred males in lake Hallwil. More recently, Christie et al. (2013) used microsatellite markers to study the effect of inbreeding on reproductive success of wild and captive rainbow trout. They found no significant effect of inbreeding, which is congruent with our findings on the sperm and breeding tubercle

characteristics of the genotyped whitefish.

The presence of the pathogen (PF) in the water had an effect on all offspring life history traits, even after correction for multiple testing. This impact did not seem to affect offspring differently depending on their sire's inbreeding level. The fact that our study included a large number of SNPs, which targeted many genes, may have hidden the effect. A study containing many traits like ours is more likely to highlight the effect on multilocus traits such as length and growth than on those linked to immune response. In addition, PF is only a mild pathogen for whitefish, so its effect may not be strong enough to notice interactions. Further study including more genotyped individuals.

Having access to the genomes of the mothers could significantly facilitate the detection of the effect inbreeding has on offspring. Indeed, maternal effects are stronger than paternal effects during the early stages of embryonic and larval development. Paternal effects increase later in the development of the fish larva (Clark et al., 2014). Having maternal genomes in addition to paternal would increase the chances of detecting the effect of inbreeding on pathogen response. In addition, having a larger sample size would increase the detection power for the effects of inbreeding. Finally, our study focused on a single population that we knew would be likely to suffer from inbreeding due to previous research with RAD sequencing (de Guttry et al., N.D.), but this resulted in all of the sires having similar level of inbreeding (0.18). A study comparing this population with a less inbred one would help strengthen our findings and possibly uncover effects that were too small to detect with our single population.

It would be interesting to see a study done that includes a larger number of genotyped individuals - both male and female - from two or more populations. With a similar experimental design for the offspring, with full factorial breeding and a controlled pathogen treatment, such a study would be able to shine a light on more attributes of inbreeding depression and how long ROHs describe it. As shown by Su et al. (1996), inbreeding seems to affect fertility traits in salmonid females, so we could expect more traits of early embryo development to be affected. Keeping a database of genotyped individuals may also help put into place breeding programs that optimize population health and help maintain genetic diversity.

Long ROHs seem to affect body size of the individuals carrying them, with more inbred males being smaller than less inbred ones. This could affect the population turnover rate by allowing smaller, more inbred individuals a second breeding season. Lake Hallwil is under high fishing pressure, with fishing limited by fish size, so this advantage to smaller males is likely to be exacerbated by artificial selection, thus worsening the average inbreeding of the population. Monitoring whitefish inbreeding and setting up effective breeding programs seem crucial in order to avoid the population size and health diminishing.

5 Acknowledgements

I wish to pay special regards to Prof. Claus Wedekind for his guidance and for giving me the opportunity to work on this amazing project. I would like to express my deepest gratitude to Christian de Guttry for his gentle guidance, immensely insightful advice and unwavering faith in my capabilities. For their constant support, the hours we spend working together and boundless camaraderie, I want to thank my colleagues from CEE Alexandros Topaloudi and Marianne Bachmann Salvy. For their encouragement, advice and support, thank you to Roberto Sasek Martins, Dariush Mollet, Julia Wildi, Cynthia Meizoso, Elettra Poli, Jasper Evans-Klock, Mirko Ondras, Catherine Atherton and Robert Atherton-Diao. Finally, for bringing cheer and comfort during the harder stretch with beautiful tales of fantasy, many thanks to Brandon Sanderson, Sir Terry Pratchett and Ilona Andrews.

6 References

A. Abdellaoui, J.-J. Hottenga, G. Willemsen, M. Bartels, T. V. Beijsterveldt, E. A. Ehli, G. E. Davies, A. Brooks, P. F. Sullivan, B. W. J. H. Penninx, et al. Educational attainment influences levels of homozygosity through migration and assortative mating. *PLoS One*, 10(3):e0118935, 2015.

G. Alvarez, C. Quinteiro, and F. C. Ceballos. Inbreeding and genetic disorder. *Advances in the study of genetic disorders. Croatia: In Tech*, pages 21–44, 2011.

S. Andrews, F. Krueger, A. Segonds-Pichon, L. Biggins, C. Krueger, and S. Wingett. FastQC. Babraham Institute, Jan. 2012. URL `https://www.bioinformatics.babraham.ac.uk/projects/fastqc/`.

G. Auwera. MS Windows NT kernel description, 2013. URL `https://gatkforums.broadinstitute.org/gatk/discussion/2806/howto-apply-hard-filters-to-a-call-set`. Online; accessed 13-June-2019.

J. Ballou. Calculating inbreeding coefficients from pedigrees. *Genetics and conservation: a reference for managing wild animal and plant populations*, 509:520, 1983.

D. Bates, M. Mächler, B. Bolker, and S. Walker. Fitting linear mixed-effects models using lme4, 2014.

M. A. Beaumont. Adaptation and speciation: what can f_{ST} tell us? *Trends in Ecology & Evolution*, 20(8):435 – 440, 2005. ISSN 0169-5347. doi: https://doi.org/

10.1016/j.tree.2005.05.017. URL http://www.sciencedirect.com/science/article/pii/S0169534705001692.

Y. Benjamini and Y. Hochberg. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57(1):289–300, 1995. doi: 10.1111/j.2517-6161.1995.tb02031.x. URL https://rss.onlinelibrary.wiley.com/doi/abs/10.1111/j.2517-6161.1995.tb02031.x.

D. Bittner, L. Excoffier, and C. R. Largiadèr. Patterns of morphological changes and hybridization between sympatric whitefish morphs (coregonus spp.) in a swiss lake: a role for eutrophication? *Molecular ecology*, 19(10):2152–2167, 2010.

J. M. Bland and D. G. Altman. Multiple significance tests: the bonferroni method. *Bmj*, 310 (6973):170, 1995.

B. W. Brook, N. S. Sodhi, and C. J. Bradshaw. Synergies among extinction drivers under global change. *Trends in Ecology & Evolution*, 23(8):453 – 460, 2008. ISSN 0169-5347. doi: https://doi.org/10.1016/j.tree.2008.03.011. URL http://www.sciencedirect.com/science/article/pii/S016953470800195X.

S. R. Browning and B. L. Browning. Identity by descent between distant relatives: detection and applications. *Annual review of genetics*, 46:617–633, 2012.

F. C. Calboli, J. Sampson, N. Fretwell, and D. J. Balding. Population structure and inbreeding from pedigree analysis of purebred dogs. *Genetics*, 179(1):593–601, 2008.

J. Catchen, P. A. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko. Stacks: an analysis tool set for population genomics. *Molecular ecology*, 22(11):3124–3140, 2013.

F. C. Ceballos, P. Joshi, D. W. Clark, M. Ramsay, and J. F. Wilson. Runs of homozygocity: windows into population history and trait architecture. *Nature Reviews Genetics*, 19: 220–234, 2018. URL https://www.nature.com/articles/nrg.2017.109.

D. Charlesworth and J. H. Willis. The genetics of inbreeding depression. *Nature reviews genetics*, 10(11):783, 2009.

M. R. Christie, R. A. French, M. L. Marine, and M. S. Blouin. How Much Does Inbreeding Contribute to the Reduced Fitness of Hatchery-Born Steelhead (Oncorhynchus mykiss) in the Wild? *Journal of Heredity*, 105(1):111–119, 11 2013. ISSN 0022-1503. doi: 10.1093/jhered/est076. URL https://doi.org/10.1093/jhered/est076.

E. S. Clark, L. G. Wilkins, and C. Wedekind. Mhc class i expression dependent on bacterial

infection and parental factors in whitefish embryos (salmonidae). *Molecular ecology*, 22 (20):5256–5269, 2013.

E. S. Clark, M. Pompini, L. Marques da Cunha, and C. Wedekind. Maternal and paternal contributions to pathogen resistance dependent on development stage in a whitefish (salmonidae). *Functional Ecology*, 28(3):714–723, 2014. doi: 10.1111/1365-2435.12214. URL https://besjournals.onlinelibrary.wiley.com/doi/abs/10.1111/1365-2435.12214.

I. Curik, M. Ferenčaković, and J. Sölkner. Inbreeding and runs of homozygosity: a possible solution to an old problem. *Livestock Science*, 166:26–34, 2014.

P. Danecek, A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, et al. The variant call format and vcftools. *Bioinformatics*, 27(15):2156–2158, 2011.

P. David. Heterozygosity–fitness correlations: new perspectives on old problems. *Heredity*, 80(5):531, 1998.

C. de Guttry, D. L. Jeffries, R. Sermier, and C. Wedekind. Genetic load affects female sexual ornaments and offspring performance in whitefish. unpublished, N.D.

R. De-Kayne, S. Zoller, and P. G. D. Feulner. A de novo chromosome-level genome assembly of coregonus sp. "balchen": one representative of the swiss alpine whitefish radiation. *bioRxiv*, 2019. doi: 10.1101/771600. URL https://www.biorxiv.org/content/early/2019/09/16/771600.

G. E. Dickerson. INBREEDING AND HETEROSIS IN ANIMALS. *Journal of Animal Science*, 1973(Symposium):54–77, 01 1973. ISSN 0021-8812. doi: 10.1093/ansci/1973.Symposium.54. URL https://doi.org/10.1093/ansci/1973.Symposium.54.

C. Enz, H. Bürgi, F. Stössel, and R. Müller. Food preference of adult whitefish in eutrophic lake hallwil (switzerland), and the question of cannibalism. *Archiv Für Hydrobiologie*, pages 81–98, 2001.

W. Forstmeier, H. Schielzeth, J. C. Mueller, H. Ellegren, and B. Kempenaers. Heterozygosity–fitness correlations in zebra finches: microsatellite markers can be better than their reputation. *Molecular Ecology*, 21(13):3237–3249, 2012. doi: 10.1111/j.1365-294X.2012.05593.x. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-294X.2012.05593.x.

J. W. Gibbons, D. E. Scott, T. J. Ryan, K. A. Buhlmann, T. D. Tuberville, B. S. Metts, J. L. Greene, T. Mills, Y. Leiden, S. Poppy, and C. T. Winne. The Global Decline of

Reptiles, Déjà Vu Amphibians: Reptile species are declining on a global scale. Six significant threats to reptile populations are habitat loss and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change. *BioScience*, 50(8):653–666, 08 2000. ISSN 0006-3568. doi: 10.1641/0006-3568(2000)050[0653:TGDORD]2.0.CO;2. URL https://doi.org/10.1641/0006-3568(2000)050[0653:TGDORD]2.0.CO;2.

C. J. Goodnight. On the effect of founder events on epistatic genetic variance. *Evolution*, 41(1):80–91, 1987. doi: 10.1111/j.1558-5646.1987.tb05772.x. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1558-5646.1987.tb05772.x.

C. Grossen, I. Biebach, S. Angelone-Alasaad, L. F. Keller, and D. Croll. Population genomics analyses of european ibex species show lower diversity and higher inbreeding in reintroduced populations. *Evolutionary Applications*, 11(2):123–139, 2018. doi: 10.1111/eva.12490. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/eva.12490.

M. Kardos, M. Åkesson, T. Fountain, Ø. Flagstad, O. Liberg, P. Olason, H. Sand, P. Wabakken, C. . Wikenros, and H. Ellegren. Genomic consequences of intensive inbreeding in an isolated wolf population. *Nature Ecology & Evolution*, 2:124–131, 2018. URL https://www.nature.com/articles/s41559-017-0375-4.

M. C. Keller, P. M. Visscher, and M. E. Goddard. Quantification of inbreeding due to distant ancestors and its detection using dense single nucleotide polymorphism data. *Genetics*, 189(1):237–249, 2011.

M. Kitano. Updating of oecd guidelines for the testing of chemicals. *Water Science and Technology*, 25(11):465, 1992.

B. J. Knaus and N. J. Grünwald. VCFR: a package to manipulate and visualize variant call format data in R. *Molecular Ecology Resources*, 17(1):44–53, 2017. ISSN 757. URL http://dx.doi.org/10.1111/1755-0998.12549.

K. L. Laidre, I. Stirling, L. F. Lowry, O. Wiig, M. P. Heide-Jørgensen, and S. H. Ferguson. Quantifying the sensitivity of arctic marine mammals to climate-induced habitat change. *Ecological Applications*, 18(sp2):S97–S125, 2008. doi: 10.1890/06-0546.1. URL https://esajournals.onlinelibrary.wiley.com/doi/abs/10.1890/06-0546.1.

P. Laurent. Lac léman: Effects of exploitation, eutrophication, and introductions on the salmonid community. *Journal of the Fisheries Board of Canada*, 29(6):867–875, 1972.

G. Leroy and R. Baumung. Mating practices and the dissemination of genetic disorders in domestic animals, based on the example of dog breeding. *Animal Genetics*, 42(1):

66–74, 2011. doi: 10.1111/j.1365-2052.2010.02079.x. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2052.2010.02079.x.

A.-L. Leutenegger, B. Prum, E. Génin, C. Verny, A. Lemainque, F. Clerget-Darpoux, and E. A. Thompson. Estimation of the inbreeding coefficient through use of genomic data. *The American Journal of Human Genetics*, 73(3):516–523, 2003.

H. Li. Aligning sequence reads, clone sequences and assembly contigs with bwa-mem. *arXiv preprint arXiv:1303.3997*, 2013.

H. Li, B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. The sequence alignment/map format and samtools. *Bioinformatics*, 25(16): 2078–2079, 2009.

J. W. MacCluer, A. J. Boyce, B. Dyke, L. R. Weitkamp, D. W. Pfenning, and C. J. Parsons. Inbreeding and pedigree structure in Standardbred horses. *Journal of Heredity*, 74(6): 394–399, 11 1983. ISSN 0022-1503. doi: 10.1093/oxfordjournals.jhered.a109824. URL https://doi.org/10.1093/oxfordjournals.jhered.a109824.

A. McKenna, M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, D. Altshuler, S. Gabriel, M. Daly, et al. The genome analysis toolkit: a mapreduce framework for analyzing next-generation dna sequencing data. *Genome research*, 20(9):1297–1303, 2010.

R. McQuillan, A.-L. Leutenegger, R. Abdel-Rahman, C. S. Franklin, M. Pericic, L. Barac-Lauc, N. Smolej-Narancic, B. Janicijevic, O. Polasek, A. Tenesa, A. K. MacLeod, S. M. Farrington, P. Rudan, C. Hayward, V. Vitart, I. Rudan, S. H. Wild, M. G. Dunlop, A. F. Wright, H. Campbell, and J. F. Wilson. Runs of homozygosity in european populations. *The American Journal of Human Genetics*, 83(3):59 – 372, 2008. doi: https://doi.org/10.1016/j.ajhg.2008.08.007. URL http://www.sciencedirect.com/science/article/pii/S000292970800445X.

R. McQuillan, N. Eklund, N. Pirastu, M. Kuningas, B. P. McEvoy, T. Esko, T. Corre, G. Davies, M. Kaakinen, L.-P. Lyytikäinen, et al. Evidence of inbreeding depression on human height. *PLoS genetics*, 8(7):e1002655, 2012.

T. Newbold, L. N. Hudson, S. L. Hill, S. Contu, I. Lysenko, R. A. Senior, L. Börger, D. J. Bennett, A. Choimes, B. Collen, et al. Global effects of land use on local terrestrial biodiversity. *Nature*, 520(7545):45, 2015.

P. Nietlisbach, S. Muff, J. M. Reid, M. C. Whitlock, and L. F. Keller. Nonequivalent lethal equivalents: Models and inbreeding metrics for unbiased estimation of inbreed-

ing load. *Evolutionary Applications*, 0(0), 2018. doi: 10.1111/eva.12713. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/eva.12713.

T. J. Pemberton, D. Absher, a. R. M. M. M. W. Feldman, and a. J. Z. L. N. A. Rosenberg. Genomic patterns of homozygosity in worldwide human populations. *The American Journal of Human Genetics*, 91(2):275–292, 2012.

S. Purcell, B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. De Bakker, M. J. Daly, et al. Plink: a tool set for whole-genome association and population-based linkage analyses. *The American journal of human genetics*, 81(3): 559–575, 2007.

R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical computing, Vienna, Austria, 2018. URL https://www.R-project.org/.

K. Ralls, K. Brugger, and J. Ballou. Inbreeding and juvenile mortality in small populations of ungulates. *Science*, 206(4422):1101–1103, 1979. ISSN 0036-8075. doi: 10.1126/science.493997. URL https://science.sciencemag.org/content/206/4422/1101.

D. H. Reed, E. H. Lowe, D. A. Briscoe, and R. Frankham. Fitness and adaptation in a novel environment: Effect of inbreeding, prior environment, and lineage. *Evolution*, 57(8):1822–1828, 2003. doi: 10.1111/j.0014-3820.2003.tb00589.x. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/j.0014-3820.2003.tb00589.x.

J. Slate, P. David, K. Dodds, B. Veenvliet, B. Glass, T. Broad, and J. McEwan. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity*, 93(3):255, 2004.

P. Stam. The distribution of the fraction of the genome identical by descent in finite random mating populations. *Genetics Research*, 35(2):131–155, 1980.

J. D. Storey. *False Discovery Rate*, pages 504–508. Springer Berlin Heidelberg, Berlin, Heidelberg, 2011. ISBN 978-3-642-04898-2. doi: 10.1007/978-3-642-04898-2_248. URL https://doi.org/10.1007/978-3-642-04898-2_248.

G.-S. Su, L.-E. Liljedahl, and G. A. Gall. Effects of inbreeding on growth and reproductive traits in rainbow trout (oncorhynchus mykiss). *Aquaculture*, 142(3-4):139–148, 1996.

M. Szulkin, N. Bierne, and P. David. Heterozygosity-fitness correlations: A time for reappraisal. *Evolution*, 64(5):1202–1217, 2010. doi: 10.1111/j.1558-5646.2010.00966.x. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1558-5646.2010.00966.x.

E. A. Thompson. Identity by descent: variation in meiosis, across genomes, and in populations. *Genetics*, 194(2):301–326, 2013.

G. A. Van der Auwera, M. O. Carneiro, C. Hartl, R. Poplin, G. Del Angel, A. Levy-Moonshine, T. Jordan, K. Shakir, D. Roazen, J. Thibault, et al. From fastq data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. *Current protocols in bioinformatics*, 43(1):11–10, 2013.

B. A. von Siebenthal, A. Jacob, and C. Wedekind. Tolerance of whitefish embryos to pseudomonas fluorescens linked to genetic and maternal effects, and reduced by previous exposure. *Fish & Shellfish Immunology*, 26(3):531 – 535, 2009. ISSN 1050-4648. doi: https://doi.org/10.1016/j.fsi.2009.02.008. URL http://www.sciencedirect.com/science/article/pii/S1050464809000527.

J. Wang. Pedigrees or markers: Which are better in estimating relatedness and inbreeding coefficient? *Theoretical population biology*, 107:4–13, 2016.

S. Wang, J. J. Hard, and F. Utter. Salmonid inbreeding: a review. *Reviews in Fish Biology and Fisheries*, 11(4):301–319, 2002.

C. Wedekind and R. Müller. Parental characteristics versus egg survival: towards an improved genetic management in the supportive breeding of lake whitefish. In *Annales Zoologici Fennici*, pages 105–115. JSTOR, 2004.

C. Wedekind, R. Müller, and H. Spicher. Potential genetic benefits of mate selection in whitefish. *Journal of Evolutionary Biology*, 14(6):980–986, 2001.

H. Wickham. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York, 2016. ISBN 978-3-319-24277-4. URL https://ggplot2.tidyverse.org.

H. Wickham, M. Averick, J. Bryan, W. Chang, L. D. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. L. Pedersen, E. Miller, S. M. Bache, K. Müller, J. Ooms, D. Robinson, D. P. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, and H. Yutani. Welcome to the tidyverse. *Journal of Open Source Software*, 4(43):1686, 2019. doi: 10.21105/joss.01686.

S. Wright. The genetical structure of populations. *Annals of Eugenics*, 15:323–354, 1949. doi: 10.1111/j.1469-1809.1949.tb02451.x. URL https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1469-1809.1949.tb02451.x.

R. Xie, L. Shi, J. Liu, T. Deng, L. Wang, Y. Liu, and F. Zhao. Genome-wide scan for runs of homozygosity identifies candidate genes in three pig breeds. *Animals*, 9(8):518, 2019.

Appendices

FDR Analysis

Table A1: FDR Analysis for 6 Models, with minimal p-value necessary for significance according to p-value rank

P-value SignificanceThreshold	Sire F_{ROH}	**Offspring** F_{ROH}	**Offpsring Treatment**
0.0083	0.054	0.122	$< 2.2e^{-16}$
0.0167	0.197	0.138	$< 2.2e^{-16}$
0.0250	0.254	0.154	$6.6e^{-13}$
0.0333	0.354	0.510	$9.1e^{-6}$
0.0417	0.962	0.610	0.0126
0.0500	0.994	0.640	0.0150

SNP and ROH Distribution

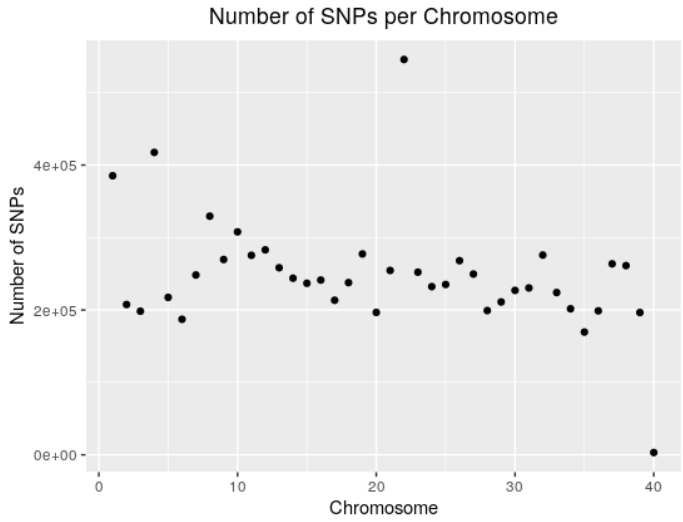


Figure A1: Number of SNPs on each of the 40 chromosomes, comprising SNPs of every genotyped individual (14 males)

Table A2: The distribution of ROHs on each chromosome, for each category. Short ROHs measure between 0.1MB and 0.5MB, medium ROHs between 0.5MB and 1MB, and long ROHs are 1MB and longer

Chromosome	Long ROHs	Medium ROHs	Short ROHs	Total ROHs
01	49	156	1473	1678
02	9	37	817	863
03	7	34	796	837
04	48	90	1076	1214
05	14	37	719	770
06	11	37	774	822
07	31	50	857	938
08	12	30	1045	1087
09	19	68	1033	1120
10	16	50	869	935
11	39	82	909	1030
12	22	92	981	1095
13	39	57	838	934
14	12	36	724	772
15	17	59	912	988
16	18	69	856	943
17	12	65	897	974
18	22	58	929	1009
19	22	69	944	1035
20	41	70	801	912
21	19	70	839	912
22	6	4	302	312
23	10	64	888	962
24	20	38	817	875
25	27	74	834	935
26	6	65	862	933
27	10	45	740	795
28	1	9	629	639
29	20	68	878	966
30	9	53	795	857
31	13	46	846	905
32	0	1	427	428
33	10	52	771	833
34	9	37	674	720
35	3	24	678	705
36	13	48	737	798
37	14	56	578	648
38	0	3	221	224
39	3	15	486	504

Table A2 Continued : The distribution of ROHs on each chromosome, for each category. Short ROHs measure between 0.1MB and 0.5MB, medium ROHs between 0.5MB and 1MB, and long ROHs are 1MB and longer

40	2	2	15	19

Table A3: The distribution of ROHs and inbreeding coefficient for every individual, for each category. Short ROHs measure between 0.1MB and 0.5MB, medium ROHs between 0.5MB and 1MB, and long ROHs are 1MB and longer

Sire ID	F_{ROH}	Total ROHs	Long ROHs	Medium ROHs	Short ROHs
1102	0.03432474	2422	49	130	2243
1103	0.03775288	2458	48	135	2275
1105	0.04936936	2479	64	153	2262
1106	0.02460141	2522	30	150	2342
1107	0.04520151	2495	59	165	2271
1108	0.05546829	2432	64	160	2208
1109	0.04075298	2427	51	160	2216
1110	0.03261868	2373	43	132	2198
1111	0.02928032	2404	40	149	2215
1112	0.01807454	2182	23	108	2051
1113	0.03930336	2410	47	127	2236
1114	0.03140822	2380	43	137	2200
1115	0.03863569	2419	51	154	2214
1117	0.03217140	2539	43	160	2336

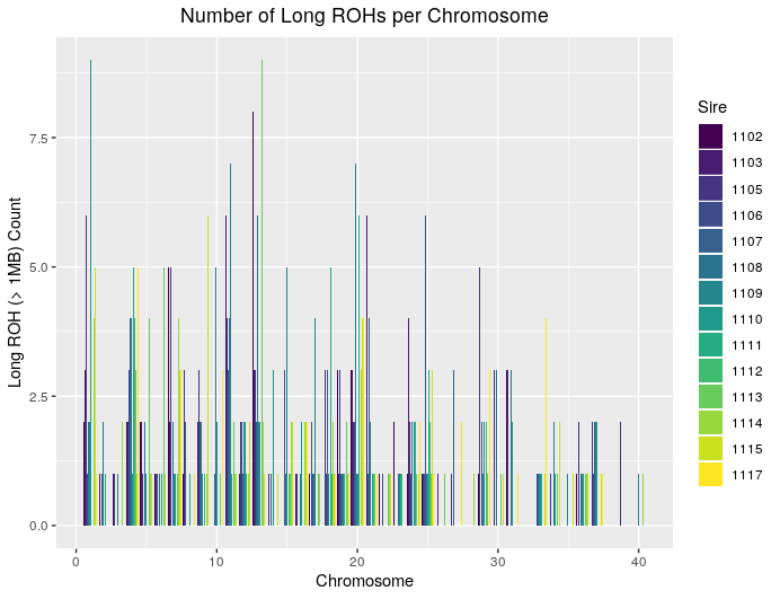


Figure A2: Proportion of chromosome covered by long ROHs for every genotyped individual, ROHs measured between 1MB and 8.1MB, each colour represents one of the genotyped individuals

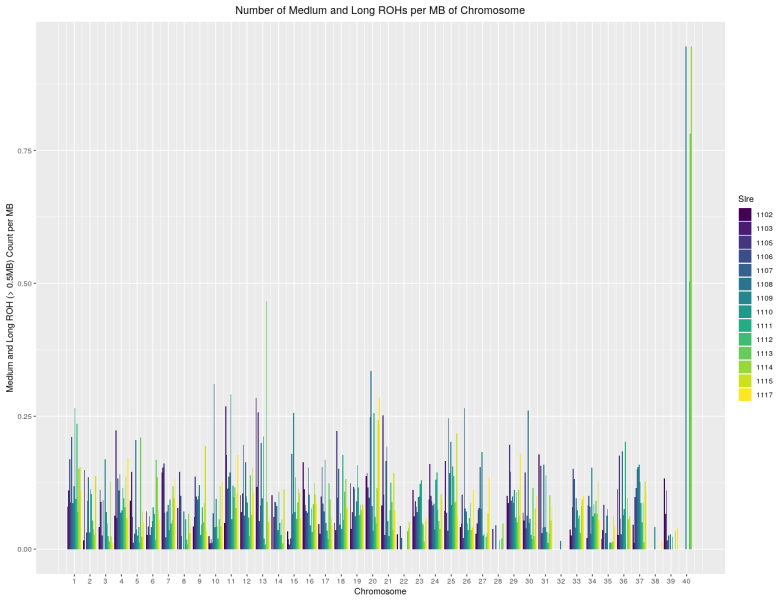


Figure A3: Proportion of chromosome covered by medium and long ROHs for every genotyped individual, ROHs measured between 0.5MB and 8.1MB, each colour represents one of the genotyped individuals

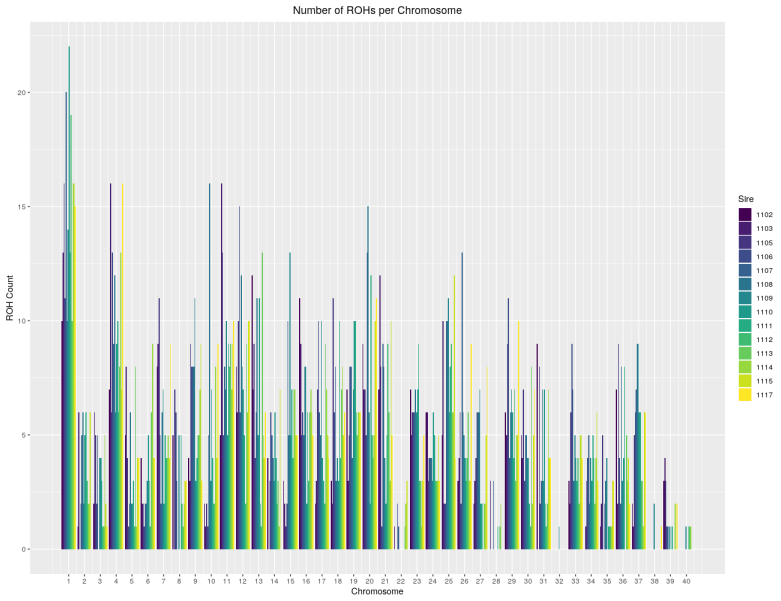
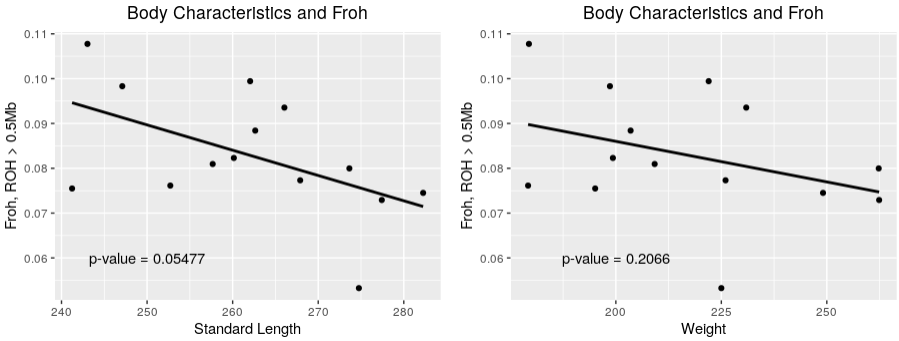


Figure A4: Number of medium and long ROH pe chromosome as averaged over all 14 genotyped individuals, ROHs measured between 0.5MB and 8.1MB, each colour represents one of the genotyped individuals

Models with medium and long ROHs

Effects on Sires

Body Characteristics

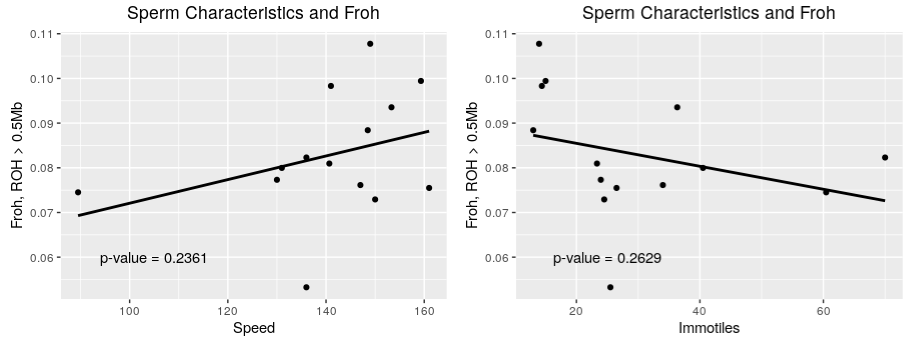


(a) Standard Length (mm)

(b) Weight (g)

Figure A5: Body characteristics of sires in relation with F_{ROH} for ROH≥0.5MB, trend line obtained through a LM

Sperm Characteristics

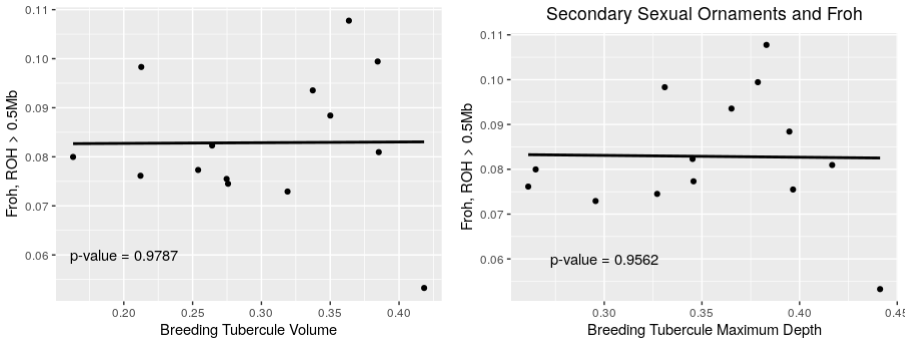


(a) Speed ($\mu m/s$)

(b) Immotiles (cells)

Figure A6: Sperm characteristics of sires in relation with F_{ROH} for ROH≥0.5MB, trend line obtained through a LM

Breeding Tubercle Characteristics



(a) Volume (mm^3) **(b)** Maximum Depth (mm)

Figure A7: Breeding tubercle characteristics of sires in relation with F_{ROH} for ROH$\geq$0.5MB, trend line obtained through a LM

Effect of paternal inbreeding on offspring early life history traits

Table A4: The effect of treatment (exposure to PF), sire F_{ROH} (medium and long ROH, ROH $\geq$ 0.5MB), sire and dam identity on : **(A)** Egg volume before hatching, **(B)** Hatching time, **(C)** Yolk sac volume, **(D)** Length on hatching day, **(E)** Length on day 21 and **(F)** Growth rate between hatching day and day 21.Likelihood ratio tests on mixed model regressions were used to compare a reference model (in bold) with models including or lacking the term of interest. Significant effects are highlighted in bold.

Model	Effect Tested	AIC	d.f.	χ^2	P-value
(A) Egg Volume Before Hatching					
Ref : F_{ROH} + Treatment + Sire + Dam		8027.172	6		
F_{ROH} + Treatment + Sire	Dam	9214.792	5	1189	**<0.001**
F_{ROH} + Treatment + Dam	Sire	8031.028	5	5.86	**0.015**
F_{ROH} + Sire + Dam	Treatment	8600.522	5	575	**<0.001**
Treatment + Sire + Dam	F_{ROH}	8028.071	5	2.90	0.089
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	8031.083	8	0.09	0.956
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	8028.173	7	0.99	0.317
(B) Hatching Time					
Ref : F_{ROH} + Treatment + Sire + Dam		19254.071	6		
F_{ROH} + Treatment + Sire	Dam	20136.10	5	884	**<0.001**
F_{ROH} + Treatment + Dam	Sire	19853.268	5	601	**<0.001**
F_{ROH} + Sire + Dam	Treatment	19357.253	5	105	**<0.001**

Table A4 : The effect of treatment (exposure to PF), sire F_{ROH} (medium and long ROH, ROH $\geq$ 0.5MB), sire and dam identity on : **(A)** Egg volume before hatching, **(B)** Hatching time, **(C)** Yolk sac volume, **(D)** Length on hatching day, **(E)** Length on day 21 and **(F)** Growth rate between hatching day and day 21.Likelihood ratio tests on mixed model regressions were used to compare a reference model (in bold) with models including or lacking the term of interest. Significant effects are highlighted in bold.

Treatment + Sire + Dam	F_{ROH}	19253.611	5	1.54	0.215
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	19257.087	8	0.98	0.611
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	19254.987	7	1.08	0.298
(C) Yolk Sac Volume					
Ref : F_{ROH} + Treatment + Sire + Dam		82.5066	6		
F_{ROH} + Treatment + Sire	Dam	696.5270	5	616	**<0.001**
F_{ROH} + Treatment + Dam	Sire	126.7390	5	46	**<0.001**
F_{ROH} + Sire + Dam	Treatment	86.7378	5	6.23	**0.013**
Treatment + Sire + Dam	F_{ROH}	80.7411	5	0.23	0.628
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	86.4252	8	0.08	0.960
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	84.4454	7	0.06	0.805
(D) Length at Hatching Day					
Ref : F_{ROH} + Treatment + Sire + Dam		4582.470	6		
F_{ROH} + Treatment + Sire	Dam	5232.014	5	651	**<0.001**
F_{ROH} + Treatment + Dam	Sire	4669.189	5	88	**<0.001**
F_{ROH} + Sire + Dam	Treatment	4632.107	5	51	**<0.001**
Treatment + Sire + Dam	F_{ROH}	4580.732	5	0.26	0.609
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	4585.814	8	0.66	0.720
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	4583.701	7	0.77	0.380
(E) Length at Day 21					
Ref : F_{ROH} + Treatment + Sire + Dam		4148.221	6		
F_{ROH} + Treatment + Sire	Dam	5477.705	5	1331	**<0.001**
F_{ROH} + Treatment + Dam	Sire	4209.945	5	63	**<0.001**
F_{ROH} + Sire + Dam	Treatment	4152.111	5	5.89	**0.01522**
Treatment + Sire + Dam	F_{ROH}	4146.552	5	0.33	0.5651
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	4151.120	8	1.10	0.5767
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	4148.247	7	1.97	0.16
(F) Growth Rate Until Day 21					
Ref : F_{ROH} + Treatment + Sire + Dam		-9154.301	6		
F_{ROH} + Treatment + Sire	Dam	-9049.239	5	107	**<0.001**
F_{ROH} + Treatment + Dam	Sire	-9150.639	5	5.66	**0.01733**
F_{ROH} + Sire + Dam	Treatment	-9136.631	5	19	**<0.001**
Treatment + Sire + Dam	F_{ROH}	-9154.858	5	1.44	0.2297
F_{ROH} + Treatment + Treatment X Sire + Dam	Treatment x Sire	-9150.305	8	0.01	0.9979
F_{ROH} + Treatment + F_{ROH} x Treatment + Sire + Dam	F_{ROH} x Treatment	-9152.319	7	0.02	0.8924

Appendix 2 - Male sexual signaling and expected effects of hatchery-induced sperm competition vary with water depth at which whitefish are caught

Giulia Perroud[1], David Nusbaumer[1], Christian de Guttry[1], Claus Wedekind[1]

[1]Department of Ecology and Evolution, Biophore, University of Lausanne, Lausanne, Switzerland

Published manuscript (peer-reviewed): 2021 Current Zoology 4 pp. zoab007, https://doi.org/10.1093/cz/zoab007

Authors contributions

GP and CW designed the study, CdG scanned the fish, GP and CdG determined breeding tubercles volumes and fish age, CW stripped the fish, GP and DN took all milt measurements, and GP and CW analysed the data and wrote the manuscript.

Current Zoology, 2021, 1–4
doi: 10.1093/cz/zoab007
Advance Access Publication Date: 30 January 2021
Letter to the Editor

Letter to the Editor

Male sexual signaling and expected effects of hatchery-induced sperm competition vary with water depth at which whitefish are caught

Giulia Perroud, David Nusbaumer, Christian de Guttry, and Claus Wedekind*

Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland

*Address correspondence to Claus Wedekind. E-mail: claus.wedekind@unil.ch.

Handling editor: Murielle Ålund

Received on 4 September 2020; accepted on 18 January 2021

Key words: sexual signaling, sperm competition, whitefish

Salmonids like whitefish (*Coregonus* spp.) are often propagated in supportive breeding. Spawners are caught from their spawning locations, their gametes mixed, and the resulting offspring reared in a protected environment before being released into the wild. This procedure can affect sexual selection, for example, by enhancing the importance of sperm competition or by reducing the relevance of sexual signals. While it is often unclear how sperm competitiveness is affected by a male's overall genetic quality, there is accumulating evidence that sexual signals reveal good genes and that mate choice based on such signals can increase offspring viability (Auld et al. 2019). Therefore, supportive breeding may affect the genetic variance and the mean genetic quality of next generations. We sampled whitefish from various locations along a depth gradient to test how male characteristics that are likely to affect sexual selection under natural conditions correlate with characteristics that affect hatchery-induced sperm competition. Whitefish are external fertilizers, and multi-male spawning and hence sperm competition is common under natural conditions. Mate choice is not sufficiently understood but could be based on breeding tubercles. These are small conical structures that grow on scales before the breeding season and fall off shortly afterwards. The size of breeding tubercles varies much among males and has repeatedly been found to correlate positively with offspring viability (Wedekind et al. 2001; Kekäläinen et al. 2010). Male–male dominance is typically dependent on body size (Auld et al. 2019) and could also be relevant in whitefish. Body size itself can reflect individual inbreeding coefficients (Su et al. 1996) and be an indicator of heritable genetic quality in small or structured populations (Neff and Pitcher 2008). In another fish with a somewhat comparable mating system, the size of breeding tubercles and male size was not correlated but could both be used to predict male reproductive success under close to natural conditions (Jacob et al. 2009). We study whitefish from Lake Hallwil (Switzerland). This lake has suffered so much from anthropogenic eutrophication that it is being artificially aerated since 1985. Three hatcheries around the lake are likely to have played a key role in maintaining the whitefish population, as concluded also from a recent mark–recapture experiment (Vonlanthen 2015). However, eutrophication combined with possible hybridization in hatcheries can have led to a speciation reversal (Vonlanthen et al. 2012) and may thereby have destroyed any genetic structure linked to water depth. Hatchery protocols now focus on maintaining overall genetic variance by pooling milt of many males before adding the mix to eggs of multiple females. Milt volume varies among sires, for example, because males often loose milt when being pulled up from deep locations (Figure 1), an effect that likely depends on how much the swim bladder is inflated by the change in pressure. This variance in milt volume is likely to affect the genetic variance that, in combination with the average genetic quality, may then affect the long-term survival of a population. The extent to which hatchery protocols affect genetic quality can be estimated by the correlations between male quality indicators and traits that affect hatchery-induced sperm competition, that is, sperm number, velocity, and longevity (summarized here as "milt potency," see also Supplementary Material). Many breeding protocols are likely to promote genetic quality if male attractiveness or dominance are positively correlated to milt potency. If there are no such correlations or negative ones because of life-history trade-offs, hatchery-induced sperm competition is likely to reduce the average genetic quality in future generations. We sampled fish from various depths and determined their age, size, breeding ornamentation, and milt potency (see methods in the Supplementary Material) to test whether and how different male characteristics affect reproductive success in supportive breeding in a heavily managed population.

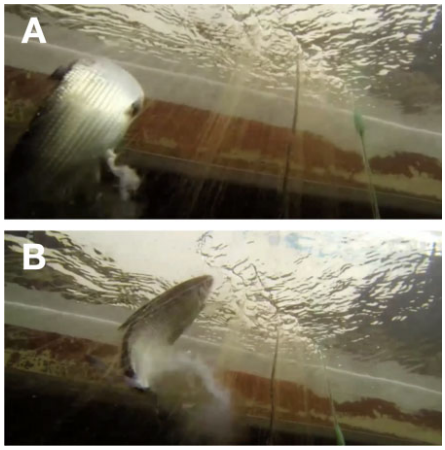


Figure 1. Male being pulled up from deep water and loosing milt just before being lifted into the boat. Photograph **A** also reveals the breeding tubercles on the male's skin. Photograph **B** was taken about half a second after photograph A (©arte tv; reproduced with permission).

The samples taken at 25, 12, and 4 m did not differ in age (Fisher's exact test, $N_1 = 54$, $N_2 = 30$, $N_3 = 14$, $P = 0.58$) nor in sex ratios ($P = 0.28$). The males caught at different depths did also not differ in size (Supplementary Figure S1A). However, the mean size of their breeding tubercles varied with depth (Supplementary Table S1 and Supplementary Figure S1B). The tubercles were significantly larger in males caught at 25 m depth than at 12 m depth (*post hoc* Tukey's HSD test, $P = 0.03$), while no such differences were observed in the other possible comparisons (P always > 0.12). Overall, larger males had larger breeding tubercles (Supplementary Table S1), but this relationship varied with location and was strongest in males caught at the deepest location (Supplementary Table S1 and Supplementary Figure S1C). Sperm velocity did not vary with water depth (measured at 12 versus 4 m for sperm characteristics), increased with body length (but not with age), and was not significantly revealed by the size of the breeding tubercles (Figure 2A–C and Supplementary Tables S2A and S3A). Mean sperm concentration did also not seem to vary with water depth, body length, or breeding tubercles (Figure 2D–G and Supplementary Tables S2 and S3B). Milt potency was significantly reduced in males caught at larger depth (Figure 2G and Supplementary Table S3B) and was overall not significantly linked to body length and breeding tubercles. However, there was a significant interaction between the effects of depth and breeding tubercles on milt potency (Figure 2H–J and Supplementary Table S2B).

The protocols of supportive breeding at Lake Hallwil and most (if not all) other Swiss lakes promote sperm competition (Wedekind et al. 2007). We studied what kind of males would be expected to profit from such hatchery-induced sperm competition. Our samples had similar size and age distributions. Nevertheless, fish caught at the deepest location had the largest breeding tubercles. It remains to be shown whether this difference in breeding ornamentation is due to genetics, environmental factors, or a combination of both. Intralacustrine diversification may have largely vanished during the eutrophication crisis (Vonlanthen et al. 2012) but could now be rebuilding itself so that phenotypic differences reflect genetic differences. Alternatively, if breeding tubercles are generally valid indicators of good genetic quality, fish caught in deeper locations could be on average of better genetic quality than fish caught in more shallow regions. However, the link between body length and breeding ornaments changed also with water depth, suggesting that either the information content of breeding tubercles does not depend on genetic quality alone, or that variance in genetic quality has location-specific effects on growth. We found that larger males had faster sperm, and that sperm velocity could not be predicted by breeding tubercles or water depth. There was no link between male size and milt potency, but we found an interaction between breeding tubercles and depth on milt potency. These observations suggest that there were no trade-offs between male dominance or mate attractiveness and investment into milt. On the contrary, males that are predicted to be successful under natural conditions, because they are large and/or well ornamented, seemed still able to invest more into high-quality sperm than small or less ornamented males, that is, there seemed to be positive correlations between the various fitness-relevant traits (Reznick et al. 2000). It remains to be tested whether such correlations are to be expected in non-managed populations or can be a consequence of hatchery-induced selection in previous generations. Our measure of milt potency was based on sperm number, velocity, and lifespan, but fertilization success can also be affected by the composition of the fertilization media (pH, ovarian fluids, etc.) and possibly further factors that may influence sperm motility. Based on our measurements, we conclude that the current protocol used in supportive breeding (mixing the milt of many males, then adding the mix to the eggs) may give large males and males caught from more shallow regions a reproductive advantage. Alternative breeding protocols would then affect hatchery-induced sperm competition differently: (i) When equalizing milt volume, hatchery-induced sperm competition would be driven by sperm velocity and sperm concentration. (ii) When equalizing cell counts, that is, taking sperm concentration into account, male reproductive success would be largely determined by sperm velocity. (iii) When equalizing milt potency, no type of male would be favored. However, minimizing the effects of milt potency, for example, in full-factorial crossings, would only minimize the loss of genetic variation over time. Genetic quality would not be promoted by such a breeding design. If breeding tubercles and body size are indeed indicators of heritable genetic quality, genetic quality could be promoted in hatcheries by giving large males and/or males with large breeding tubercles a reproductive advantage over small and/or poorly ornamented males. However, with declining effective population sizes (N_e), minimizing the loss of genetic variance become increasingly important. Small N_e therefore require an optimization between minimizing the loss of genetic variance and minimizing the loss of genetic quality in order to maximize a population's long-term survival probability. In conclusion, the consequences of hatchery-induced sperm competition are different for whitefish males caught at different water depths and at different body sizes. If variance in milt potency is ignored in the breeding protocols (as currently in the study population), males caught from more shallow locations are favored over males caught from deeper locations. Breeding protocols that would be based on equalized milt volume or even equalized sperm counts per male would give males with high sperm velocity a reproductive advantage. In our study population, these would be the large males. Any hatchery-induced variance in male reproductive success could be avoided, for example, in full-factorial breeding designs that minimize mean kinship within the next generation. However, minimizing the loss of genetic variance can reduce average genetic quality, because elevating the reproductive success of large and/or well

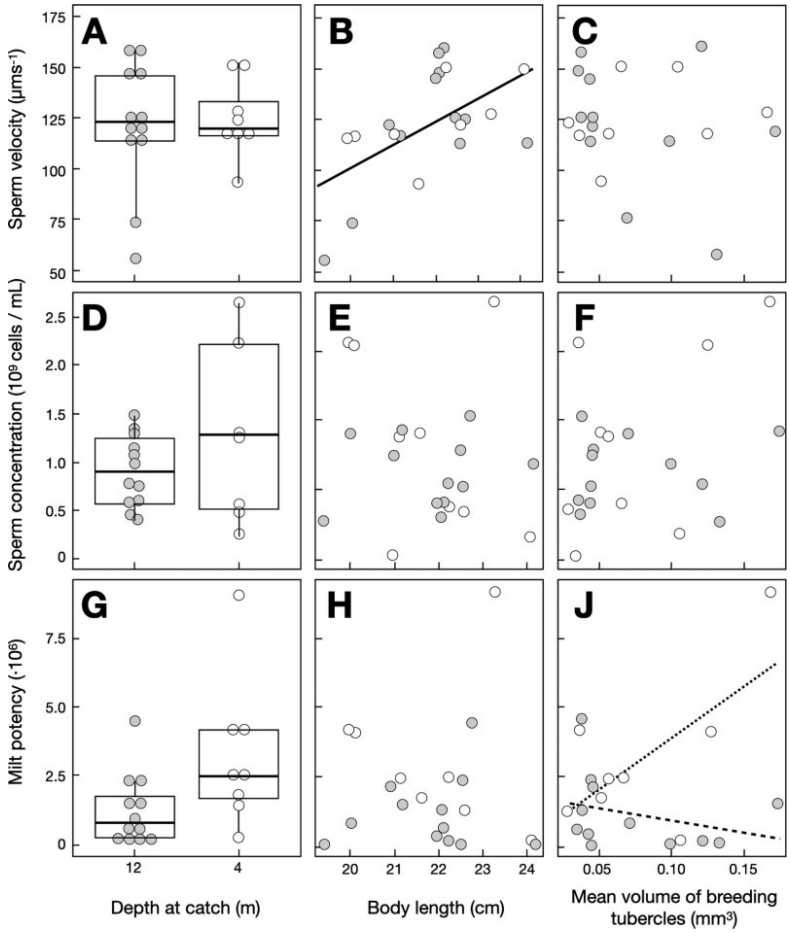


Figure 2. Sperm velocity (**A**–**C**), sperm concentration (**D**–**F**), and milt potency (**G**–**J**) versus water depth, body length, and mean breeding tubercles volume for fish caught at 12 m (gray symbols, dashed regression line) and fish caught at 4 m (open symbols, dotted line). Panels **A**, **D**, and **G** show Tukey boxplots with quartiles and whiskers. The regression line in panel **B** shows the significant relationship between body length and sperm velocity at both depths. The regression lines in panel **J** illustrate the significant interaction between depth and mean breeding tubercles volume on milt potency. No significant links could be observed in panels **C**, **E**, **F**, and **H**. See text for statistics.

ornamented males can positively affect the mean viability of the next generation.

Funding

This work was supported by the Swiss National Science Foundation [31003A_182265].

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgments

The author thank Richard and Noah Stadelmann for catching the fish; David Bittner (Fishery Inspectorate of the Aargau canton) for permission; Oliver Selz for providing some nets; Dany Buffat, Jonas Bylemans, Laura Garaud, Eliane Longange, and Hugo Palejowski for assistance; and Zhi-Yun Jia, Murielle Ålund, and four reviewers for constructive comments.

References

Auld HL, Noakes DLG, Banks MA, 2019. Advancing mate choice studies in salmonids. *Rev Fish Biol Fish* **29**: 249–276.

Jacob A, Evanno G, Renai E, Sermier R, Wedekind C, 2009. Male body size and breeding tubercles are both linked to intrasexual dominance and reproductive success in the minnow. *Anim Behav* **77**: 823–829.

Kekäläinen J, Huuskonen H, Tuomaala M, Kortet R, 2010. Both male and female sexual ornaments reflect offspring performance in a fish. *Evolution* **64**: 3149–3157.

Neff BD, Pitcher TE, 2008. Mate choice for non-additive genetic benefits: a resolution to the lek paradox. *J Theor Biol* **254**: 147–155.

Reznick D, Nunney L, Tessier A, 2000. Big houses, big cars, superfleas and the costs of reproduction. *Trends Ecol Evol* **15**: 421–425.

Su GS, Liljedahl LE, Gall GAE, 1996. Effects of inbreeding on growth and reproductive traits in rainbow trout *Oncorhynchus mykiss*. *Aquaculture* **142**: 139–148.

Vonlanthen P, 2015. *Otolithenmarkierung Der Felchen Vom Hallwilersee – Erfolgskontrolle 2 (1+)*. Aarau, Switzerland: Kanton Aargau, Sektion Jagd und Fischerei.

Vonlanthen P, Bittner D, Hudson AG, Young KA, Müller R et al., 2012. Eutrophication causes speciation reversal in whitefish adaptive radiations. *Nature* **482**: 357–362.

Wedekind C, Müller R, Spicher H, 2001. Potential genetic benefits of mate selection in whitefish. *J Evol Biol* **14**: 980–986.

Wedekind C, Rudolfsen G, Jacob A, Urbach D, Müller R, 2007. The genetic consequences of hatchery-induced sperm competition in a salmonid. *Biol Conserv* **137**: 180–188.