Master's thesis

The role of parental effect and relatedness on early development of vendace and whitefish in short and long winter

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ABSTRACT

In this study, parental effect on embryonic mortality, embryonic growth, hatching time and hatching size of vendace (*Coregonus albula*) and whitefish (*C. lavaretus*) was studied under different climate change conditions. The aim was to distinguish the maternal, paternal and maternal-paternal interaction effects by crossbreeding individual females and males of vendace and whitefish separately. Moreover, the relatedness of the parent pairs of vendace was examined genetically to find out if inbreeding depression could explain the variation in reproduction success between different pairs. The eggs were incubated under short and long winter conditions. Whitefish had significantly lower embryonic mortality rate than vendace, but the different winter conditions did not affect the embryonic mortality of either species. In the full cross-fertilization experiment, the mortality rate of vendace was clearly higher than the mortality in the earlier experiment with the fertilization design simulating mass spawning. In whitefish experiments there was no difference in mortality between the two designs. This supports the possibility that vendace have some kind of post-spawning fertilization control mechanism. Water temperature was the main factor influencing the hatching time of larvae, but within the time range set by the temperature, parental origin and especially the female also had an effect. All the vendace parents that had very low reproduction success also had very high or low inbreeding coefficients (F). Therefore, it seems that for vendace, the individuals' inbreeding coefficient is related to its reproduction success.

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TIIVISTELMÄ

Tutkimuksessa testattiin ilmastonmuutoksen vaikutuksia muikun (*Coregonus albula*) ja siian (*C. lavaretus*) alkioiden kuolevuuteen, alkioiden kasvuun, kuoriutumisaikaan ja kuoriutumiskokoon. Koejärjestely toteutettiin sekä muikulla että siialla yksilöllisinä ristiinhedelmöityksinä tavoitteena selvittää naaraan ja koiraan vaikutusta sekä niiden yhdysvaikutusta mitattaviin muuttujiin. Sen lisäksi muikun emokalojen sukulaisuutta tutkittiin mikrosatelliittien avulla, jotta nähtäisiin voiko sisäsiitosdepressio selittää eroja jälkeläisten kuolevuudessa eri emoparien välillä. Munia haudottiin lyhyen talven sekä pitkän talven lämpöoloissa. Siian alkioiden kuolleisuus oli huomattavasti alhaisempaa kuin muikun alkioiden, mutta eri lämpötilaolot eivät vaikuttaneet kummankaan lajin alkioiden kuolleisuuteen. Täydessä ristihedelmöityskokeessa muikun alkioiden kuolleisuus oli huomattavasti korkeampaa kuin aiemmassa massakutua (yksi naaras ja useita koiraita) jäljitelleessä koejärjestelyssä. Siialla tällaista eroa kuolleisuudessa ei eri koejärjestelyiden välillä ollut. Tulokset antavat viitteitä, että muikulla on jonkinlainen kudun jälkeen tapahtuva hedelmöittymisen kontrollointimekanismi. Poikasten kuoriutumisajankohta määräytyi lähinnä veden lämpötilan perusteella, mutta lämpötilan määrittämän kuoriutumisjakson sisällä myös vanhemmat, erityisesti naaras, vaikuttivat poikasten tarkkaan kuoriutumisajankohtaan. Kaikilla muikkuvanhemmilla, joiden lisääntymismenestys oli alhainen, oli myös joko hyvin korkea tai hyvin matala sisäsiitoskerroin (F), joten vaikuttaa siltä, että muikulla kutevien kalojen sukulaisuusaste voi vaikuttaa sen lisääntymistulokseen.

Contents

1. INTRODUCTION

Embryonic and larval periods are the most sensitive life stages of fish (Miller *et al*. 1988). In many fish populations, large proportion of natural mortality takes place in these early stages (Bradford and Cabana 1997). During embryogenesis mortality is mostly due to developmental disorders or pathogen infections (Wedekind *et al*. 2001), and after hatching larval mortality is mainly due to starvation or predation (Fuiman and Magurran 1994, Fuiman and Cowan Jr 2003). Fish are also more vulnerable to environmental interactions in the early life-stages than in adulthood (Finn 2007). Many different physiological and parental factors can affect fertilization success, early development of embryos, and larval survival (Alderdice and Velsen 1978, Brooks *et al*. 1997, Kamler 2005). Because of the high selection pressures in the early development of fish, an understanding of the individual variability in offspring responses is useful for predicting how populations may respond or adapt to environmental change (Hutchings 2004).

Development rate during the egg and yolk-sac larval stages is mostly determined by the water temperature (Alderdice and Velsen 1978, Luczynski and Kirklewska 1984). Temperature affects the duration of these vulnerable ontogenetic stages and also many important traits, such as the size at hatching and feeding onset, efficiency of yolk utilization, deformities, gender and survival of the larvae (Polo *et al*. 1991, Koumoundouros *et al*. 2001, 2002; Baird *et al*. 2002, Kavanaugh *et al*. 2010). Parental genotype and phenotype can also influence the early development of fish (Bernardo 1996, Wedekind *et al*. 2001, Wedekind and Müller 2004). Parental effect can cause large variation among individual larvae in important traits, such as size and performance (Probst *et al*. 2006, Kekäläinen *et al*. 2010). Such variation within a population can lead to large divergence in survival probability among individual larvae, and therefore, parental effect can have a significant influence on recruitment (Miller *et al*. 1988). Maternal effect is usually more pronounced in fish than is paternal effect, especially on fertilization success, embryonic survival, and size and composition of yolk (Nagler *et al*. 2000, Kennedy *et al*. 2007, Huuskonen *et al*. 2011). It can also influence the temperature tolerance of offspring (Huuskonen *et al*. 2011). However, paternal effects have been shown to influence, for example, larval length, egg survival, and immunity against bacterial infections (Wedekind *et al*. 2001, Wedekind and Müller 2004; Bang *et al*. 2006). Also, maternal-paternal interaction can play a significant role in influencing embryonic survival and offspring quality (Trippel *et al*. 2005, Kekäläinen *et al*. 2010, Huuskonen *et al*. 2011).

The importance of water temperature on hatching time of fish has been well acknowledged. In autumn-spawning fish, such as vendace (*Coregonus albula*) and whitefish (*C. lavaretus*), an increase in water temperature in spring determines the onset of hatching, which in the lakes is also well synchronized with the ice-out (Karjalainen *et al*. 2002, Urpanen *et al*. 2005). The start of the water warming under ice also synchronizes many processes related to feeding, such as the start of accelerated spring plankton production (Aberle *et al*. 2012). Therefore, hatching of larvae shortly after ice-out is beneficial for the year-class strength of autumn-spawning species (Nyberg *et al*. 2001). The convergence of hatching with water warming adjusts at least part of offspring hatching to optimal conditions. In contrast, the influence of parental effect on the hatching time of individuals have not been studied much in fish.

This study complements the study of Karjalainen *et al*. (2014). They tested experimentally effects of different climate change scenarios on the hatching time, hatching size, larval growth rate and tolerance against starvation after hatching of both vendace and

whitefish. In their experiments, eggs from the same families (one female x three males per family) were incubated in nine different temperature scenarios predicted by emission scenarios of IPCC (2007). The results showed high flexibility in the egg development and hatching of both species. The results also showed an among-family effect on the hatching time of both vendace and whitefish. However, the effect of females and males could not be separated because of the experimental design (one female x three males) so the significance of the variation between individuals on fertilization success, egg development and hatching time remained unclear.

The aim of this study was to distinguish the maternal, paternal and maternal-paternal interaction effects by crossing individual females and males of vendace and whitefish in a full-factorial breeding design. Parental effect on embryonic mortality, embryonic growth, hatching time and hatching size was studied. The experiment was initially planned to test mainly the parental effect on hatching time, but it also gave an opportunity to study the role of parental relatedness and inbreeding on embryonic survival. The relatedness of the parent pairs of the vendace was examined genetically in order to find out if inbreeding depression could explain the variation in reproduction success of the different pairs, which in some instances was quite large. In the incubation experiment, the eggs were incubated under short and long winter conditions to examine if the parental effects were similar in both temperatures and to continue the climate change study of Karjalainen *et al*. (2014).

2. BACKGROUND

2.1. Parental influence on reproduction success of fish

2.1.1. Parental effect

The parental effects on offspring survival can be divided to genetic influences and to the direct influences of the parent's phenotype on the offspring (Bernardo 1996). Phenotypic influence can be, for example, the nourishment that a female provides to its eggs. In many instances, if genetic and non-genetic effects are not separable the term 'parental effect' refers more generally to any detectable offspring variation attributable to maternal or paternal identity. These effects have been widely studied in fish mainly associated to artificial breeding programs in order to achieve highest possible survival and fitness of the offspring (Wedekind and Müller 2004). A full-factorial breeding design is often used in experimental studies in order to distinguish all, maternal, paternal and maternal-paternal interaction effects (Lynch and Walsh 1998). In a full-factorial breeding design, n number of sires are each mated with n number of dams.

Maternal effect is usually the main determinant of fertilization success and embryonic size and survival (Heath *et al*. 1999, Nagler *et al*. 2000, Huuskonen *et al*. 2011). It has been widely acknowledged that there is a positive correlation between maternal size and egg size of mother and larger egg size usually predicts higher embryonic survival and larger offspring (Chambers and Leggett 2005 Brooks *et al*. 1997, Vandeputte *et al*. 2002, Tamada and Iwata 2005, Kennedy *et al*. 2007). Variation between female spawners has also been shown to cause variation in yolk size and composition (Kamler 2005, Kennedy *et al*. 2007), growth rate (Green and McCormick 2005), metabolic physiology (Pakkasmaa *et al*. 2006) and swimming ability (Garenc *et al*. 1998). Female whitefish can have breeding tubercules that indicate the larvae swimming performance, size and yolk volume and embryonic mortality (Kekäläinen *et al*. 2010, Huuskonen *et al*. 2011). Female age has also been shown to influence the egg size and thus embryonic survival and growth. Typically

females spawning for the first time produce the smallest eggs, females of average age produce the largest eggs and old spawners lay again smaller eggs (Kamler 1992). This relationship is shown for example in vendace (Kamler *et al.* 1982).

In species without male parental care, paternal effects to offspring are mainly genetic, since the only significant contribution of the sperm is DNA (Bang *et al.* 2006). Although there is variation between different species and studies, the general pattern is that female effects are displayed throughout development and are most pronounced in the early life stages of offspring, affecting for example the survival, size and yolk volume of the embryo. Paternal effects usually appear later in development and males have been shown to influence for example later embryonic survival (Wedekind *et al*. 2001), carcass and yolk weight of larvae (Bang *et al*. 2006, Ottesen and Babiak 2007, Kekäläinen *et al*. 2010) and the swimming ability of larvae (Kekäläinen *et al*. 2010).

In some species males advertise their genetic quality, for example via ornamentation, and these qualities can be used to indicate the paternal effects (Wedekind *et al*. 2001, Wedekind and Müller 2004, 2008). Reynolds *et al*. (1992) observed that larger Trinidadian guppy (*Poecilia reticulata*) males sired offspring with higher growth rates. Females also preferred these large-bodied males over small-bodied ones. Wedekind *et al*. (2001) studied optimal mate selection in whitefish and found that males with strong breeding ornamentation sired offspring that better survived bacterial infections during development. Other male characteristics that have been noticed to correlate with embryonic survival are for example melanin-based colors in brown trout (Wedekind *et al.* 2008) and fluctuating asymmetry in whitefish (Wedekind *et al*. 2004). Fluctuating asymmetry is non-directional deviation from perfect bilateral symmetry in traits that are on average bilaterally symmetrical (Palmer and Strobeck 1986), and in the Wedekind and Müller's *et al*. (2004) study, high fluctuating asymmetry indicated low embryonic survival.

The importance of maternal-paternal interactions on the embryonic survival and offspring quality has also been demonstrated in several studies (Trippel *et al*. 2005, Kekäläinen *et al*. 2010, Huuskonen *et al*. 2011, Saillant *et al*. 2001). Such interactions have been shown to influence for example swimming ability, length and survival of larvae. In the study of Trippel *et al*. (2005), embryonic survival, yolk-sac utilization and resistance to starvation of Baltic cod (*Gadus morhua*) were significantly influenced by the female parent and the interaction between both parents.

Temperature is the single most important factor regulating the early development of fish. Besides affecting the quality of embryos and larvae, parental genetic effect can influence the temperature tolerance of the embryos and larvae (reviewed in Burt *et al*. 2011). Beacham and Murray (1985) and Beacham (1988) studied among-family variation in development of chum salmon (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*) under different thermal regimes. They found that the egg and fry survival rates varied between different incubation temperatures according to the family. Huuskonen *et al.* (2003) incubated eggs of Arctic char (*Salvelinus alpinus*) at two temperatures, 7 °C and 2 °C, and found that variability in the embryonic survival could be attributed to the female at both incubation temperatures, whereas the male effect was apparent only at the lower temperature.

The condition of adults and the process of gametogenesis can also be affected by the environment during their reproductive development, providing a means for non-genetic parental effects to be transferred to their offspring (Rossiter 1996). Water temperature experienced by females during oogenesis can affect egg biochemical composition (Atse *et*

al. 2002) and exposure to acute stress during gametogenesis can reduce the gamete and hence larval quality (Campbell *et al*. 1992, McCormick 1998).

2.1.2. Inbreeding and relatedness

Inbreeding refers to a situation where mating occurs among related individuals, which leads to an increase in homozygosity (reviewed in Keller and Waller 2002). It is well recognized that offspring of closely related individuals are less fit than those of less closely related individuals. Inbreeding might also weaken the immune response of the inbred individuals (Reid *et al.* 2007, Spielman *et al.* 2004). This lower fitness of inbred individuals is called inbreeding depression.

Magnitudes of inbreeding and relatedness are estimated with the coefficient of inbreeding, F, that is the probability that two alleles at any locus in an individual are identical by descent and with the coefficient of relatedness, r, that is defined as the proportion of all alleles that two individuals share that are identical by descent due to recent common ancestry. F is therefore an intra-individual measure whereas r is measured between two individuals. In practice, coefficients of inbreeding and relatedness for populations and individuals are often estimated using microsatellite markers. Microsatellites are tandem repeats of 1–6 nucleotides found in the genomes of most taxa. Many microsatellites have high mutation rates and are therefore highly polymorphic, which is necessary for genetic studies of processes acting on ecological time scales (Schlötterer 2000).

Many different estimators have been developed to evaluate inbreeding and relatedness, with different assumptions and uses (Rousset 2002, Blouin 2003, Wang 2014). Some commonly used estimators for inbreeding coefficient are developed by Loiselle *et al*. (1995) and Ritland (1996) and for relatedness Queller and Goodnight's (1989) r and Lynch and Ritland's (1999) r. Inbreeding and relatedness estimators are calculated in reference to an implicit (or explicit) reference population in which all homologous genes within and between individuals are assumed to be not identical by descent (IBD). However, in practice, F and r are often estimated relative to the current sample or population due to lack of information about the true reference population. Wang (2014) investigated some of the relatedness and inbreeding estimators and showed that in marker-based analysis when the current sample is used as a reference population, F and r are better interpreted as correlation coefficients than as the probability of IBD relative to a reference. In such a case, the F and r estimates fall between -1 and 1 and negative values have biological significance.

In fish, effects of inbreeding depression have been studied in several species. Fessehaye *et al*. (2007) studied the effects of different levels of inbreeding (F =0–0.25) on Nile tilapia (*Oreochromis niloticus*) and they found that high levels of inbreeding significantly decreased the survival and body weight of early fry and increased the proportions of deformed fry. Kincaid *et al*. (1976) studied inbreeding in rainbow trout (*O. mykiss*) and they found an increased frequency of crippled fry and decreased feed conversion efficiency, as well as decreased survival and growth rate of early fry when F = 0.25 compared to non-inberd individuals. In zebrafish (*Danio rerio*) full-sib matings (F = 0.125 and 0.25) led to decreased fertility and survival and length at 30 days age (Mrakovčič and Haley 1979), whereas in guppy (*P. reticulata*) full-sib matings led to a decrease in survival and salinity tolerance of the offspring (Shikano and Taniguchi 2003).

2.1.3. Inbreeding avoidance

Harmful effects of inbreeding have led many species to develop different mechanisms to avoid mating among closely related individuals. These mechanisms include for example dispersal, promiscuity, kin recognition and avoidance (Cockburn *et al*. 1985, Pusey 1987, Blouin and Blouin 1988, Pusey and Wolf 1996, Kempenaers 2007). Kin recognition and the kin recognition mechanisms have been studied widely in fish and many species have been reported to recognize kin or familiars (Ward and Hart 2003). However, most of these studies have been conducted on species that choose their mate, not on mass-spawning species. Trippel *et al*. (2009) studied if haddock (*Melanogrammus aeglefinus*), a broadcast spawning species, could recognize kin, but they did not observe preference for mating with unrelated individuals. However, in mass- and group-spawning species, different post-spawn mechanisms, such as sperm competition and cryptic female choice, might exist to inhibit inbreeding (Zeh and Zeh 1997, Birkhead and Pizzari 2002).

Olsén (1992) reviewed the kin recognition mechanisms known in fish and summarized them into four categories: kin recognition via 1) spatial distribution 2) familiarity and previous associations; individuals become familiar with the features of their clutch mates, 3) phenotypic matching; individuals learn the phenotype of close relatives or itself during early development and later recognize kin by comparing this learned phenotype and 4) recognition alleles; both the genotype and it's recognition have genetic bases. Juvenile kin recognition using olfactory or visual cues has been recorded mainly in salmonid species (Stabell 1987, Olsén *et al*. 1998, Courtenay *et al*. 1997, Brown and Brown 1992), but also in zebrafish (*D. rerio*) (Mann *et al*. 2003) and the three-spine stickleback (*Gasterosteus aculeatus*) (Fitzgerald and Morrissette 1992). Recognizing kin can be highly beneficial for juvenile fish because many fish species form shoals with close relatives, which increases an individual's inclusive fitness. However, this behavior also includes the risk of inbreeding. Kin recognition among adult fish has not been studied as widely as it has in juveniles, but there is evidence of kin recognition among adults in several species, including fathead minnows (*Pimephales promelas*) (Brown and Smith 1994) and rainbow fish (*Melanotaenia eachamensis*) (Arnold 2000). Arnold (2000) demonstrated that rainbow fish females prefer to shoal with female relatives but avoid male relatives, and so are able to get the benefits of relative altruism but avoid the harmful effects of inbreeding. Gerlach and Lysiak (2006) reported similar behavior in zebrafish.

There is also evidence that fish can recognize kin by olfactory cues determined by the major histocompability complex (MHC). MHC is an extremely polymorphic large chromosomal region containing several closely linked genes with alleles that convey specific resistance against parasites and pathogens (Edwards and Hedrick 1998). Because MHC genes are highly polymorphic, individuals sharing MHC alleles are likely to be related (Penn and Potts, 1999). For example, juvenile Arctic char, adult Atlantic salmon (*Salmo salar*) and three-spine stickleback choose their shoal or breeding mates according to MHC heterozygosity (Olsén *et al*. 1998, Landry *et al*. 2001, Reusch *et al*. 2001). The study of Landry *et al*. (2001) showed that in Atlantic salmon, mate choice was driven more by MHC heterozygosity than by parental relatedness.

2.2. The effects of changing temperature on fish reproductive success

Water temperature is one of the most important factors regulating habitat availability and the reproduction of fish. The development of fish during the embryo and yolk-sac larval stages is mostly determined by the water temperature (Alderdice and Velsen 1978, Luczynski and Kirklewska 1984). Temperature affects the duration of these vulnerable ontogenetic stages and also a variety of important characteristics, such as the size at

hatching and feeding onset, efficiency of yolk utilization, deformities, gender and survival of fish (Polo *et al*. 1991, Koumoundouros 2001, 2002, Baird *et al*. 2002, Kavanaugh *et al*. 2010).

Each fish species has their specific thermal range and requires different temperature conditions for spawning and embryonic, larval and juvenile development (Herzig and Winkler 1986). Kamler (1992) reviewed effects of incubation temperature on the larval size at hatching and reported different temperature responses in different species. Some species had the maximum size in the coldest or warmest temperatures used and some at intermediate temperatures. The most probable explanation for this discrepancy is different coefficients of developmental and metabolic rates in different species and temperature conditions, which together determine the allocation of energy from yolk to tissue production and respiration (Kamler 1992). Divergence from the optimal temperature conditions during early development can result in lower survival rates, metabolic rates and structural asymmetry, (for example Beacham and Murray 1985, Huuskonen *et al*. 2003, Turner *et al*. 2007). According to Fuiman *et al*. (1998), elevated water temperatures generally accelerate the rate of development more than the rate of growth resulting in different larval sizes at certain stages of development. It has been shown in different fish species that as the temperature rises, the total hatching length of fish (Ryland and Nichols 1975, Batty *et al*. 1993), the notochord flexion and the appearance of fins, swimbladder and teeth (Fuiman *et al*. 1998) are hampered.

2.2.1. Climate change

Freshwater organisms are physically restricted to lakes and rivers and must therefore deal directly with the variation and changes in the climate (Roberts *et al*. 2013). Several studies have also shown that the developmental temperature tolerance (Hubbs and Amstrong 1962, Hubbs and Strawn 1963, Kavanaugh *et al*. 2010) and developmental rates (Eckmann 1987, Baird *et al*. 2002, Kavanaugh *et al*. 2010) can vary between different local populations of many fish species. These differences have been suggested to be the result of genetic adaptation to certain thermal environments (Hubbs and Strawn 1963, Eckmann 1987, Baird *et al*. 2002, Kavanaugh *et al*. 2010). Rapid climate change is likely to impose strong selection pressures on traits important for the fitness of many organisms (Gienapp *et al*. 2008). This selection pressure can induce micro-evolutionary changes in the populations with selection favoring new genotypes with different thresholds of response to environmental conditions, or individuals can adapt to the different conditions within the means of phenotypic plasticity (Bradshaw and Holzapfel 2006, Karjalainen *et al*. 2014). According to Gienapp *et al*. (2008), reviews made so far suggest plastic responses prevail over genetic responses.

Cingi *et al*. (2010) studied the effects of high temperatures on the fertilization success and early development of whitefish and observed that temperatures higher than 7 ºC increased the proportion of unfertilized and abnormally dividing eggs, deformed embryos and resulting mortality. When embryos were exposed to high temperatures at a developmental stage later than fertilization or the four-cell stage, fewer developmental abnormalities and a lower cumulative mortality rate were observed. Taranger and Hansen (1993), Huuskonen *et al*. (2003) and Turner *et al*. (2007) have also found that high temperatures in the incubation period result in lower survival of cold-adapted species, such as Atlantic salmon, arctic char and rainbow trout. However, in these studies, the incubation temperatures used were not very realistically observed in natural conditions and the temperature was kept constant during the whole incubation period. Karjalainen *et al*. (2014) studied the effects of elevated spring water temperatures on vendace and whitefish

and their results showed these coregonid species being highly flexible in adjusting their reproductive cycles to inter-annual changes. Incubation temperature was observed to affect hatching time, hatching size and growth rate but despite the highly divergent temperature conditions, the embryonic survival did not differ between the scenarios.

3. MATERIALS & METHODS

3.1. Study species

Parental effect and parent relatedness were tested on the early development of two cold-water adapted coregonid species – vendace and whitefish. Vendace and whitefish typically spawn in the autumn and the hatching of larvae takes place close to ice-break. Both species are presumed to be communal-spawning species that do not provide parental care to their offspring. The mating systems of vendace and whitefish are not well known. Females produce thousands of eggs per breeding season which are externally fertilized. Whitefish males develop breeding tubercules during the breeding season, which have been proposed to be sexual signals that reveal good genes (Wedekind *et al*. 2001). This indicates the possible existence of female choice. The mating system of whitefish has been proposed to be similar to that of roach (*Rutilus rutilus*), another group-spawning fish that also develops breeding tubercules shortly before mating (Wedekind 1996). Roach have a lek-like mating system with different male reproductive strategies, and with females differing in their spawning preferences (Wedekind 1996).

3.2. Incubation experiment

The parental effect on egg development in two different temperature treatments was tested experimentally at Konnevesi research station in the winter 2013–2014. Eggs were incubated in two separate tanks. In the long winter tank, water came directly from Lake Konnevesi so the water temperature in the tank followed the lake water temperatures. Water temperature of the short winter tank was regulated by a thermometer-adjusted warming system, which produced the predicted future temperature scenario. Future daily vertical profiles of water temperature were generated with MyLake-simulation model (Saloranta and Andersen 2007), as described by Karjalainen *et al*. (2014) (Figure 1). Styrofoam covers were used on the tanks to simulate the snow on ice cover, and then removed in the spring according to the situation in Lake Konnevesi or according to the predicted scenario.

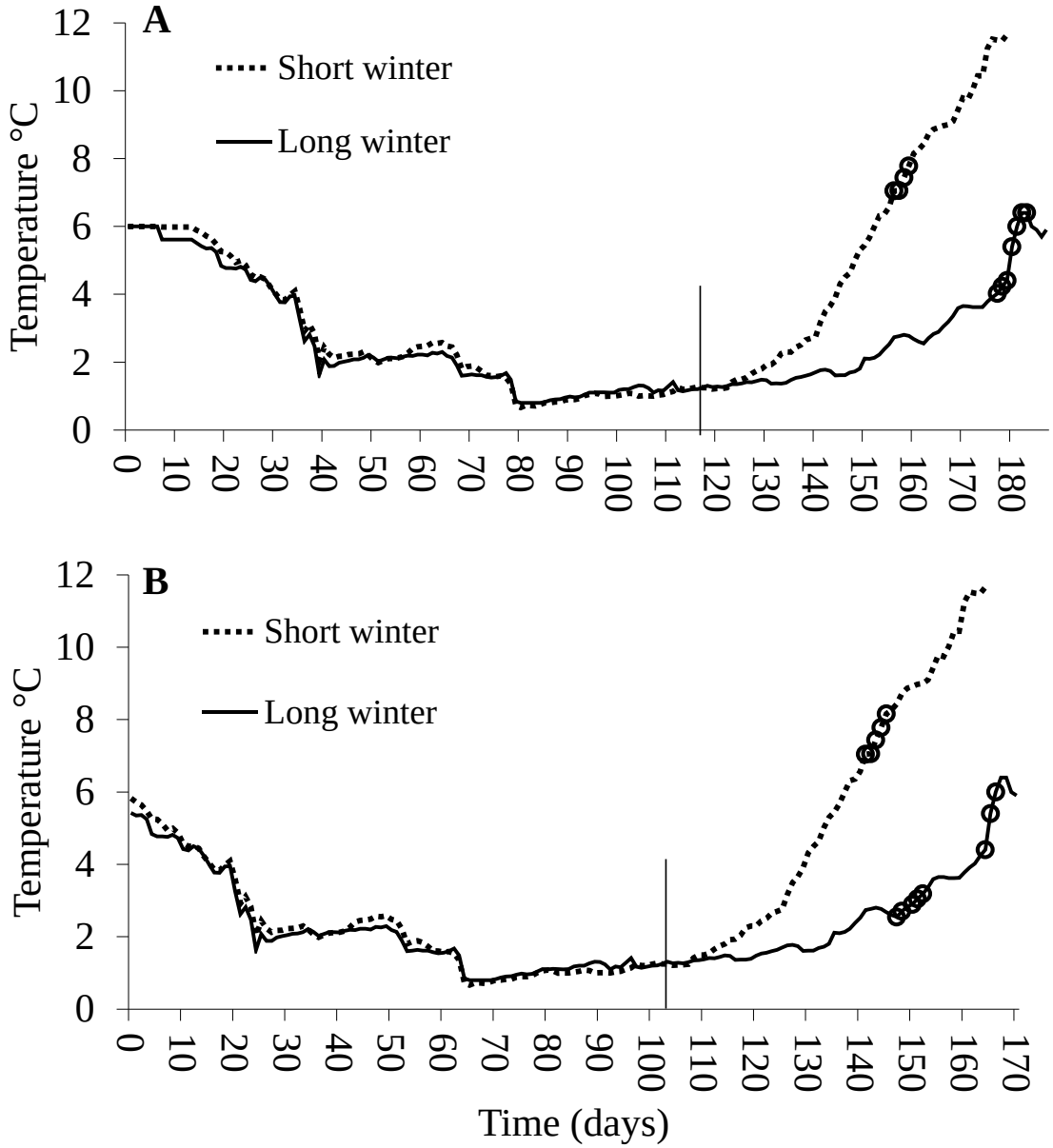

Figure 1. Water temperature curves of short and long winter conditions used in the experiment for vendace (A) and whitefish (B). Vertical bar represents the start of spring water warming and open circles represent 50 % hatching time of different parent pairs. Time is measured as days from fertilization, 24.10.2013 for vendace and 8.11.2013 for whitefish.

All the fish used in the incubation experiment were caught by gill nets by local fishermen from Lake Konnevesi during the middle of the spawning season. Vendace females and males were caught in the end of October and the whitefish in the beginning of November. For vendace two fertilization sessions were carried out to get a sufficient number of parent pairs. Parental effect was studied only using the pairs of the first session. In the first fertilization session in total six and three ripe female vendace and whitefish, respectively, and six and three males were chosen from the catch (Table 1). In addition, two vendace males were taken to fertilize the eggs of the first three females. These pairs were only used in the relatedness analyses. In the second fertilization session three females and three males of vendace were used. Parent fish were chosen randomly, but many

individuals had to be discarded because of an insufficient amount of eggs or milt or because they were not ready for spawning.

Table 1. Mean total lengths (cm), wet masses (g), gonad wet masses (g) and egg dry masses (mg) with standard deviation for the parent fish used in the incubation experiment.

Species	Sex	Date	N	Total length + SD	Wet mass + SD	Gonad wet mass + SD	Egg dry mass + SD
Vendace[a,b]	Female	24.10.	6	156.0 ± 5.5	27.4 ± 2.2	6.3 ± 1.5	0.6 ± 0.05
Vendace[a,b]	Male	24.10.	6	158.0 ± 4.8	25.4 ± 3.0	Not measured	
Vendace[b]	Male	24.10.	2	160 ± 4.2	23.8 ± 5.7	Not measured	
Vendace[b]	Female	29.10.	3	163.0 ± 7.1	26.3 ± 1.5	4.4 ± 2.8	0.5 ± 0.00
Vendace[b]	Male	29.10.	3	156.0 ± 8.5	22.5 ± 3.3	0.7 ± 0.1	
Whitefish[a]	Female	8.11.	3	299.7 ± 61.9	254.4 ± 182.8	34.1 ± 30.9	1.8 ± 0.30
Whitefish[a]	Male	8.11.	3	300.3 ± 27.2	193.2 ± 43.3	4.7 ± 1.3	

[a] = fish used to study parental effect, [b] = fish used in the genetic analyses

All the fish used in the experiment were weighed and measured before and after removal of the gonads. All the male whitefish had breeding tubercules. Eggs were stripped to Petri dishes and milt to Eppendorf-tubes. For all the fertilizations a full-factorial breeding design was used. Eggs of each female were divided to separate dishes according to the number of males, leaving minimum of 40 eggs per Petri dish. Eggs were then fertilized with an equal amount of milt from a male and activated with lake water. Eggs of each female were fertilized with milt of all the males separately. In addition, each vendace pair was replicated two times and each whitefish pair three times in the fertilization. Fertilized vendace eggs were divided to two tanks filled with lake water to allow testing for the different climate scenarios. Whitefish eggs were incubated in only one tank until the start of spring water warming.

Eggs were incubated in acrylic plastic enclosures (7×7 cm) with gentle vertical water flow through their mesh bottom (Figure 2). The number of dead eggs was recorded daily in the first week, three times per week during the first month of the incubation period and thereafter weekly. Water temperature was measured every half hour throughout the experiment and the oxygen concentration and pH were measured monthly. Maximum variation of the within-tank water temperature was fairly small, 0.1 ºC, but the places of the plates in the tanks were circulated weekly throughout the incubation period anyway to eliminate possible place specific effects. During the spring water warming period the numbers of dead eggs and hatched larvae were recorded daily. Embryonic survival and mortality were calculated as proportions of hatched and unhatched larvae out of the total number of eggs. Eggs were washed once with Malachite Green in the winter to prevent fungal infections.

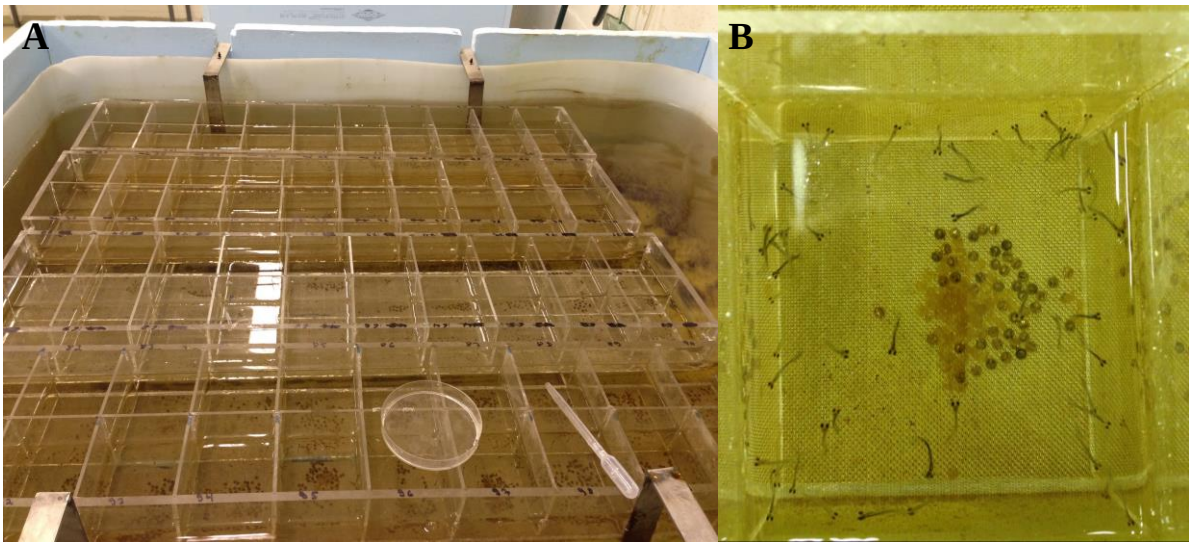

Figure 2. Pictures of the tank with the acrylic plastic enclosures used in the experiment (A) and of a single enclosure with whitefish eggs and hatched larvae (B).

Before the start of the spring water warming (19.2.2014) eggs were divided to replicates. The three replicates of each whitefish pair were divided to the two tanks so that both tanks had the same pairs and replicates. Vendace eggs of both tanks were divided to two replicates if there were enough eggs. Minimum of 30 eggs were left in every enclosure. On the same date, five eggs per replicate were sampled and preserved in 4 % formalin solution and stored immediately at -20 ºC. At the 50 % hatching peak 8–10 hatched larvae were also sampled and stored as above. Carcasses and yolk sacs of the sampled embryos and larvae were measured and weighed separately using a dissecting microscope and microbalance scale (Sartorius CP2-P, accuracy 1 μg). Carcasses and yolk-sacs were dried 24 h in an oven at 40 ºC and dry weights were also measured.

Statistical analyses were performed with IBM SPSS Statistics 22. Maternal, paternal and maternal-paternal interaction effects and the temperature conditions on the 50 % hatching time, embryonic instantaneous total mortality (Z = -ln(survival)) and dry weights of carcasses and yolk-sacs of embryos and hatched larvae were tested with general linear model of two-way ANOVA. The model was customized to consider only the main effects of females, males and treatment and the female-male interaction.

3.3. Genetic analyses

To investigate the variation in the fertilization success and weight of the larvae between different parent pairs, relatedness of the vendace parents was also studied. All the parents from both fertilization sessions were used, making in total 9 females and 11 males. Genetic analyses were carried out in the evolutionary genetics laboratory at University of Jyväskylä in autumn 2014.

In addition to the parent fish, 50 individuals caught from the same area in the end of October 2013 were genotyped for calculating population allele frequencies of the reference population. DNA was extracted from an approximately 5x5 mm cube of muscle cut from a frozen fish. Extraction was performed using DNeasy tissue kit reagents (Qiagen Nordic, Helsinki) following the manufacturer's protocol modified for use with a Kingfisher magnetic particle processor (ThermoFisher Scientific, Waltham MA, USA). Relatedness was estimated using 13 polymorphic microsatellite markers designed for coregonid species and found suitable for vendace (Præbel *et al*. 2013) (Table 2). Markers were amplified in 3

multiplex PCR reactions to reduce the time and expenses of genotyping (Neff *et al*. 2000). Multiplex groups, marker size ranges and observed allele numbers were derived from Præbel *et al*. (2013). PCR reagent concentrations and amplification cycles were performed as described by Præbel *et al*. (2013) but the annealing time was shortened from 3 minutes to 90 seconds. PCR amplification was performed using S1000 and C1000 Thermal Cyclers (Bio-Rad Laboratories, Carlsbad CA, USA). PCR products were denaturated in formamide together with GeneScan™ 500 LIZ™ Size Standard and then run on an ABI Prism 3130xl Genetic Analyzer (both Applied Biosystems, ThermoFisher Scientific, Waltham MA, USA). Alleles were scored using GeneMapper 4.0. software (Applied Biosystems, ThermoFisher Scientific, Waltham MA, USA). Scored alleles were checked and corrected manually. Genotype data was summarized using GenAlEx v.6.5 (Peakall and Smouse 2006). Two individuals from the reference population sample were deleted because of unscorable alleles in more than three loci. Because of possible null alleles, ML-Relate (Kalinowski *et al.* 2006) was used to estimate allele frequencies, parent relatedness and relationships. F-values were calculated using Ritland's inbreeding coefficient (Ritland 1996) in SPAGeDi (Hardy and Vekemans 2002).

Table 2. Details of the 13 microsatellite loci used in the study, including the original publication references. T_a = PCR annealing temperature, Mlpx = PCR Multiplex group, Dye = fluorescent label of the F primer.

Locus ID	Ta (°C)	Mplx	Dye	Repeat	Primer Sequences 5' - 3'
Bwf1[a]	57	I	PET	$(GA)_{16}(N)_{19}(TG)_{13}$	F: TACAGAGAAATACACACAACGCATCAA
					R: GAGAGGTTCCATTACTGAGCAC
ClaTet13[c]	57	I	6-FAM	$(GACA)_7$	F: TGATACATTTTTTGGCCTTTC
					R: GGACCTGCCCTATCTGTC
Cocl-Lav4[d]	57	I	6-FAM	$(CA)_{13}$	F: TGGTGTAATGGCTTTTCCTG
					R: GGGAGCAACATTGGACTCTC
Cocl-Lav6[d]	57	I	NED	$(GT)_{22}$	F: GCCATCATCCTCCCAGGAAAC
					R: CAGGGAATCTGCACTGGAGC
Cocl-Lav10[d]	57	I	NED	$(GT)_8$	F: CAGTGGAGTTAATGAGTGCC
					R: GTGGAAATTGAATACTGCGG
Cocl-Lav27[d]	57	I	VIC	$(GT)_6$	F: TGACTCTTCCCCATTCATCC
					R: CCGAGAGGTGGAGAAAACAG
BFRO018[b]	60	II	PET		F: AGAGGGGTCCAGCAACATCA
					R: GGGGAACCAGTCTAAAGCCT
Cocl-Lav49[d]	60	II	NED	$(GT)_{17}$	F: AGCCAGTTGGAGGCTATTTG
					R: AGGGCTGCTGTTGAAGTCAT
Cocl-Lav52[d]	60	II	6-FAM	$(GT)_{52}$	F: GGCGAGTTGGAGGCTATTTG
					R: ACAGAGCCCCAGATGGTAAC
Cisco-157[e]	60	II	VIC	$(GT)_{17}$	F: CTTAGATGATGGCTTGGCTCC
					R: GGTGCAATCACTCTTACAACACC
Bwf2[a]	57	III	PET	$(CA)_{25}$	F: CGGATACATCGGGCAACCTCTG
					R: AGACAGTCCCCAATGAGAAAA
ClaTet6[c]	61	III	6-FAM	$(TGTC)_{17}$	F: GAATCGGCATCTCCTGAGTCA
					R: GCTTGGGGCATAATAACCACC
ClaTet9[c]	61	III	VIC	$(TGTC)_{17}$	F: GCAAGGTGAGCCTGTGTGAGT
					R: GGTGGTTAGGTGTCTTGTGGC

[a] = Patton *et al* . 1997, [b] = Susnik *et al* . 1999, [c] = Winkler & Weiss 2008, [d] = Rogers *et al* . 2004, [e] = Turgeon *et al* . 1999

4. RESULTS

4.1 Embryonic mortality

The instantaneous total mortality of whitefish was significantly lower than the mortality of vendace (*t*-test: $t = 10.867$, df = 124, $p < 0.001$) (Figure 3). Maternal, paternal and maternal-paternal interaction effects influenced embryonic mortality of both species significantly (Table 3). Mortality of neither species differed between different winter scenarios (Table 3, Figure 3). Mean mortality (arithmetic mean) was 82.5 % for vendace and 6.2 % for whitefish. There was high variability in embryonic survival between different parent pairs of vendace (Figure 4).

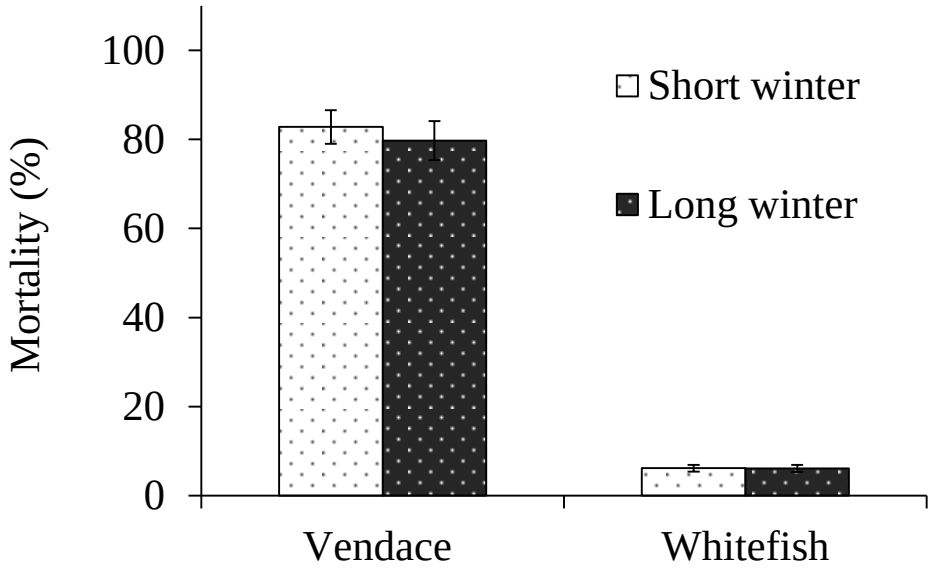

Figure 3. Vendace and whitefish mean embryonic mortality rates (%) under the short and long winter scenarios. Vertical bars represent the standard errors.

Table 3. ANOVA results of maternal and paternal effects on the embryonic instantaneous total mortality (Z) in the breeding experiment of the vendace females 1–6 and males 1–6 and whitefish females 1–3 and males 1–3. df: degrees of freedom, SS: sum squares, MS: mean squares, F and p: critical values and probability of significance tests. Treatment refers to the short and long winter scenarios.

Species	Source	df	SS	MS	F	p
Vendace	Female	5	98.202	19.640	57.21	<0.001
	Male	5	122.508	24.502	71.37	<0.001
	Treatment	1	0.202	0.202	0.59	0.448
	Female * Male	25	76.060	3.042	8.86	<0.001
	Error	35	12.016	0.343		
Whitefish	Female	2	0.077	0.039	143.83	<0.001
	Male	2	0.005	0.002	8.69	<0.001
	Treatment	1	0.000	0.000	0.01	0.909
	Female * Male	4	0.005	0.001	4.27	<0.010
	Error	44	0.012	0.000		

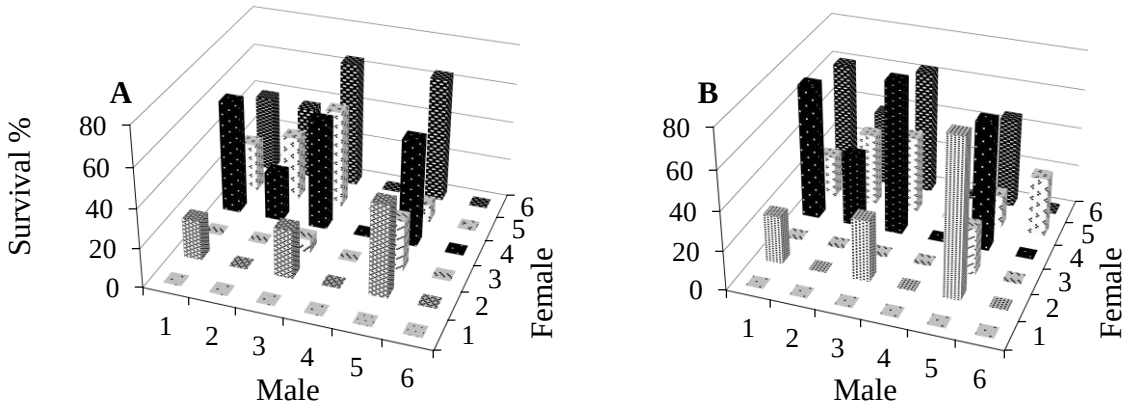

Figure 4. Arithmetic means of embryonic survival (%) of each vendace parent pair in short (A) and long (B) winter conditions.

4.2. Parental effect on hatching time

The difference between vendace pairs in the time from fertilization to 50 % hatching was 6 days in the short winter treatment and 8 days in the long winter treatment. Whitefish pairs had a wider range, 3 and 18 days in the short and long winter treatments, respectively. In vendace, both female and male affected the hatching time significantly (Table 4, Figure 5). In whitefish, male did not affect the hatching time significantly, but female and female-male interaction did have a significant effect (Table 4, Figure 6).

Table 4. ANOVA results of maternal and paternal effects on the time from fertilization to 50 % hatching in the breeding experiment of the vendace females 2–6 and males 1–5 and whitefish females 1–3 and males 1–3. df: degrees of freedom, SS: sum squares, MS: mean squares, F and p: critical values and probability of significance tests. Treatment refers to the short and long winter conditions.

Species	Source	df	SS	MS	F	p
Vendace	Female	4	25.62	6.41	6.19	<0.010
	Male	4	18.30	4.58	4.42	<0.050
	Treatment	1	4081.07	4081.07	3943.34	<0.001
	Female * Male	8	4.63	0.58	0.56	0.796
	Error	16	16.56	1.04		
Whitefish	Female	2	478.78	239.39	15.91	<0.001
	Male	2	7.00	3.50	0.23	0.793
	Treatment	1	1472.67	1472.67	97.88	<0.001
	Female * Male	4	285.56	71.39	4.75	<0.010
	Error	44	662.00	15.05		

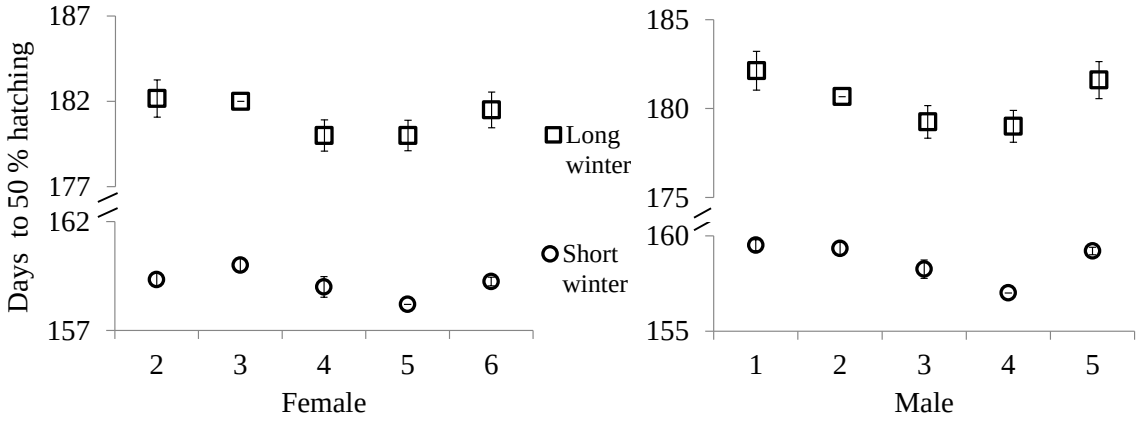

Figure 5. Mean days from fertilization to 50 % hatching of each vendace female and male with standard error.

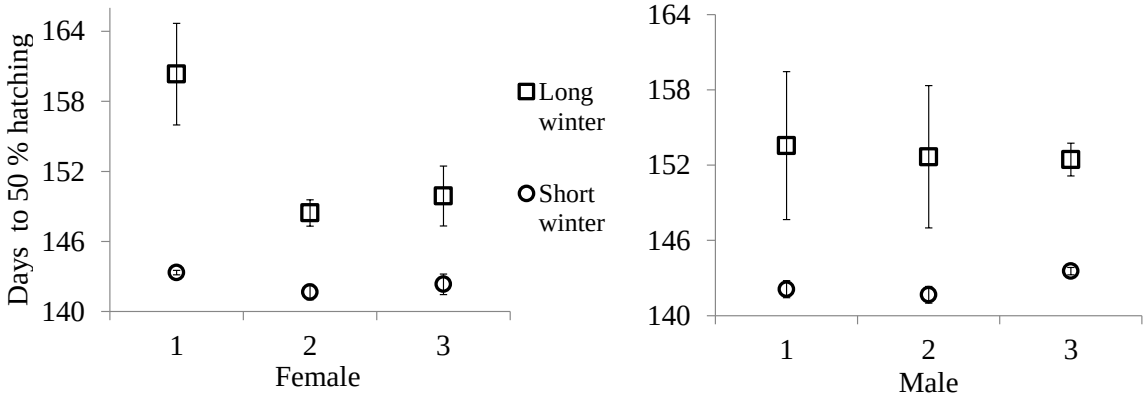

Figure 6. Mean days from fertilization to 50 % hatching of each whitefish female and male with standard error.

4.3. Parental effect on the size of embryos and larvae

In vendace, maternal effect was significant on weights of carcass and yolk in both embryos and larvae (Table 5, Figure 7). Paternal effect was significant on the carcass weights of hatched larvae but not the yolk weights. Maternal-paternal interaction was significant on both carcass and yolk weights of hatched larvae.

In whitefish, maternal effect was significant on dry weights of carcass and yolk of both embryos and hatched larvae (Table 6, Figure 8). Paternal effect was significant on the carcass dry weights of embryos and on the carcass and yolk dry weights of hatched larvae. Maternal-paternal interaction was significant on the carcass weights of hatched larvae.

Table 5. ANOVA results of maternal and paternal effects on dry weights of embryos and hatched larvae (mg) of vendace in the breeding experiment of the females 1–6 and males 1–6. df: degrees of freedom, SS: sum squares, MS: mean squares, F and p: critical values and probability of significance tests. Treatment refers to the short and long winter scenarios.

Variable	Source	df	SS	MS	F	p
Carcass of embryo	Female	2	0.91	0.45	165.62	<0.001
	Male	2	0.02	0.01	4.40	<0.050
	Female * Male	4	0.01	0.00	1.21	0.308
	Error	258	0.71	0.00		
Yolk of embryo	Female	2	1.48	0.74	154.99	<0.001
	Male	2	0.01	0.01	1.45	0.236
	Female * Male	4	0.03	0.01	1.30	0.272
	Error	258	1.24	0.01		
Carcass of larvae	Female	2	2.61	0.31	550.06	<0.001
	Male	2	0.01	0.00	1.67	<0.050
	Treatment	1	0.14	0.14	57.79	<0.001
	Female * Male	4	0.05	0.01	4.99	<0.010
	Error	341	0.81	0.00		
Yolk of larvae	Female	2	0.01	0.01	4.52	<0.050
	Male	2	0.04	0.02	15.52	<0.001
	Treatment	1	0.85	0.85	623.73	<0.001
	Female * Male	4	0.01	0.00	2.36	0.053
	Error	339	0.46	0.00		

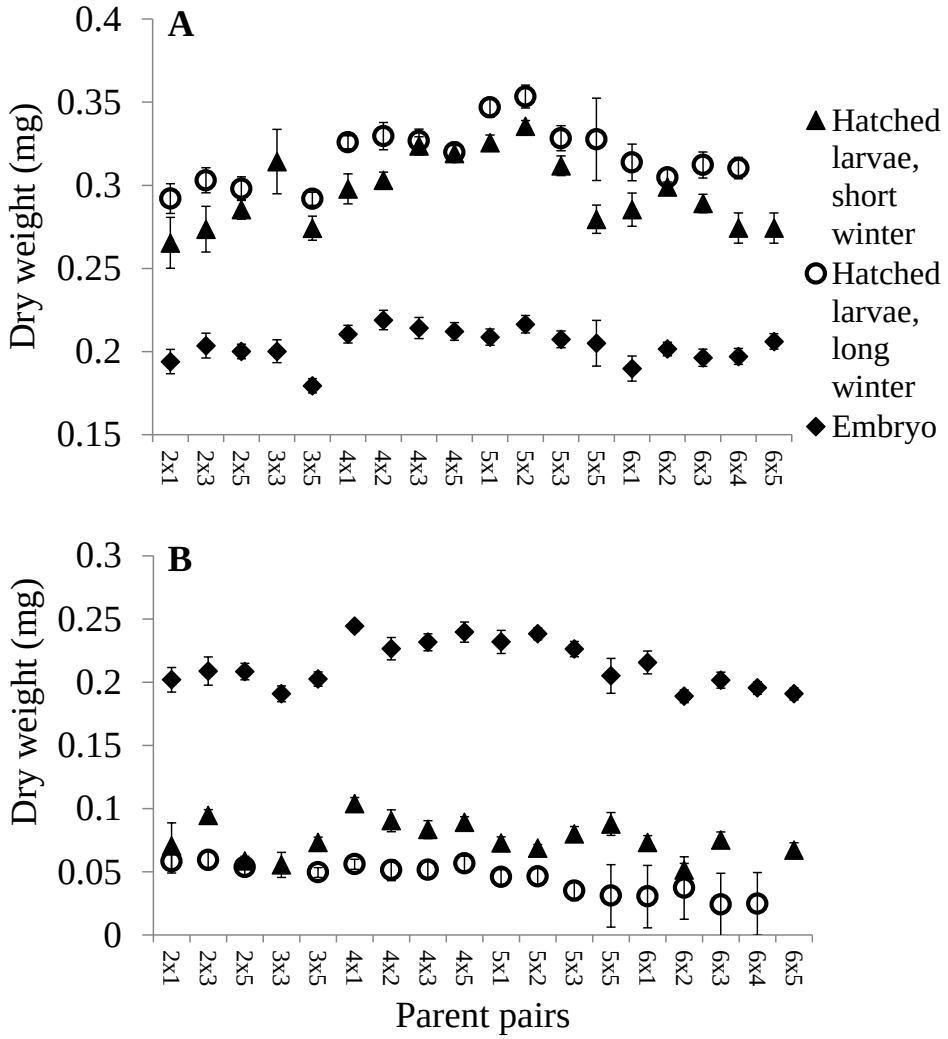

Figure 7. Dry weights of carcasses (A) and yolk sacs (B) of embryos and hatched larvae of vendace in both winter conditions by parent pairs (female x male). Vertical bars represent standard error. Data points without standard error bars have such a small error it is not visible in these figures.

Table 6. ANOVA results of maternal and paternal effects on dry weights of embryos and hatched larvae (mg) of whitefish in the breeding experiment of the females 1-3 and males 1-3. df: degrees of freedom, SS: sum squares, MS: mean squares, F and p: critical values and probability of significance tests. Treatment refers to the short and long winter conditions.

Variable	Source	df	SS	MS	F	p
Carcass of embryo	Female	2	0.91	0.45	165.62	<0.001
	Male	2	0.02	0.01	4.40	<0.050
	Female * Male	4	0.01	0.00	1.21	0.308
	Error	258	0.71	0.00		
Yolk of embryo	Female	2	1.48	0.74	154.99	<0.001
	Male	2	0.01	0.01	1.45	0.236
	Female * Male	4	0.03	0.01	1.30	0.272
	Error	258	1.24	0.01		
Carcass of larvae	Female	2	2.61	0.31	550.06	<0.001
	Male	2	0.01	0.00	1.67	<0.050
	Treatment	1	0.14	0.14	57.79	<0.001
	Female * Male	4	0.05	0.01	4.99	<0.010
	Error	341	0.81	0.00		
Yolk of larvae	Female	2	0.01	0.01	4.52	<0.050
	Male	2	0.04	0.02	15.52	<0.001
	Treatment	1	0.85	0.85	623.73	<0.001
	Female * Male	4	0.01	0.00	2.36	0.053
	Error	339	0.46	0.00		

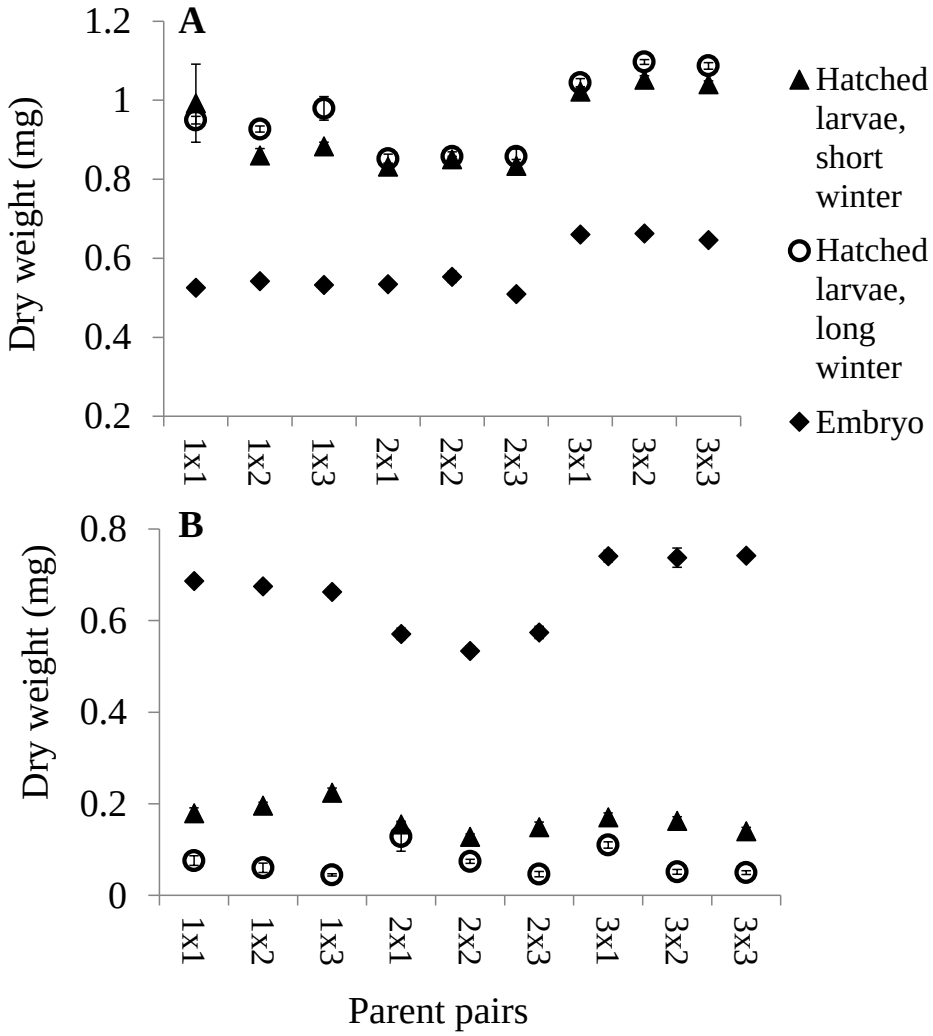

Figure 8. Dry weights of carcasses (A) and yolk sacs (B) of embryos and hatched larvae of whitefish in both winter conditions by parent pairs (female x male). Vertical bars represent standard error. Data points without standard error bars have such a small error it is not visible in these figures.

The mean dry weight of the eggs of vendace females before fertilization during the spawning season was related to the dry weights of carcasses and yolk-sacs of both embryos and hatched larvae (Table 7, Figure 9). Larger egg size led to larger embryos with larger yolk reserves and larger hatched larvae in both treatments. The mean dry weight of the whitefish females' eggs before fertilization during the spawning season was related to the dry weights of carcasses and yolk-sacs of embryos and carcasses of hatched larvae in both treatments, but not to the yolk-sacs of hatched larvae (Table 8, Figure 10).

Table 7. Results of the linear regressions between female-specific mean egg dry weight and mean dry weight of carcass or yolk-sac for both vendace embryos and larvae. r2: coefficient of determination, df(1,2): between-groups and within-groups degrees of freedom, F and p: critical values and probability of significance tests.

Variable	r^2	df(1,2)	F	p
Carcass of embryo	0.101	1,267	30.064	<0.001
Yolk of embryo	0.269	1,266	97.843	<0.001
Carcass of larvae[a]	0.254	1,132	44.952	<0.001
Carcass of larvae[b]	0.263	1,118	42.033	<0.001
Yolk of larvae[a]	0.185	1,132	29.951	<0.001
Yolk of larvae[b]	0.089	1,118	11.500	<0.010

[a] = short winter conditions, [b] = long winter conditions

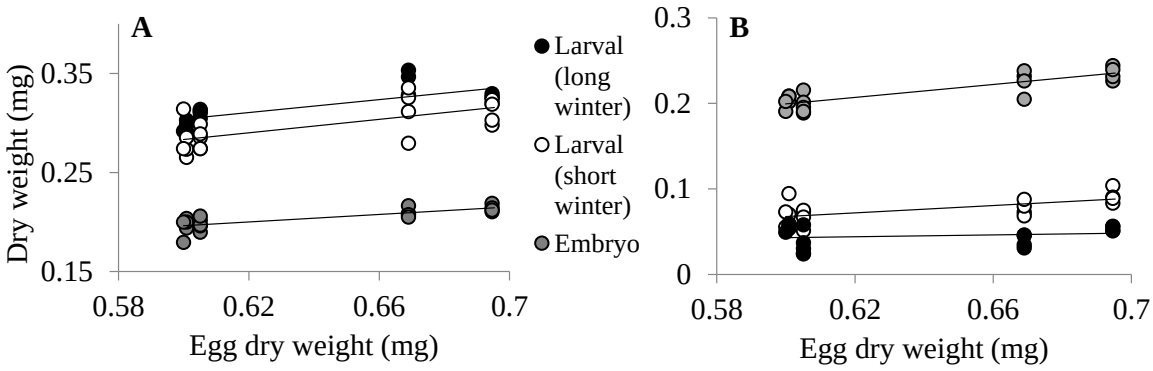

Figure 9. Mean dry weights of carcass (A) and yolk (B) (mg) of embryos and larvae in relation to the mean egg dry weights (mg) with linear regression lines for vendace. Each symbol represents a mean value of the dry weights of each parent pair.

Table 8. Results of the linear regressions between female-specific mean egg dry weight and mean dry weight of carcass or yolk-sac for both whitefish embryos and larvae. r^2: coefficient of determination, df(1,2): between-groups and within-groups degrees of freedom, F and p: critical values and probability of significance tests.

Variable	r^2	df(1,2)	F	p
Carcass of embryo	0.533	1,265	302.308	<0.001
Yolk of embryo	0.416	1,265	188.681	<0.001
Carcass of larvae[a]	0.796	1,128	498.040	<0.001
Carcass of larvae[b]	0.730	1,219	592.990	<0.001
Yolk of larvae[a]	0.003	1,128	0.331	0.566
Yolk of larvae[b]	0.000	1,217	0.086	0.770

[a] = short winter conditions, [b] = long winter conditions

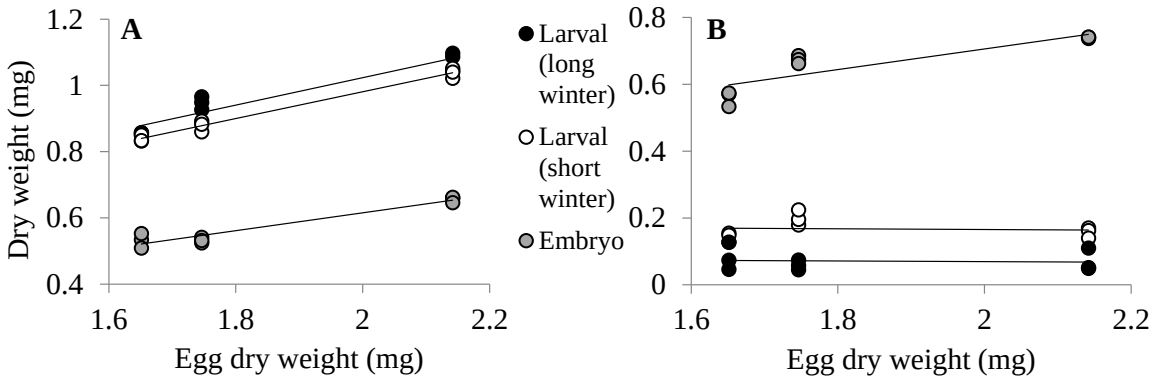

Figure 10. Mean dry weights of carcass (A) and yolk (B) (mg) of embryos and larvae in relation to the egg dry weights (mg) with linear regression lines for whitefish. Each symbol represents a mean value of the dry weights of each parent pair.

4.4. The effects of inbreeding and relatedness on the embryonic survival of vendace

All the microsatellites used in the study were polymorphic with allele numbers varying from 3 to 30. Size ranges and allele numbers were in some cases different in comparison to the results of Præbel *et al*. (2013) (Table 9). Allele frequencies did not differ significantly between the parent sample and reference sample (Contingency *G*-test: G = 4.432, df = 18, p = 1.000). The mean r-values of the parent population and reference population were -0.010 and -0.008 (Lynch and Ritland's r) and -0.036 and -0.013 (Queller and Goodnight's r), respectively. A permutation test in GenAlEx did not find difference between these r-values.

Mean F-values were 0.033 in the parent sample and 0.115 in the reference sample and did not differ significantly (*t*-test: t = -1.928, df = 65, $p > 0.05$). Intra-individual inbreeding coefficients (F) of the parents ranged from -0.22 to 0.25. Offspring of the parents with the lowest and highest inbreeding coefficient values also had the lowest survival rates (r^2 = 0.383, F = 4.657, df = 2, $p < 0.05$) (Figure 11). Pairwise kinship coefficients between parents ranged from -0.045 to 0.052. Offspring of parents with a higher pairwise kinship coefficient than 0.011 had a close to zero survival rates.

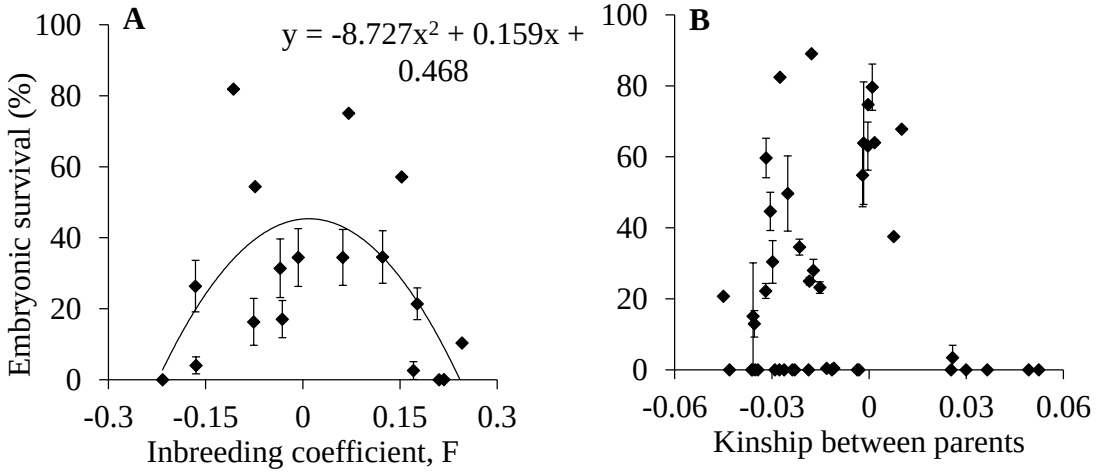

Figure 11. Embryonic survival of vendace (%) in relation to the inbreeding coefficient of the parents (A) with a quadratic regression model trendline and its equation and to the kinship coefficient between parents (B). Vertical bars represent standard error.

ML-Relate detected significant heterozygote deficiency in four loci: Bwf1, ClaTet6, ClaTet9 and Cocl-Lav52, which could have been caused by null alleles. In Bwf1, ClaTet9

and Cocl-Lav52 estimated null allele frequencies were relatively low, but in ClaTet6 it was estimated to be 0.2028. When estimating relationships with ML-relate, the potential null alleles in locus ClaTet6 were estimated and taken into account, but due to their low frequencies, potential null alleles in the other loci were ignored. For the relevant parent pairs, ML-Relate estimated half-sib relationships for pairs F1+M5 and F1+M7. A likelihood ratio test performed in ML-Relate showed that the proposed half-sib relationship was significantly more likely than no relationship between these individuals (p = 0.02 and p = 0.037 for F1+M5 and F1+M7, respectively). A likelihood ratio test was also performed for other parent pairs with low survival rate, and in all cases half-sib relationship was not more likely than no relationship between individuals ($p > 0.05$). Of the total sample 92 % of the pairs were estimated to be unrelated, 8 % half-siblings and 0.2 % full-siblings.

Table 9. Size ranges and allele numbers (N_A) for 13 microsatellite loci of lake Konnevesi vendace comparing the results of Præbel *et al*. (2013) ([a]) and this study ([b]). H_o (= observed heterozygosity) and H_e (= expected heterozygosity) presented for each loci.

Locus ID	Size range [a]	Size range [b]	N_A [a]	N_A [b]	H_o	H_e
Bwf1	195–225	206–228	10	11	0.682	0.801
ClaTet13	218–290	219–281	13	18	0.851	0.860
Cocl-Lav4	127–153	127–157	4	6	0.265	0.242
Cocl-Lav6	124–190	124–197	24	24	0.887	0.911
Cocl-Lav10	260–264	260–264	3	3	0.284	0.287
Cocl-Lav27	181–187	181–187	3	4	0.217	0.200
BFRO018	191–219	193–233	7	10	0.590	0.598
Cocl-Lav49	174–224	144–213	16	24	0.848	0.893
Cocl-Lav52	94–130	93–124	9	13	0.731	0.838
Cisco-157	119–159	118–162	8	11	0.397	0.395
Bwf2	160–232	152–233	18	30	0.896	0.929
ClaTet6	181–205	182–198	7	8	0.492	0.726
ClaTet9	174–326	165–307	24	27	0.750	0.933

5. DISCUSSION

5.1. Parental effect

The experiments showed distinct parental effects on the hatching time, mortality and size of embryos and larvae of both study species. Maternal, paternal and the maternal-paternal interaction affected the embryonic mortality of both species. In previous studies, Nagler *et al*. (2000) have found only the female to affect embryonic survival of rainbow trout. However, in other studies in addition to the maternal effect, maternal-paternal interaction effects (whitefish, Wedekind *et al*. 2001, Huuskonen *et al*. 2011) or paternal effects (whitefish, Wedekind and Müller 2004, clownfish, Green and McCormick 2005) on embryonic survival have also been reported. In this experiment, embryonic mortality was calculated as a percentage of unhatched larvae out of the total number of eggs chosen from a female. Therefore it contains also the eggs that were not successfully fertilized. For both species the maternal-paternal interaction was a significant factor on the embryonic mortality which, supports the possibility that mate compatibility affects the reproduction success.

The among-family variation in the 50 % hatching time was larger for vendace and smaller for whitefish than that reported in the study of Karjalainen *et al*. (2014). That might be caused by chance due to random sampling error because in this experiment the number of parents was smaller than in the study of Karjalainen *et al*. (2014). In warm spring water temperature conditions hatching happens much earlier and within a shorter time period than under cold conditions. The rise in the water temperature is the main influence on the larval hatching time, but within the time range set by the temperature, parental origin can also have an effect. In vendace, both parents affected the hatching time, but in whitefish only the maternal effect and maternal-paternal interaction were significant. Karjalainen *et al.* (2014) also noticed an among-family effect on hatching time of both species, but the female and male effects could not be separated. This parental effect on hatching time can provide the plasticity needed for a population to adjust to inter-annual changes in temperature. Flexibility at a population level ensures that at least part of a year class hatches in optimal conditions.

Maternal effects were more pronounced than paternal effects on the size of embryos and larvae in both species. Paternal effects or maternal-paternal interactions were only significant on the weights of carcasses and yolk sacs of hatched larvae, as expected. The females provide the egg and its nutrition, which is the main influence on the weight of the embryo and its yolk reserves (Brooks *et al*. 1997). In the species without parental care the male only provides its DNA, which usually influences the offspring only in the later stages of development (Bang *et al*. 2006). Sire can influence the metabolism and growth rate of an embryo or a larva and this way have an effect on the larval size at hatching and its yolk reserves (Green and McCormick 2005, Probst *et al*. 2006). Female egg size also correlated with the weights of carcasses and yolk-sacs of embryos and carcasses of larvae. Larger eggs resulted in larger embryos with larger yolk reserves which grow to be larger larvae. This relationship has been well established in earlier studies (Brooks *et al*. 1997, Vandeputte *et al*. 2002, Heath *et al*. 1999, Kennedy *et al*. 2007).

Equal to the study of Karjalainen *et al.* (2014), whitefish had significantly lower average embryonic mortality rate than vendace and the different winter conditions did not affect the embryonic mortality of either species. The mean mortality rate of vendace was high, 82.5 %, compared to the study of Karjalainen *et al*. (2014), where the mean mortality rate of vendace was 48.7 %. This could be due to the different breeding systems used in the experiments. Vendace is considered to be a communal-spawning species that does not have a distinct mate choice. However, there is a possibility that it does have some kind of a post-spawn fertilization control mechanism. Non-directional cryptic female choice predicts females to favor the sperm of the males with compatible genotypes regardless of their phenotype (Birkhead and Pizzari 2002). The mechanisms underlying cryptic female choice are not well known, although a number of potential physiological and biochemical mechanisms have been identified (Zeh and Zeh 1997). In externally fertilizing teleost fishes, one such mechanism can be for example the ovarian fluid that is released by the female with her eggs during spawning. The ovarian fluid can differentially enhance the swimming speed of sperm from different males (arctic char, Urbach *et al*. 2005; chinook salmon, Rosengrave *et al.* 2008). In the study of Karjalainen *et al*. (2014) the eggs of one female were fertilized with a mixture of milt of three males which leaves a possibility for a sperm competition and cryptic female choice to increase fertilization success. In this study, however, eggs of each female were fertilized separately with the milt of individual males. Therefore, if the egg and the sperm were incompatible, fertilization rate stayed low. In whitefish there was no considerable difference in mortality between the design used in this experiment (6.2 %) and the communal-spawn simulating design (7.9 %). Whitefish is

presumed to have some kind of pre-spawn mate choice because the males develop breeding tubercules during the breeding season (Wedekind *et al*. 2001). This can mean an absence of post-spawn fertilization control mechanism and explain the much higher fertilization success compared to vendace. Whitefish also reach maturity later than vendace (Sandlund *et al*. 2013) and whitefish females probably invest more to their eggs than vendace females.

A full-factorial breeding design should be conducted in such a way that there will be sufficient amount of replicates for sufficient power for the 2-way ANOVA analysis. However, this is not always possible. For example, in this incubation experiment it was difficult to obtain enough eggs and milt for a sufficient amount of replicates, especially for vendace. In whitefish, it was difficult to obtain a sufficient amount of parent fish for the experiment. The reliability of the results could be increased by obtaining more replicates for vendace and more parent fish for whitefish. However, the analyses showed a strong parental effect on the early development of the studied fish and the results can be considered valid.

5.2. Parental relatedness and inbreeding

In addition to the parental effect on the egg development dynamics, relatedness of the vendace parents used in the incubation experiment was estimated using 13 coregonid specific microsatellite loci. Both full- and half-sibs were found from the sample population, but of the parents used in the experiment, only two pairs were found to be half-sibs. Female 1 was found to be half-sib with males 5 and 7. Since all the eggs of female 1 stayed unfertilized or died shortly after fertilization, the effect of relatedness on fertilization and survival and growth of offspring cannot be separated. Female 1, however, had the highest inbreeding coefficient of all females.

Inbreeding is breeding of individuals that are genetically closely related. The relationship between increasing inbreeding coefficient and declining fitness has been noticed both in wild populations (reviewed in Keller and Waller 2002) and in controlled environments (Fessehaye *et al*. 2007). The lowered fitness of the inbred individuals often appears as lowered survival and reproduction success. These harmful effects of inbreeding depression have been observed also in several fish species (Kincaid 1976, Mrakovčič and Haley 1979, Fessehaye *et al*. 2007). In this study, offspring of the individuals with the highest inbreeding coefficients had low survival rates. Also offspring of the parent pairs with highest pairwise kinship coefficients had low survival rates. In most of the cases, the highly inbred individuals had very low fertilization success or their offspring died already in the very early stages of the development.

Also offspring of the individuals with the lowest inbreeding coefficients had low survival rates. Outbreeding can be advantageous in some cases but it can also lead to outbreeding depression, the reduction in fitness that occurs following breeding of two distantly related or unrelated individuals (Lynch 1991). McClelland and Naish (2007) reviewed outbreeding studies in fishes and found the responses of outbred individuals differing between the studies widely from negative to positive. For example, Monson and Sadler (2010) found reduction in mating frequencies and clutch sizes when crossing distantly related lines of zebrafish. In this study, individuals with the lowest inbreeding coefficients (lowest F = -0.22) also had low reproduction success, similar to highly inbred individuals. This result indicates that outbreeding can have similar harmful effects as does inbreeding in vendace.

In this study the individuals with an inbreeding coefficient closest to zero had the highest reproduction success. According to the optimal outbreeding hypotheses, there is an optimal genetic relatedness between mates that stands somewhere between kin and hybrid genes (Bateson 1978). Mating with kin leads to inbreeding depression and with genetically distant individuals to outbreeding depression. Studies with salmonid species have shown that their mate choice is often driven by the optimal genetic dissimilarity, usually in the major histocompatibility complex (Forsberg *et al*. 2007, Evans *et al*. 2012).

Some of the markers used to estimate genetic relationships among vendace were shown to have possible null alleles. Null alleles are alleles that fail to amplify in PCR, either because of non-ideal PCR conditions or because the primer-binding region contains mutations that inhibit binding (Selkoe and Toonen 2006). Because of the null alleles, some heterozygotes are genotyped as homozygotes, which leads to a heterozygote deficit. Some individuals also may fail to amplify any alleles. ML-Relate detected heterozygote deficiency in four loci; Bwf1, ClaTet6, ClaTet9 and Cocl-Lav52; but it was difficult to determine if the heterozygote deficiency was due to null alleles or for other reasons. Other possible explanations for the heterozygote deficiency can be for example the so-called Wahlund effect. The Wahlund effect refers to heterozygosity deficiency in a population caused by a subpopulation structure (Wahlund 1928). In this study, parent fish and the samples used as a reference population for the parents were caught at different times. The samples are assumed to be part of the same spawning population because they were caught from the same area within a short time period (1 week), but because sample sizes were fairly small, allele frequencies might differ between the samples due to random sampling error. However, according to the goodness of fit -test, the allele frequencies did not differ between the two populations. Præbel *et al*. (2013) also detected significant heterozygote deficits in four loci including Cocl-Lav52 and ClaTet9, which indicated presence of possible null alleles.

A few of the specimens from the reference population had low signal, which made scoring the alleles impossible in some cases. Some of the specimens were re-amplified and re-analyzed because of the low signal. Some of the specimens had had low signal most probably because of pipetting error in PCR amplification process or in moving the samples to the sequencing plate. Some of them, however, had low signal probably because of unsuccessful DNA extraction. In the first DNA extractions, larger pieces of muscle were cut out and used in the extraction than in the later ones, which made the solutions too viscous. This could have led to samples with an insufficient amount of extracted DNA. In total, 17 individuals had unscorable alleles due to low signal in one or more loci. Two of these individuals were deleted from the analysis because of unscorable alleles in more than three loci.

When estimating the relationship categories, in some cases the log likelihoods of other relationships were not much lower than the log likelihood of the relationship that was assigned to the pair. This can mean that the data were not powerful enough for the test. Genotyping more loci and a larger sample size of the reference population could improve the estimates. Allele size ranges and allele numbers were observed to be larger than what was observed in the study of Præbel *et al*. (2013) even though a larger sample size was analyzed in their study. Præbel *et al*. (2013) analyzed a sample that was collected from one Norwegian and one Finnish lake. Different populations can have very different size ranges for the same markers.

5.3. Conclusions

The mean survival of vendace was much lower in this study, where a full-factorial breeding design was used, in comparison to the earlier study of Karjalainen *et al*. (2014), where eggs of one female were fertilized with a mixture of milt of three males. These results support the possibility that vendace have some kind of post-spawn fertilization control mechanism even though it is assumed to be a communal-spawning species. This would be an interesting objective for future research. Overall, results of this study verify the prediction, that maternal effects have a strong influence on the early development of fish. However, the results also show that the paternal origin and mate compatibility can also influence many traits, especially in the later stages of larval development and on the embryonic mortality. The parental effect on hatching time of fish has not been studied much but this study indicates that especially maternal effects can be an important influence on hatching time and that paternal effect and maternal-paternal interaction might also be important. The flexibility in hatching time between the larvae of a year class can provide plasticity for a population to adjust to the inter-annual changes in temperature by ensuring that at least part of the year class hatching in optimal conditions.

The experiment was designed initially to study the parental effect on the hatching time, but it gave an opportunity to also study if the parental relatedness or the inbreeding coefficient of the parents could explain differences in embryonic survival. In this study all the parents that had close to zero reproduction success also had very high or low inbreeding coefficients (F). Therefore, it seems that for vendace, the inbreeding coefficient of the individual is related to its reproduction success. The kinship coefficient between the parents also seemed to affect the reproduction success of the pair, especially when the values were positive. The kinship between the parents might be one factor that affects the post-spawn fertilization control, if there is a mechanism for the gametes to recognize non-compatible genotypes. This would also be an interesting subject for future research. Also, it would be interesting to see if a similar relationship exists in whitefish.

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